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# RELATION BETWEEN DNA SYNTHESIS AND GERMINATION OF *VACCARIA PYRAMIDATA* SEEDS

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**Key Word Index**—*Vaccaria pyramidata*; Caryophyllaceae; dormant seeds; after-ripened seeds; DNA biosynthesis; cytokinin; ethylene.

**Abstract**—Dormant seeds of *Vaccaria pyramidata* are characterized by a high level of [$^{14}$C]leucine incorporation into proteins, a high level of [$^3$H]uracil incorporation into RNA and an undiminished synthesis of polyA$^+$ RNA; however, DNA synthesis is very much reduced. Germination of dormant *Vaccaria* seeds can be induced either partially by cytokinins or more completely by cytokinins and ethylene. Cytokinins cause a drastically increased [$^3$H]thymidine incorporation in cotyledons of imbibing seeds. This effect is enhanced by ethylene. The incorporation of [$^3$H]thymidine in radicles is also stimulated, but to a lesser extent. Cytokinin-dependent stimulation of [$^3$H]thymidine incorporation can be referred to as an activation of nuclear DNA synthesis.

## INTRODUCTION

Phytohormones decisively interfere with the physiological processes of seed germination and may determine ways preceding the involvement of transcriptional and translational nucleic acid control of enzyme formation at the ribsomal level [1–3]. Indeed, a major question in dormancy seems to be the degree of involvement of this regulation. Frequently ethylene [4], different gibberellins [5] or cytokinins [6] are involved in the breaking of dormancy, whereas abscisic acid can induce or maintain dormancy. Further characteristic changes in the phytohormone dynamics and inhibitor contents were observed during the naturally occurring after-ripening processes [7–12]. However, details of these regulations, starting from the initial reactions of the phytohormones (i.e. binding at receptors) up to the complex regulation of physiological processes (i.e. overcoming of seed dormancy and germination) are not fully understood. Therefore, all processes detectable before the emergence of the radicle in the seeds should be of particular interest, because they may be important in this reaction sequence. It can be assumed that they may be involved directly or indirectly in the regulation of activity changes.

We have found that dormancy of seeds of *V. pyramidata* (Caryophyllaceae) can be broken by simultaneous application of cytokinins and ethylene. It was shown that this physiological activation of the seeds was preceded by a striking stimulation of the [$^3$H]thymidine incorporation in cotyledons.

## RESULTS AND DISCUSSION

### [$^3$H]*Thymidine incorporation in cotyledons and axes of dormant and after-ripened seeds of V. pyramidata*

Immediately after harvest the seeds of *V. pyramidata* are in a natural state of dormancy; they are not able to germinate at 20° (Fig. 1). Only after a period of after-ripening for some weeks, during which the seeds were stored in a dry state at room temperature were they capable of germination (Fig. 1; cf. ref. [13]). A few hours before protrusion of the radicle the [$^3$H]thymidine incorporation in the axes increased, whereas it remained at a far lower level in the cotyledons (Fig. 2). In contrast to the synthesis of RNA and proteins, the synthesis of DNA in imbibing seeds did not start immediately after soaking [13–18] and the incorporation of [$^3$H]thymidine into DNA showed a clear dependence upon the physiological state of the seeds. Dormant seeds incorporate far less [$^3$H]thymidine than after-ripened ones. This depression of DNA synthesis was limited to the axes. In cotyledons of dormant as well as after-ripened seeds, a similarly low level of [$^3$H]thymidine incorporation was measured (Fig. 2; see also ref. [13]). After the radicle has protruded (i.e. after 20 hr) a significantly

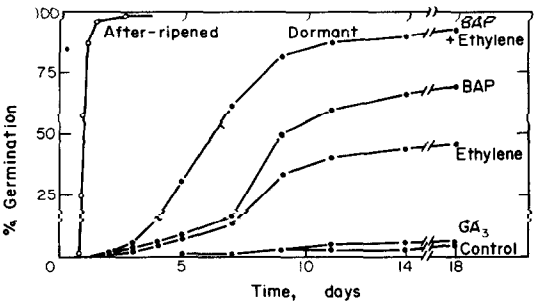


Fig. 1. Germination of dormant (●) and after-ripened (○) seeds of *V. pyramidata*. The influence of gibberellic acid ($GA_3$; 50 ppm), ethylene (10 ppm) and benzylaminopurine (BAP; 50 ppm) on the germination of dormant seeds. Abscissa: Days of germination at 20°.

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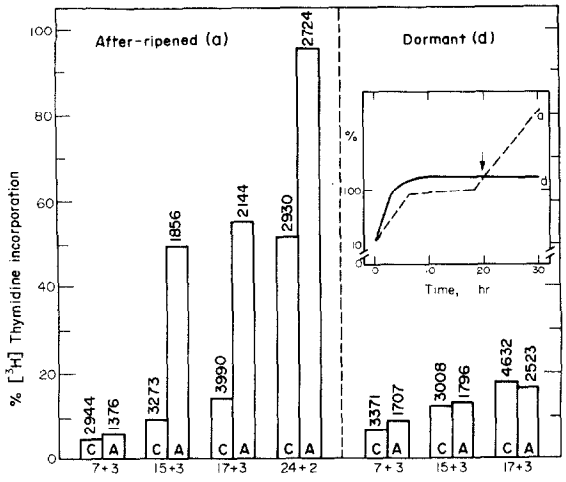


Fig. 2. Incorporation of [$^3$H]thymidine (as a percentage of the rate of [$^3$H]thymidine uptake) by axes (A) and cotyledons (C) of dormant and after-ripened seeds of *V. pyramidata* during the imbibition period (7, 15, 17 hr imbibition, 3 hr incubation in 20 $\mu$Ci [$^3$H]thymidine). The uptake rates (cpm and per embryo part) are indicated on the top of the columns. After germination the incorporation in the cotyledons rises; it remains at a low level in dormant ones (of Fig. 3). Included in the figure: Water uptake of dormant (d) and after-ripened (a) embryos as a percentage of the initial weight. The arrow indicates the radicle protrusion of after-ripened seeds. Abscissa: Imbibition time in hr (for importance of water see ref. [26]).

enhanced [$^3$H]thymidine incorporation began in the cotyledons of the after-ripened seeds (Fig. 2). This was observed in seeds of *V. pyramidata* [19] as well as in seeds of *Agrostemma githago*. This observation is remarkable in that in cotyledons of germinating seeds of *A. githago* and *V. pyramidata* cell divisions have not been found [19]. This thymidine incorporation is mainly due to nuclear DNA synthesis [19].

### *The influence of phytohormones on the incorporation rate of dormant seeds*

Dormant seeds stored at $-20°$ maintained dormancy, and at room temperature they germinated even after a very long period of imbibition only to a maximal amount of 5–10% (see also ref. [13]). Gibberellic acid did not enhance germination, whereas ethylene applied as a gas and above all benzylaminopurine were more effective. Most effective in breaking dormancy was a combined application of benzylaminopurine and ethylene (Fig. 1).

### *The influence of phytohormones on the incorporation of* [$^3$H]*thymidine in dormant embryos of* V. pyramidata

Neither gibberellic acid [unpublished data] nor benzylaminopurine markedly affected the incorporation of [$^3$H]thymidine in after-ripened embryos. This phenomenon may be due to the already very high rates of incorporation in untreated after-ripened controls. However, the action of phytohormones on the incorporation of [$^3$H]thymidine in dormant embryos was parallel to the dormancy breaking effect, i.e. applied in combination, a cytokinin and ethylene, but not gibberellic acid, stimulated DNA synthesis.

After treatment with benzylaminopurine, a significant enhancement of the [$^3$H]thymidine incorporation, mainly in the cotyledons, was obtained. Compared with the untreated controls after 24 hr imbibition the increase was to 100%; it advanced further to 300% after 72 hr.

The most pronounced stimulation of [$^3$H]thymidine incorporation was seen after a combined treatment with ethylene and benzylaminopurine (Fig. 3). Gibberellic acid has no effect on the incorporation of [$^3$H]thymidine, the amounts were identical with the controls and therefore were not demonstrated separately (Fig. 3).

Particularly striking was the finding that the stimulation of [$^3$H]thymidine incorporation was first detectable in the cotyledons and only at a significantly later period and much less clearly in the radicles (Fig. 3). In contrast, the phytohormones had no influence on the incorporation of L-[$^{14}$C]leucine [20]. Furthermore, the results indicate that the DNA synthesis occurring after germination in cotyledons (Fig. 2) is triggered in dormant embryos by phytohormones (BAP, ethylene) even before the radicle protrudes (see also Figs. 1 and 3).

The amount of [$^3$H]thymidine incorporation measured in imbibing embryos significantly reflects the physiological activity of the seeds: a high degree of [$^3$H]thymidine incorporation is a characteristic marker for the imminent protrusion of the radicle, whereas in physiologically blocked seeds the DNA synthesis remains at a low level (seeds of *A. githago* [19]); seeds of *V. pyramidata* [13]). The dormancy of the *V. pyramidata* seeds can be broken by phytohormones, especially by treatment with cytokinins

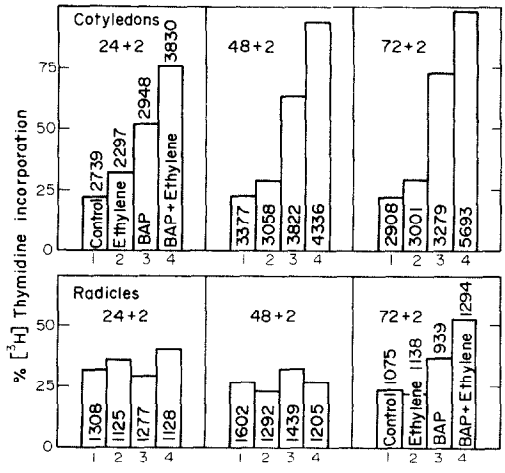


Fig. 3. Incorporation of [$^3$H]thymidine (as a percentage of the rate of [$^3$H]thymidine uptake) by cotyledons and radicles of dormant seeds of *V. pyramidata* during the imbibition period under the influence of 10 ppm ethylene and 50 $\mu$g/ml benzylaminopurine (BAP) (24, 48, and 72 hr imbibition, 2 hr incubation in [$^3$H]thymidine). The uptake rate (cpm and per embryo part) is indicated in the columns.

and ethylene. This dormancy breaking action of the phytohormones is preceded by a significant stimulation of [$^3$H]thymidine incorporation, which was first detected in cotyledons and later in radicles. The effect of benzylaminopurine on the measurable [$^3$H]thymidine incorporation in dormant seeds could be detected even after 16–24 hr soaking. After a prolonged imbibition it increased from 300 to 600% in comparison to the control.

It can generally be reported that the DNA synthesis of cotyledons is affected only by those phytohormones which are able to break seed dormancy. These results may indicate a causal relationship between the activation of DNA synthesis in cotyledons and the breaking dormancy.

[$^3$H]Thymidine incorporation in cotyledons of dormant seeds initiated by cytokinin is caused by DNA synthesis, as was shown by MAK-CC of radioactively labeled nucleic acids prepared from the cotyledons (Fig. 4). Radioactivity was found mainly in the DNA peak. Therefore metabolization (degradation) of [$^3$H]thymidine and an incorporation of the [$^3$H] activity into RNA could be excluded [21]. Investigations on the nature of the DNA synthesized after application of ethylene are in progress (cf. refs. [22, 23]).

## EXPERIMENTAL

For the investigation dormant (i.e. seeds stored at −20° to prevent after-ripening) and after-ripened seed (i.e. dry storage at room temp.) of *Vaccaria pyramidata* Med. var. *typica* Gürke harvested in 1975 were used. The seeds were soaked on moistened filter paper in Petri dishes to which 30 μg/ml chloramphenicol was added in the dark. After a definite period of imbibition for each sample 20 embryos were isolated from the seeds and incubated with a soln of 10 μCi methyl-[$^3$H]thymidine (sp. act. 9 Ci/mmol, vol. 1 ml) for 2 or 3 hr with the addition of 30 μg chloramphenicol (see also ref. [13]).

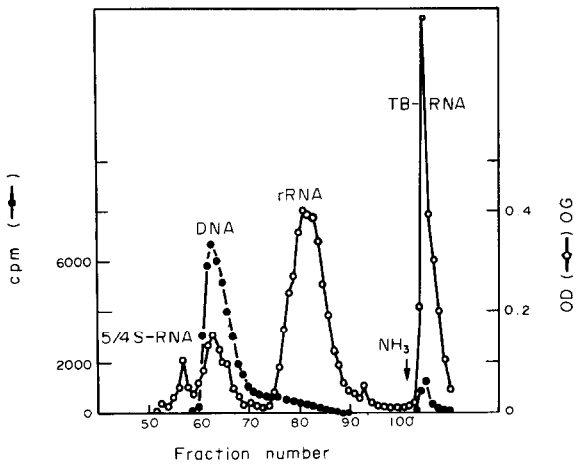


Fig. 4. Separation of nucleic acids by MAK-CC from cytokinin-activated *V. pyramidata* seeds. Dormant seeds of *V. pyramidata* were treated with benzylaminopurine (50 μg/ml; 48 hr, 20°) during the imbibition period; isolated embryos were fed with [$^3$H]thymidine. Nucleic acids were prepared from cotyledons and separated by MAK-CC. The label is found in the DNA fraction.

In most cases the incorporation by cotyledons and axes was determined separately by excision of the parts from the intact embryos after incubation. The determination of the uptake and of the incorporation of [$^3$H]thymidine into substances precipitable by TCA was performed according to ref. [24] modified in refs. [23, 25]. The rates of uptake and incorporation were expressed as cpm per embryo part and as percentage incorporation of the total precursor uptake.

Preparation of nucleic acids and their separation by MAK-CC were carried out as described earlier [26].

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