

Biochemical changes in embryos of dormant and after-ripened seeds of *Caryophyllaceae*

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Abstract

The dependence of ^{14}C -leucine, ^3H -uracil and ^3H -thymidine incorporation in dormant and after-ripened seeds is demonstrated and discussed together with other metabolic events. Striking differences in dependence on the physiological state in both types of seeds could only be found in the axes of after-ripened seeds.

INTRODUCTION

Recent investigations on seed germination revealed that plant embryos are a suitable material for the analysis of processes of growth and development, because their physiological activity can be changed or modified in several ways (i.e. phytohormones, temperature, light, aeration, osmotic pressure). Freshly harvested seeds of *Vaccaria pyramidata* and *Agrostemma githago*, when soaked, show a developmental arrest preventing growth of radicles and thus germination. After-ripened, i.e. physiologically activated, seeds, however, germinate normally under favorable conditions often a few hours after soaking. It should be said, that even such general terms like dormancy and germination have been rarely defined to the satisfaction of everyone (Côme, 1970; Khan, Tao, 1978).

We use the most widely accepted definition of germination, which is based on an easily detectable event and is characterized by the visible protrusion of the radicle through the seed coat. Another definition, according to Khan and Tao, (l.c.), considers resumption of growth of the embryo or the root-shoot axis which may or may not result in protrusion of the testa as germination. Based on the four phases imbibition, biochemical activation, mitotic activity and elongation of radicles (Evnari, 1957, 1961) or also those of Hecker (1978) the latter definition assumes an event before radicle protrusion. So Khan and Tao, (l.c.) conclude that it has not been definitely resolved whether the beginning or absence of biochemical and structural changes is related to germina-

tion or dormancy. According to Côme (l.c.) we also refer to the frequently used definition for dormancy, i.e. dormant seeds or embryos are incapable of germination even under favorable conditions. Though the reasons for dormancy of seeds can be very different, in the case of *Caryophyllaceae* the embryos themselves exhibit dormancy, which is after harvest a primary dormancy. Further as a result of unfavorable conditions, e.g. far-red or high temperatures, respectively, after-ripened seeds can again be prevented from germination (i.e. thermodormancy).

Investigations on the biosynthesis of macromolecules of after-ripened and dormant embryos should lead to a better understanding of the rules involved in the biochemical changes of activity of seeds, because differences may have causal relations to the physiological activity of both the states of embryos. The metabolism of proteins and nucleic acids during seed germination has been extensively analyzed (Spiegel et al., 1975; Payne, 1976; Bewley, Black, 1978) nevertheless the mechanisms regulating activation or maintainance of dormancy are incompletely understood. Results of some authors demonstrate that seed germination may be regulated by a repression of large parts of the genome, expressed by a diminished synthesis of RNA in the arrested embryos. During after-ripening by means of a derepression of parts of the genome physiological activation should take place. Our investigations on *Caryophyllaceae* seeds, however, show no increase of either protein or RNA synthesis in after-ripened seeds and this is true also for some other species (*Oryza sativa*, *Avena fatua*). Thus, the above mentioned hypothesis (Jarvis et al., 1968; Khan et al., 1968) may be valid only for some seeds requiring stratification (*Corylus avellana*).

MATERIAL AND METHODS

Dormant (stored at -20°C to prevent after-ripening) and after-ripened seeds (dry storage at room temperature) of *Vaccaria pyramidata* Med. var. *typica* Gürke and of *Agrostemma githago* L. were used. For comparison fruits of *Fraxinus excelsior* L. were investigated (cf. stratification: Krauss et al., 1980).

For measurement of the ^{14}C -leucine, ^3H -uracil and ^3H -thymidine incorporation see to Hecker and Köhler (1979) and Hecker et al. (1981). Affinity chromatography with poly(U)sepharose — Hecker and Köhler (1979).

RESULTS AND DISCUSSION

The germination of *Vaccaria pyramidata* seeds (Köhler, Borriß, 1967; Hecker, Köhler, 1979), is illustrated in Fig. 1, including dormancy, thermodormancy and environmental influences. After-ripened

seeds germinate very well under the favorable conditions of 20°C and in the presence of activated charcoal in Petri dishes (1). None or only few seeds, however, germinate under the same conditions, if the seeds were dormant (2). Thermodormancy was induced in after-ripened seeds if temperatures of 30°C were applied (3). It is remarkable, that *Vaccaria* seeds without charcoal in the medium are capable of germination at 10°C but not at 20°C (5). Moreover from curve 4 may be seen that charcoal increases and accelerates the germination of these seeds (10°C).

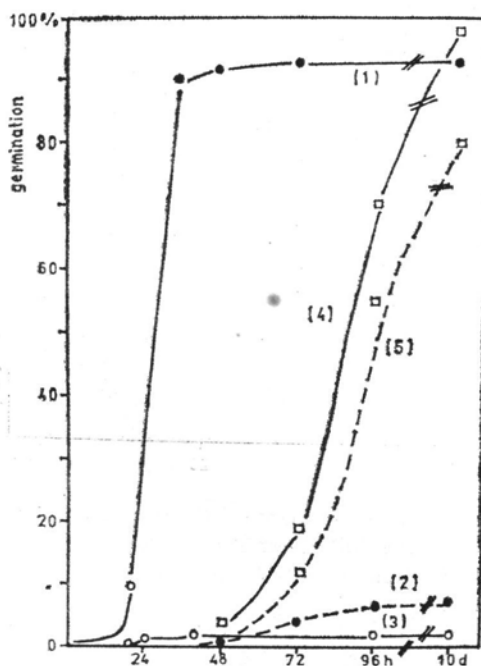


Fig. 1. Germination of *Vaccaria pyramidata* seeds under different conditions (1) — after-ripened, $+20^{\circ}\text{C}$, with charcoal; (2) — dormant, 20°C , with charcoal; (3) — after-ripened, $+30^{\circ}\text{C}$, with charcoal; (4) — after-ripened, $+10^{\circ}\text{C}$, with charcoal; (5) — after-ripened, $+10^{\circ}\text{C}$ without charcoal.

The rapid uptake of water (within a few hours) by the seeds and the above mentioned imbibition phase according to Evenari (1957, 1961) are clearly visible in Fig. 2. A further remarkable phenomenon seems to be that imbibition of dormant seeds during the first hours of soaking occurs more rapidly than in after-ripened ones. The easier way to isolate the embryos from the seeds during this time proves true for this finding, too. As regards the results of Knypl and Janas (1979) were to clarify whether this phenomenon is caused by different characteristics of the membranes in the dormant seeds or by a different imbibition behaviour of the proteins in the embryo. In this connection it must be em-

phasized that dormant seeds were stored in desiccators at -20°C . To exclude the possibility that differences in the degree of imbibition were caused by chilling, we equilibrated the dormant seeds for 1, 2, 4 or 6 days, respectively at a temperature of $+20^{\circ}\text{C}$ (and vice versa the after-ripened ones were stored for comparison for the same time at -20°C). In Fig. 3 no effect of equilibration on the water content during the imbibition process of dormant after-ripened seeds under changed tem-

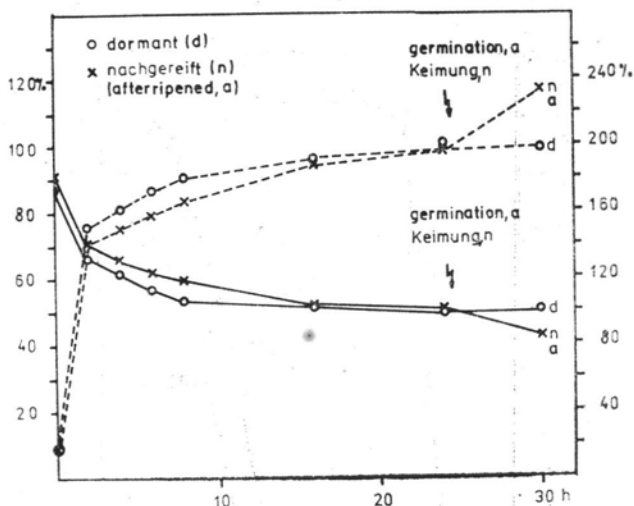


Fig. 2. Uptake of water of dormant and after-ripened *Vaccaria* seeds. Left: dry weight in % of the initial embryo weight; right: uptake of water as percent of initial water content.

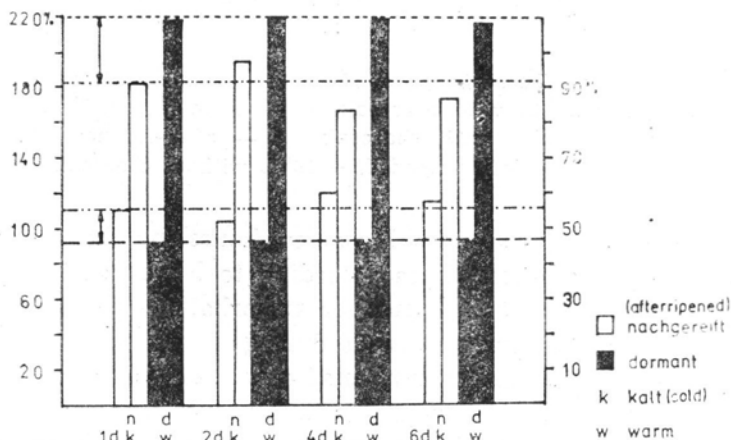


Fig. 3. Influence of different times of temperature equilibration on the uptake of water of dormant and after-ripened *Vaccaria* seeds (determined after 10 h of imbibition)

peratures can be seen, i.e. the difference between dormant and after-ripened seed demonstrated in Fig. 2 is not caused by a simple effect of chilling on the dormant seeds. As mentioned, the imbibition phase proceeds to the phase of activation of biochemical processes.

In earlier investigations (Köhler, 1965; Köhler, Borriß, 1967) we showed an increase of the free amino acid content within the imbibition phase up to 48 h, at 1-3°C, 10°C, 20°C and also 30°C. As compared with 20°C the rise of free amino nitrogen was faster at 30°C. Further the increase in free amino acids was independent of the physiological state of the seeds. After germination, of course the expected dramatic increase in free amino acid content in the seedlings followed, but in those seeds which were incapable for germination (dormant, thermodormant) the amount of free amino acids declined after about 1 (30°C) or 2 (20°C) days of imbibition.

The development of patterns of enzyme activity should also be mentioned here briefly. Dormant embryos differ from after-ripened ones, besides their prevented growth processes, also by their developmental enzyme pattern and competence on phytohormone treatment (Borriß, 1977). In dormant embryos *de novo* synthesis is totally blocked despite the observed general protein and RNA synthesis. Thus, we could detect no activity of amylase (Abraham, Köhler, unpublished) or threonine-deaminase activity (Schmidt, Köhler, in preparation) in dormant, or in after-ripened seeds. Borriß (l.c.) discusses the changing activity patterns of different enzymes in dependence on the physiological state and on environmental conditions or on phytohormone treatment.

Analysing the genetic basis of dormancy in *Agrostemma* and *Antirrhinum* seeds Borriß (1977) concluded that the developmental block is regulated by only a few genes.

The synthesis of both protein and RNA is activated at the beginning of the imbibition phase (Hecker, 1978). In *Vaccaria pyramidata* (Fig. 4) and *Agrostemma githago* seeds (results not shown here) we obtained no correlation between the level of ³H-uracil and L-¹⁴C-leucine incorporation into macromolecules and the physiological state of the seeds. A comparison of RNA fractions (using double-labeling for dormant and after-ripened embryos) after electrophoretic separation of RNA on polyacrylamide gels (Hecker, Köhler, 1979) revealed that there were no differences in the two ribosomal RNA fractions (tRNA and heterogeneous RNA). Moreover, the RNA from dormant and after-ripened embryos can hybridize with poly(U)-sepharose to the same degree (Fig. 5). The fraction eluted with a buffer of lower ionic strength at 20°C should contain poly(A) mRNA with shorter poly(A) chains, while the fraction eluted at 50°C should contain poly(A) mRNA with longer

poly(A) sequences (Harris, Dure, 1974). It may be concluded that: in dormant (and also thermodormant) *Vaccaria* seeds poly(A) mRNA and also other RNAs are transcribed with undiminished intensity. It may be concluded further that the small differences in the imbibition behaviour were without detectable influences. On the other hand, it is well known that drastic application of osmo-conditioning reduces the time of imbibition required for the onset of RNA and protein synthesis as well as polyribosome formation (Khan et al., 1978).

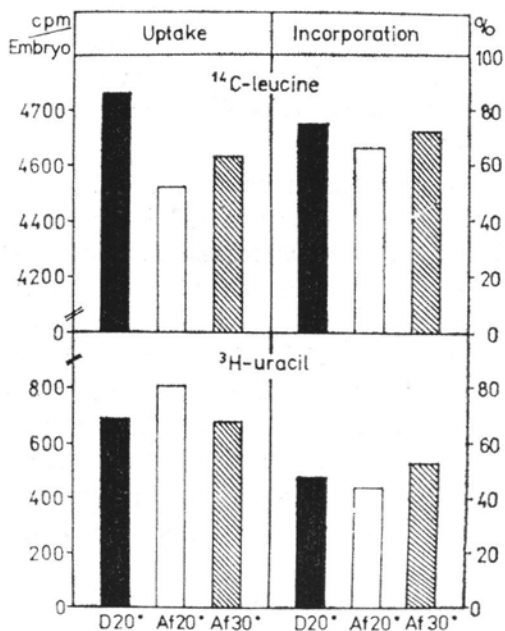


Fig. 4. Uptake and incorporation of ^{14}C -leucine or ^3H -uracil into proteins or nucleic acids, respectively, of dormant (D) and after-ripened (Af) seeds at 20°C or 30°C (= thermodormant)

Incorporation of ^3H -thymidine into the DNA of *Vaccaria* embryos, however, shows a striking correlation to the developmental state (Fig. 6), especially in the axes (but not in the cotyledons). This is in accordance with results of Robinson and Bryant (1975) concerning the onset of nucleic acid synthesis during germination of *Pisum sativum* L. It is of course of outstanding interest to know the enzymes involved in these regulatory processes, and there are many attempts to correlate these syntheses with the activity of polymerases. Jenns and Bryant (1978), however, have recently proved that a rather dramatic increase in chromatin-bound DNA with its endonucleolytic activity in the embryonic axes immediately prior to the start of DNA replication may regulate DNA synthesis. Evidence has been obtained in this case for an

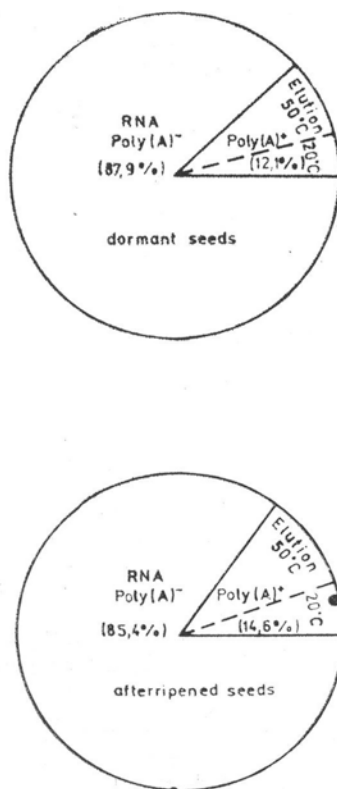


Fig. 5. Hybridization on poly(U)-sepharose of RNA synthesized in dormant and after-ripened embryos of *Vaccaria* during the period of imbibition

inhibitor of DNase in the chromatin preparations from embryo axes not undergoing DNA replication.

This may be a further hint in favour of the hypothesis that it is not the general synthesis of proteins and RNA, but rather the appearance of specific proteins that may play a crucial role in the development or maintenance of developmental blocks (Hecker, Köhler, 1979; Borriß, 1977). The effect of phytohormones (GA_3 , BAP = benzylaminopurine as a cytokinin, ethylene) and their combinations on the overcoming of dormancy is shown in Fig. 7. In the figure an insert is included with the effect of these phytohormones on 3H -thymidine incorporation into DNA. BAP as well as ethylene, however, and especially the combined application of BAP and ethylene increase dramatically DNA synthesis (Hecker et al., in preparation).

In this connection we will make a brief comparison with the processes occurring during stratification of ash-seeds (Fig. 8). Only fruits transferred from the warm (22°C) to the cold (3-5°C) are able to grow

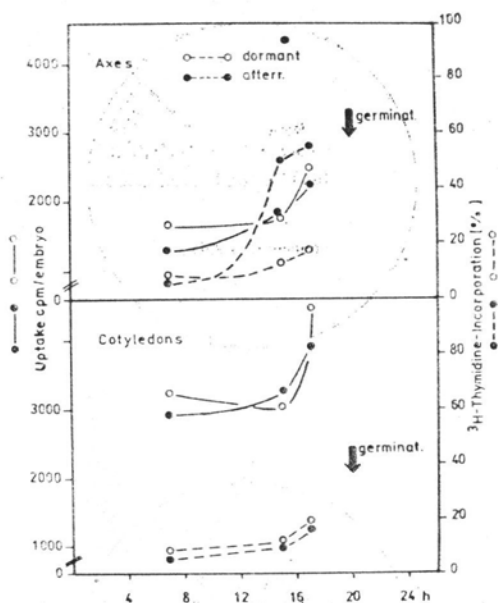


Fig. 6. Uptake and incorporation of ^3H -thymidine into DNA in axes or cotyledons, respectively, of dormant and after-ripened seeds

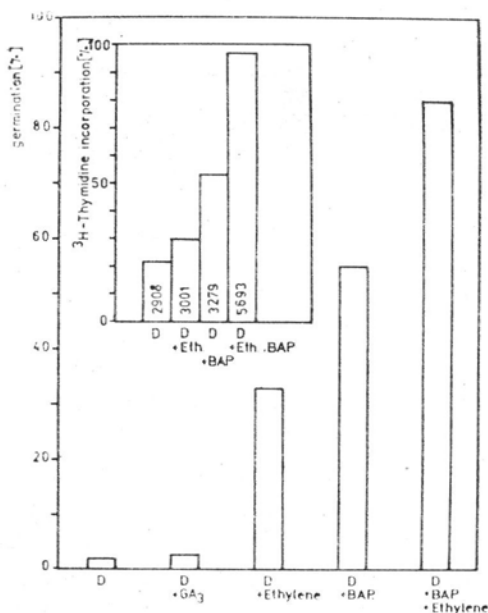


Fig. 7. Influence of phytohormones (gibberellic acid = GA_3 (50 $\mu\text{g/l}$); benzylaminopurine = BAP (50 $\mu\text{g/ml}$); ethylene (10 p.p.m)) on the germination of *Vaccaria* seeds (% germination after 8 days)

Insert: uptake (c.p.m./embryo; in the bars) and incorporation (%) of ^3H -thymidine under the influence of the phytohormones.

(see fresh weight = FG) and to germinate. ^{14}C -leucine incorporation takes place with nearly equal intensity and time dependence in both these variants, also; the incorporation of uracil reveals a nearly equal pattern. ^3H -thymidine incorporation, however, increases drastically only in the warm/cold-variant.

Abb. 4 Prozeßabläufe während der Stratifikation (*Fraxinus excelsior* L.)

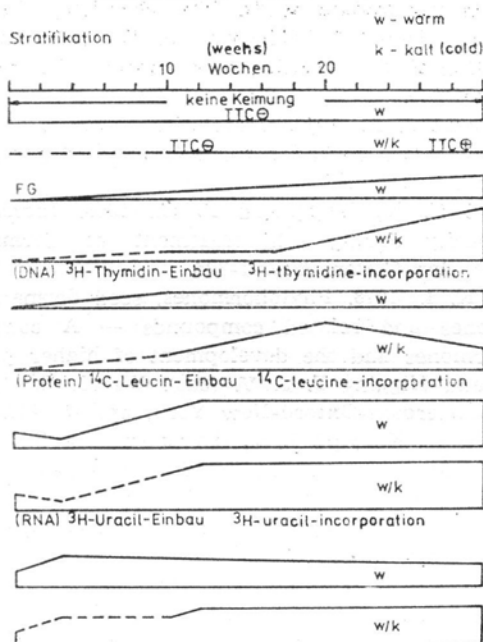


Fig. 8. Diagram of the processes occurring during stratification of ash embryos
— = warm; — — — = cold; FG = fresh weight; TTC = reduction of triphenyl-tetrazolium-chloride.

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