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# Polyamine Accumulation in Aged Wheat Seeds

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**Abstract.** The present work was conducted to evaluate the content of the main polyamines (Spm, Spd, Put) in a series of naturally aged durum wheat seeds as well as the activities of the enzymes ODC and ADC involved in their biosynthesis. In dry seeds the content of polyamines, especially that of Spd, rose during ageing till 6 years and then declined sharply. However, an increase of PA content upon imbibition was observed only with the youngest seeds, while a decrease was found in the older ones.

The activities of ODC and ADC differed in aged seeds, the ODC activity being constant and lower than the ADC in the course of seed ageing. The ADC increased till the early ageing and decreased then in the very old, ungerminating seeds. Imbibition increased both enzyme activities in the youngest seeds only, in the older ones rather a decrease and changed ADC/ODC ration was found.

The obtained results are discussed in relation to the participation of these enzymes in the biosynthesis of polyamines during seed ageing and in the course of plant senescence or stress.

*Additional index words: Triticum durum*: spermine ; spermidine ; putrescine ; arginine decarboxylase ; ornithine decarboxylase ; ageing.

Recent investigations indicate that polyamines (PA) may play an important regulatory role in a variety of growth and developmental plant processes such as seed germination and growth, response to stress, senescence (GALSTON 1983, SLOCUM *et al.* 1984). Data were reported that reveal that exogenous PA may retard the progressive senescence of oat leaf protoplasts (ALTMAN *et al.* 1977), cause the inhibition of RNase activity and solute leakage from tuber disc of *Solanum* by stabilizing membranes against leakage (ALTMAN 1982a), inhibit protease activity (KAUR-SAWHNEY *et al.* 1982a, BALESTRIERI *et al.* 1987) and prevent chlorophyll breakdown in detached, dark-incubated leaves of several plants (ALTMAN 1982b, CHENG and KAO 1983).

The diamine putrescine (Put) and, to a lesser extent, spermidine (Spd) accumulate in cereal leaf segments exposed (YOUNG and GALSTON 1983, FLORES and GALSTON 1982) to osmotic stress. This in parallel with an increase in the activity of

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arginine decarboxylase (EC 4.1.1.19; ADC), while the ornithine decarboxylase (EC 4.1.1.17; ODC) remained unaffected. In spite of PA analysis in several senescing plant organs and tissues that reveals a correlation between senescence and low level of Put, Spd and spermine (Spm) (ALTMAN and BACHRACH 1981), some reports indicate that PA concentration can increase dramatically during incubation of senescing leaves (KAUR-SAWHNEY et al. 1982b) and flowers (ROBERTS *et al.* 1984) this being in correlation with the increase of ADC activity mainly.

Nevertheless no detailed studies as to the relation of whole plant senescence to PA titers are available and very few data exist as regards the PA metabolism in ageing seeds (ANGUILLESI *et al.* 1974, ANGUILLESI and FLORIS 1975, MUKHOPADHYAY *et al.* 1983).

Loss of vigour in ageing wheat grains is accompanied by the degradation of r-RNA in dry embryos (GRILLI *et al.* 1982) and by changes in phospholipid composition resulting in membrane damage (PETRUZZELLI and TARANTO 1984). Since PA may stabilize both nucleic acid and membranes during senescence, the present study was carried out to determine PA titre and the activity of their biosynthetic enzymes in the embryo from ageing *Triticum durum* seed.

## MATERIALS AND METHODS

### Plant Material and Germination Conditions

*Triticum durum* cv. Cappelli seeds from crops 1979, 1980, 1983, 1984, 1985 and 1986 harvested in open field at Plant Science Department of Pisa University (S. Piero a Grado) were stored in sealed glass containers and naturally aged in the dark at laboratory conditions. Dry hand-isolated embryos were used for all the experimental procedures.

The viability of seeds of different age was evaluated by testing germination pattern in sterile conditions, at 25 °C in the dark, in Petri dishes with filter paper imbibed with distilled water. The seed germination was recorded twice daily and at 72 h from the beginning of imbibition the shoot and root lengths of seedlings were determined.

Germination percentage was calculated at 16, 24, 40, 48, 64 and 72 h; 6 replicates of 50 seeds each for the different crops were considered.

### Polyamine Determination

PA were extracted from 50 isolated embryos from dry and 24 h imbibed seeds. The embryos were homogenized in 5% $HClO_4$, centrifuged and the crude extracts were dansylated according to the Seiler's method (SEILER 1971). Dansylated PA

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*Abbreviations used*: ADC = arginine decarboxylase; DTT = dithiothreitol; EDTA = ethylene diamino tetracetic acid; ODC = ornithine decarboxylase; PA = polyamine(s); PLP = pyridoxal-5'-phosphate; Put = putrescine; Spd = spermidine; Spm = spermine.

were then extracted with 0.5 ml benzene and separated on Silicagel TLC plates with cyclohexane : ethyl acetate (5 : 4 v/v) as solvent.

Fluorescent spots, corresponding to comigrated PA standards, were scraped from plates, eluted by 3 ml acetone and quantified in a spectrophotofluorimeter with the excitation and emission wavelengths at 365 and 500 nm, respectively.

#### Extraction and Measurement of ADC and ODC

The enzymes were extracted from 300 isolated embryos homogenized with cold Tris–HCl buffer at pH 8.1, containing EDTA 50 μM, PLP 25 μM and DTT 2.5 mM. After centrifugation, the supernatant clear fraction was used as crude enzyme and for protein determination. Aliquots of crude enzyme, corresponding to 50 embryos, were placed in glass vials containing 18.5 kBq of [U-$^{14}$C]-L-arginine or [U-$^{14}$C]-L-ornithine, sealed with rubber caps and equipped with little wells containing filter paper (1.5 × 2 cm) imbibed with 300 μl Picofluor to trap $^{14}CO_2$ liberated. As a control assay the crude enzyme boiled prior to test was used.

The reactions in vials incubated at 37 °C for 2 h, were stopped by injecting 0.5 ml of 25% TCA. The vials were then incubated for an additional 30 min. The liberated $^{14}CO_2$ was determined by placing the filter paper into 10 ml of Econofluor with 50 μl of acetic acid and by counting the radioactivity by a scintillation counter. Enzyme activity was expressed in nmol ($^{14}CO_2$ liberated) $mg^{-1}$ (protein) $h^{-1}$.

#### Protein Content

Protein content of the crude enzyme extracts was measured according to the modified method of Lowry (BENSADOUN and WEINSTEIN 1976).

The presented data are the mean of 4 replicates.

## RESULTS AND DISCUSSION

#### Loss of Vigour during Seed Ageing

We tested the viability of a series of progressive naturally aged wheat grains (from 1 year old to 8 year old seeds), from 1986 (1 year old seeds), 1985 (2 years), 1984 (3 years), 1983 (4 years), 1980 (7 years) and 1979 (8 years) crops, respectively.

Ageing of dry wheat seeds results in a progressive loss of their vigour (ROBERTS 1960, ROBERTS and ABDALLA 1968) as may be seen from both the decrease of their germination energy and capacity. The 1985 seeds exhibit (Table 1) at 16 h and 24 h higher germination energy than the 1986 seeds. These latter in fact maintain a residual effect of the relative dormancy which characterizes this cultivar (MELETTI 1968). The first signal of the loss of viability appears in the 3 year old 1984 seeds which strongly reduce their germination energy and capacity and, as a consequence, their growth. The strong decrease of seed viability appears in this cultivar (FLORIS

TABLE 1

Germination and seedling growth of a series of progressive aged *Triticum durum* seeds

| Harvest year | Age [years] | % germination at time [h] | | | | | | Growth [mm] at 72 h | |
|---|---|---|---|---|---|---|---|---|---|
| | | 16 | 24 | 40 | 48 | 64 | 72 | Shoot | Root |
| 1986 | 1 | 34 ± 2.1 | 58 ± 1.8 | 78 ± 0.9 | 88 ± 0.5 | 94 ± 0.1 | 100 | 20 ± 0.8 | 58 ± 2.1 |
| 1985 | 2 | 42 ± 2.4 | 75 ± 1.2 | 82 ± 0.7 | 90 ± 0.4 | 96 ± 0.1 | 100 | 21 ± 0.4 | 57 ± 1.8 |
| 1984 | 3 | 6 | 26 ± 3.6 | 70 ± 1.7 | 74 ± 1.6 | 77 ± 1.6 | 78 ± 1.6 | 13 ± 1.1 | 38 ± 2.3 |
| 1983 | 4 | 0 | 2 | 12 ± 2.8 | 14 ± 2.5 | 16 ± 2.5 | 16 ± 2.5 | 10 ± 1.7 | 34 ± 1.3 |
| 1980 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1979 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

± SE = Standard error of means

1970) when the 4 year old 1983 seeds are considered: they exhibit a very low germination capacity but are capable of reaching, at 72 h, a growth similar to younger 1984 seeds. This means that the 1983 seed population can be distinguished into two groups: the ungerminating group and the germinating one still having a growth power similar to the younger 1984 seeds.

**Polyamine Content**

The imbibed embryos from aged seeds exhibit (Fig. 1) a lower content of free PA than dry embryos – mainly when the 1984 and 1983 and the oldest seeds are considered – whereas the 1986 imbibed embryos have a much higher one than the dry seeds. Most pronounced changes were found in the level of Spd, while changes in the Put and Spm levels were much lower. This fact suggests the occurrence of a quick leakage and/or a rapid catabolism of these molecules in the early old seed imbibition, while in dry seeds, the PA content/embryo ratio increases with ageing and appears to drop, mainly for Spd, only in the oldest seeds incapable of germinating.

When the values are expressed on the protein content basis (Fig. 2), the above described increase is more pronounced. Also the oldest 1980 and 1979 dry seeds

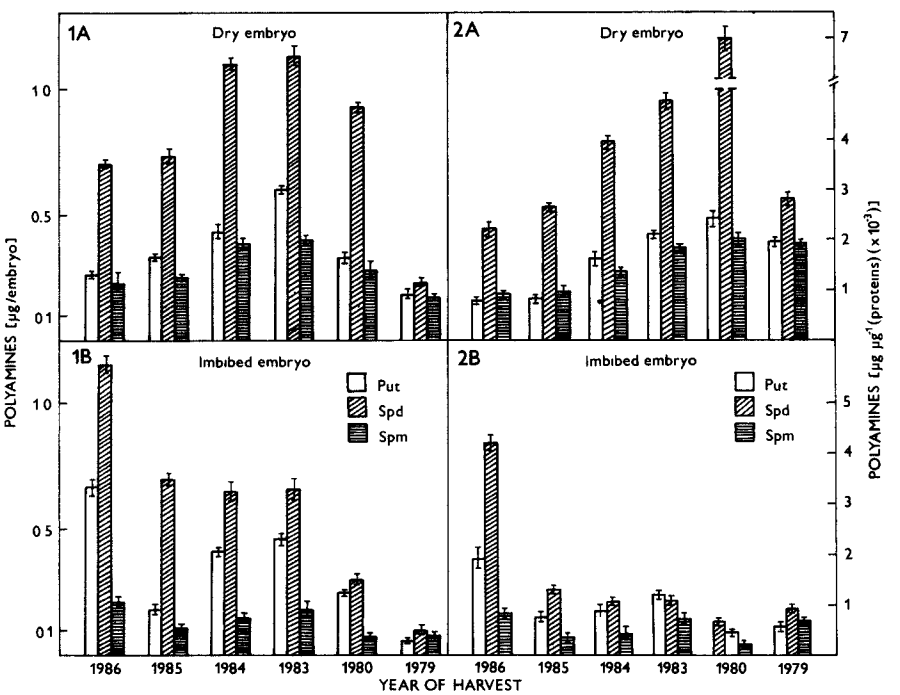


Fig. 1. Changes in polyamine amounts of dry (A) and imbibed (B) embryos from a series of aged *Triticum durum* seeds. Put – putrescine; Spd – spermidine; Spm – spermine. Bars are ± SE of 4 replicates.

Fig. 2. Changes in polyamine amounts of dry (A) and imbibed (B) embryos from a series of aged *Triticum durum* seeds. Put – putrescine; Spd – spermidine; Spm – spermine. Bars are ± SE of 4 replicates.

contain very high relative amounts of PA, a fact not seen when the values were expressed on the embryo basis. After 24 h of imbibition only the youngest 1986 embryos show PA content higher than the older imbibed ones. In this way their high vigour and perfect reactivation of the germination process and of biosynthetic enzymes machinery were confirmed. The older seeds lose these capabilities and exhibit very low content of PA, irrespective of their age.

**ADC and ODC Activity**

In dry seeds the activity of embryo ADC, tested *in vitro* as liberation of $^{14}CO_2$, differs significantly from that of ODC (Fig. 3). In the dry young 1986 embryos the ODC activity is very low and remains at low values in all the embryos studied. The ADC activity, in spite of its low level in 1986 dry young embryos, increases sharply after one year of storage when seeds are still showing full vigour. This activity declines again in the oldest seeds but with the exception of the very old 1979 embryos remains higher than in the young 1986 embryos. This suggests that during the ageing

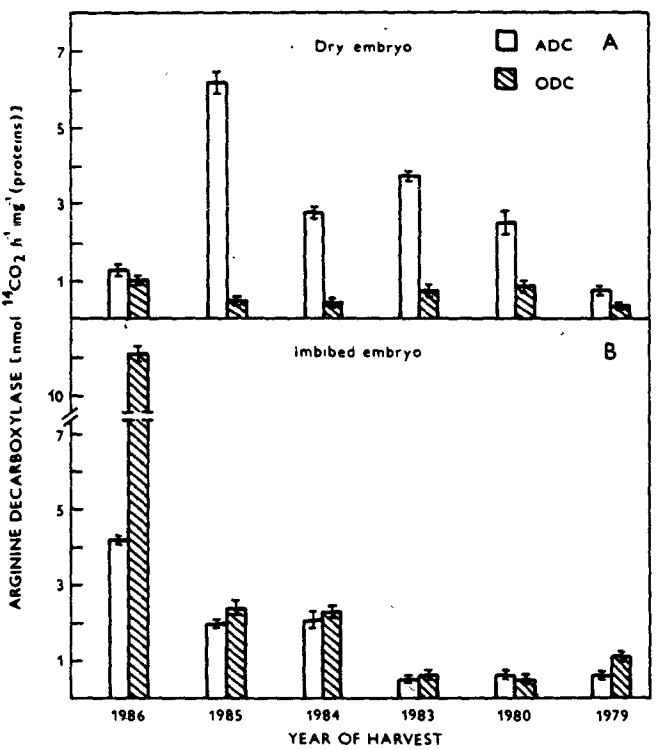


Fig. 3. Activities of arginine decarboxylase (ADC) and of ornithine decarboxylase (ODC) in dry and imbibed embryos of a series of aged seeds of *Triticum durum*. Bars are ± SE of 4 replicates.

of wheat in dry conditions, the increase of PA level, as shown in Figs. 1 and 2, is mainly due to the increase of ADC activity.

The imbibition quickly induces a rise in the activity of both enzymes in young 1986 seeds and this dramatic increase, mainly for ODC, parallels the increase in PA titer recorded in the youngest 1986 embryos (Figs. 1 and 2). In the imbibing seeds, after the first year of storage, the embryos reduce or loose their ability to react by increasing ADC and ODC activities; the 1983 embryos reach the lowest enzymatic activity whereas they still are capable of exhibiting a 16% germination capacity. Moreover it can be noted that, in imbibed embryos, the ODC is the enzyme which shows higher specific activity in the *in vitro* tests. Consequently, it appears to be probably the main responsible enzyme of Put biosynthesis in young rapidly germinating wheat seeds in which, after 24 h of imbibition, the apical meristems are known to undergo the first cycle of mitotic division (TAGLIASACCHI and VOCATURO 1977).

The obtained results confirm previous reports (ANGUILLESI *et al.* 1974, ANGUILLESI and FLORIS 1975, MUKHOPADHYAY *et al.* 1983) on the increase of these "antisenescing" molecules during ageing, although these data contrast with other reports on declining PA titer in senescing tissues or organs (ALTMAN and BACHRACH 1981, KAUR-SAWHNEY *et al.* 1982b). It is possible that this discrepancy might be due to the fact that the term "ageing" is utilized for indicating quite different processes as, for instance, the development of the first leaf from 4 to 21 d in oat seedlings (KAUR-SAWHNEY *et al.* 1982b). In these experiments not fully expanded "ripe" leaves were used but meristematic, expanding ones, which have very high PA titer. Moreover, the variations of both PA titer and enzymatic activities are only one aspect of the complex biochemical systems leading to senescence and death, which are, in turn, controlled by various factors which can act for very different periods: years for ageing of seeds but hours for detached leaf segments or tuber discs (ALTMAN and BACHRACH 1981, KAUR-SAWHNEY *et al.* 1982b).

Since senescence exhibits several characteristics in common with the various kinds of stress, the rise of PA content and ADC activity in ageing but not necrotic tissues or organs, could be considered as the first physiological response to ageing stress: the plant tissues, to be able to oppose the free radical mediated lipid peroxidation, increase the production of substances, such as polyamines, capable of scavenging activated oxygen (DROLET *et al.* 1986). The deleterious effects quickly induced by the tissue wounding can alter natural PA metabolism in the leaf segments or tuber discs.

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## BOOK REVIEW

DAVIS, T. D., HAISSIG, B. E., SANKHLA, N. (ed.): ADVENTITIOUS ROOT FORMATION IN CUTTINGS. – Advances in Plant Sciences Series Vol. 2. Dioscorides Press/Timber Press, Portland, Oregon 1988. 315 pp. US $ 39.95 + 3.00.

Root formation in cuttings has been of interest to plant scientists and horticulturists for many years. The ability of cuttings to form adventitious roots and to restore the whole plants provides a means of propagation of plants with unique properties. Many of the procedures increasing the rooting capacity are still used without understanding why they are effective, although adventitious root formation attracted much attention of research workers interested in morphogenesis. The need of a better understanding of the control of plant regeneration from cuttings stimulated fundamental investigation in physiology, biochemistry and genetics. The editors succeeded in preparation a book which brings together and evaluates the involvement of physiological and morphogenetic factors which interact in the process of regeneration. The book is an excellent example of plant physiology focussed on a single problem of morphogenesis. It is a comprehensive and well balanced treatment of the problem, informing the reader about the literature data, but also pointing to the controversies and to the areas which need further investigation. It contains 22 chapters written from different aspects by 26 contributors from 10 countries. Some of the chapters are devoted to the physiological status of the stock plant. Environmental conditions for plant cultivation and cutting storage are discussed. Much attention is given to the relationship between maturation of the stock plant and reduction of rooting capacity. About one-third of the book deals with hormonal regulation of root formation. Attention is paid mostly to auxins and their commercially used analogs. Convincing evidence of root-promoting properties of auxins and the confusing data on the variations in levels of and sensitivity to auxins point to gapes in understanding the regulatory mechanisms. The multistage nature of rooting is shown in all chapters devoted to phytohormones. About one-third of the book concerns other areas of plant physiology in relation to rooting behaviour of cuttings. Chapters on mineral nutrition, carbohydrate metabolism, enzyme activities, photosynthesis and water relations are included. There is also a chapter briefly summarizing literature on root formation in tissue culture and another one on root formation in plants transformed with T-DNA of *Agrobacterium rhizogenes*. Perspectives and outlooks are added in conclusion. There is a reasonable amount of references given at the end of each chapter and an adequate subject index at the end of the book.

The book is very important to horticulturalists which rely on adventitious root formation. It is useful to plant scientists working on any of the problems of morphogenesis, and teaching plant physiology. It should be available in the libraries of institutions engaged in research programs of plant development and morphogenesis.

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