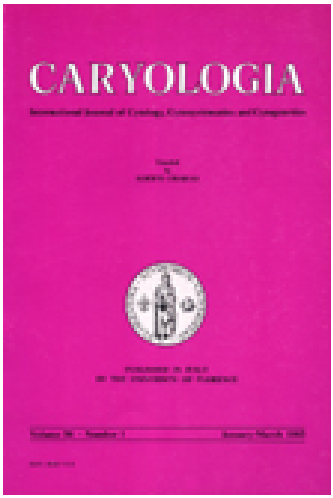

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Cytophotometric Analyses and in Vitro Culture Test in the Embryo First Node of Old Triticum Durum Caryopses

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CYTOPHOTOMETRIC ANALYSES AND *IN VITRO* CULTURE TEST IN THE EMBRYO FIRST NODE OF OLD *TRITICUM DURUM* CARYOPSES *

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SUMMARY — The nuclei of the first node embryo of *Triticum durum* show the same amount of histone bound to DNA in young and in aged seeds in line with earlier findings of remaining metabolic activity. Furthermore the explants of the first node from the old seeds analyzed, are still able to generate calli and plantlets and might be usefull to recover embryos from aged seeds.

INTRODUCTION

During storage not all the embryo areas of *Triticum durum* seeds response with the same sensitivity against the damaging effects of ageing. Namely, in the embryo of old non-germinating seeds the first node cells retain a DNA, RNA and protein synthetic activity similar to that found in young viable seeds, whereas the cells in the root apex reduce or loose their metabolic activity as soon as the seeds fail to germinate (INNOCENTI and FLORIS 1979).

Since meristematic root nuclei alone in *Triticum durum* caryopses had a higher amount of fast-green stainable histones in aged than in young embryos (INNOCENTI and BITONTI 1979) the loss of the metabolic activity might be related, in some way, to the inactivation of the chromatin by the nuclear histones.

As the cells of the first node in the aged seeds of *T. durum* still show a marked synthetic activity (INNOCENTI and FLORIS 1979) it seems of interest to verify in this embryo area:

1) if the fast-green stainable histones increase with ageing;

2) if the explants of aged seeds are able to grow in *in vitro* culture.

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MATERIAL AND METHODS

Young (crop 1979) and old (crop 1977) caryopses of *Triticum durum* cv. Cappelli stored at room temperature were used. Previously the germination rate of young and old seeds was determined on a lot of 100 seeds of each crop; the result was 100% and 3% respectively.

A) *Cytophotometric analyses*: seven old and seven young dry caryopses were fixed in 10% neutral formalin and washed overnight. One portion of the embryo first nodes from old and one from young caryopses were excised under a dissecting microscope and squashed separately on one single slide in 45% acetic acid. The root apices from the utilized embryos were squashed as control. The squashes were stained by the Feulgen method, replacing 1 mol. dm^{-3} trichloracetic acid (TCA) for 1 mol dm^{-3} hidrochloric acid (HC1), and mounted in Canada balsam. Measurements of DNA content in individual nuclei were carried out with a microscope photometer Zeiss 01, at 565 nm (McLEISH and SUNDERLAND 1961). The nuclei were thereafter stained with fast-green FCF (at pH 8.1) and scanned for histones at 635 nm (ALFERT and GESCHWIND 1953; BLOCH and GODMAN 1954; PILLER 1977); for each sample at least 100 nuclei were analyzed. The double measurement of DNA and histones in the same nuclei was made possible by locating the individual nuclei in microphotographs.

B) *In vitro culture*: Dry caryopses were surface sterilized by immersion into 0.5% mercuric chloride solution for 3 minutes. After three washes in sterile deionized water, the seeds were sown on water imbibed filter paper in sterilized Petri dishes and stored in the dark at 25° C for 20 h. The embryos were excised in a sterile roo munder dissecting microscope and the first node zones were separated and transplanted to flasks (5-6 per flask) containing 0.03 dm^{-3} of agar-solidified Smith's medium supplemented with (mg. dm^{-3}) casein hydrolysate 1000, inositol 100 and naphtalene acetic acid (NAA)[5] (MASCARENHAS *et al.* 1975; BENNICI and D'AMATO 1978). The cultures were incubated in the growth room under continuous illumination of 2500 lux delivered by daylight fluorescent tubes.

RESULTS AND CONCLUSIONS

The cytophotometric analyses clearly demonstrate that the distribution of histone/DNA ratios in the embryo first node nuclei is practically the same in young (crop 1979) and in old (crop 1977) seeds (Fig. 1 A). Though some

TABLE 1 — *Callus formation and regenerated plantlets from the first node of « durum » wheat embryos in young seeds (crop 1979) and in old seeds (crop 1977: two experiments).*

crop	number of explants	calli %	regenerated plantlets %
1979	56	85.7	29
1977 a	148	50	6.7
1977 b	58	55	6.9

authors demonstrated that, with the loss of viability in aged embryos, DNA lesions occur (CHEAH and OSBORNE 1978), we found in the nuclei of the first node (which are all in the presynthetic phase of the mitotic cycle, G_1, 2C) DNA values per nucleus are rather homogeneous (mean DNA value: 0.19 ± 0.02; standard error 10%). Thus, the fast-green measuraments show a distribution very much like the one shown in Fig. 1A. The variability within

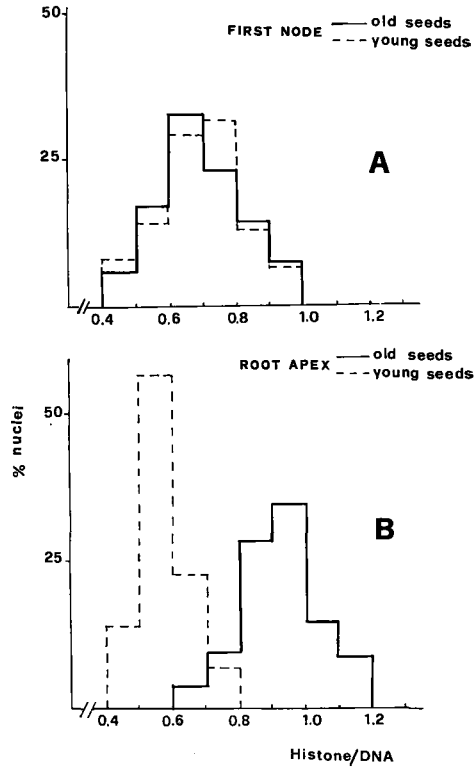

Fig. 1. — Frequency distribution of histone/DNA ratios in young (crop 1979) and old (crop 1977) caryopses of *T. durum*. In the first node nuclei (Fig. 1A) and in the 2C meristematic root nuclei (Fig. 1B). Broken-line histogram: young seeds; unbroken line histogram: old non-germinating seeds.

each distribution probably depends on the moment of the G_1 phase at the time of seed ripening (GURLEY *et al.* 1974).

If we now compare these results with the data collected in the 2C (G_1) meristematic root nuclei analyzed as control, in line with the results shown in a previous paper (INNOCENTI and BITONTI 1979), it appears clearly that: i) in the young seeds the histone/DNA ratio in the root apex nuclei is lower than in the first node nuclei (Fig. 1A and 1B); ii) in the old seeds, the histone/DNA ratio increases significantly in the root apex nuclei while remains unchanged in the first node nuclei (Fig. 1B). In accordance with our knowledge on the

function of histones (HUANG and BONNER 1962; BILLEN and HNILICA 1964), our results indicate that nuclei with the same DNA content (2C) show a different portion of the chromatin repressed by the histones when considered in different embryo areas of the resting seeds.

Furthermore, as the histone/DNA ratios distribution, during seed storage, undergoes variations in the root nuclei but remains practically the same in the first node nuclei, we may suggest that the quantity (HUANG and BONNER 1962; BERLOWTIZ 1965) and/or quality (SWIFT 1964) of the proteins bound to DNA might influence the ageing pattern of the single embryo areas.

To better clarify the described differences let us now consider the 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 σo and σy are the standard errors of the corresponding distribution) as defined by INNOCENTI and BITONTI 1981. We have calculated that Ap = 0.53 in the embryo first node while Ap = 11.33 in the root apex. This parameter gives a quantitative view of the cytological changes produced in the nuclear components during ageing, which are imperceptible in the embryo first node and conspicuous in the root apex. Therefore in the first node the nuclei retain their synthetic activity (INNOCENTI and FLORIS 1979) and undergo the ageing process slowly. Also the « *in vitro* » results support the assumption that the first node cells in old seeds are still in good condition and able to perform their metabolic activity. Namely, the *in vitro* culture results demonstrate that: after seven days of culture, whitish, friable calli were produced from the first node explants and after fifteen days some shoots with or without roots were observed. As shown in Table 1 the cells from old non-germinating seeds, were still able to divide and generate, in good percentages (about 7%) plantlets. These results provide useful informations for the recovery of embryos from aged seeds.

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