

Regulation of Bud Rest in Tubers of Potato, *Solanum tuberosum* L.

VII. EFFECT OF ABSCISIC AND GIBBERELIC ACIDS ON NUCLEIC ACID SYNTHESIS IN EXCISED BUDS¹

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ABSTRACT

The effect of gibberellin A₃ (10⁻⁴M) and abscisic acid (10⁻⁴M), applied separately and together, on incorporation of ³H-thymidine and ³H-uridine into DNA and RNA of buds from freshly harvested potatoes was investigated. In some treatments apical buds in intact tubers were treated three times daily for 3 days with test solution before the buds were excised and treated an additional 12 hours in Petri dishes. In other treatments, untreated buds were excised and treated 12 hours. Irrespective of length of treatment, gibberellin A₃ slightly promoted synthesis of DNA and RNA, and abscisic acid essentially blocked such synthesis, in both the presence and absence of gibberellin A₃.

Cumulative evidence favors the concept that the rest period of many buds and seeds is under control of growth promoters and inhibitors (1, 33). Thomas, Wareing, and Robinson (29) indicated that the dormancy of buds of *Betula* and *Acer* is regulated by a balance of ABA³ and gibberellin-like substances (GAs). Chrispeels and Varner (6) reported that ABA inhibited GA₃-induced α -amylase synthesis, and Nitsch (19) and Khan (15) showed that GA₃-induced germination of lettuce seeds was completely inhibited by ABA.

Hemberg (11) was the first to suggest a direct correlation between the rest period and the occurrence of growth-inhibiting substances in the potato tuber. A later investigation (12) with extracts of potato peelings revealed that the concentration of inhibitor β -complex decreased and essentially disappeared at the time rest terminated. In contrast, GAs in potato buds increased in concentration near the end of rest (26, 27). Boo (3) reported a low concentration of GAs in freshly harvested potatoes but found that concentration increased to a maximum some weeks before rest terminated.

In excised buds from resting potato tubers, prolonged inhibition of sprouting by inhibitor β -complex or ABA was partly reversed by exogenous GA₃ (2, 18). In intact tubers, GA₃ only slightly reversed the inhibition by ABA (9).

The emergence of potato buds from rest appears not to be controlled qualitatively by any environmental factor, but requires only the passage of time (24). For example, field-grown potatoes

(cv. White Rose) stored under favorable conditions sprout about 20 to 25 days after a harvest made 100 days after planting. Thus, emergence from rest is regulated by an internal timing mechanism, a conclusion indicated by results of Tuan and Bonner (30) and Rappaport and Wolf (22, 23). This internal regulation was associated with the initiation of nucleic acid synthesis. Tuan and Bonner (30) showed that RNA and DNA increased prior to the increase in fresh weight of potato buds the rest period of which had been terminated with ethylene chlorhydrin. Deoxyadenosine, actinomycin D, and puromycin—inhibitors of nucleic acid and protein syntheses—reduced sprouting of buds in excised potato plugs, indicating that these syntheses are associated with the end of rest (18). Using histoautoradiographic techniques, Rappaport and Wolf (22, 24) related timing of GA₃ action, changes in concentration of nucleic acids, and the onset of growth in excised potato buds. They found that the application of GA₃ to excised buds dramatically enhanced synthesis of DNA and RNA within 12 hr, well before onset of cell elongation or division. This paper presents information on the effects of ABA and GA, alone and in combination, on synthesis of DNA and RNA in excised potato buds.

MATERIALS AND METHODS

Resting White Rose potato tubers harvested 80 to 100 days after planting were used in all experiments. The tubers were washed with tap water and surface-sterilized with 1% sodium hypochlorite (Purex) for 10 min. After they had been rinsed twice with distilled water, they were dried overnight at room temperature. The treatment solutions contained ³H-thymidine or ³H-uridine (20 μ C/ml) as well as ABA or GA₃, or both. For controls, the treatment solutions were prepared without hormones. In some experiments 5 μ l of test solution were applied to apical buds on intact tubers daily for 3 days, after which the buds were excised and treated with test solutions in Petri dishes for 12 hr. In other experiments excised buds only were treated. Buds were excised with a 2-mm "de-eyer" (20). The plugs, 10 per treatment, were placed in sterilized Petri dishes containing one disk of Whatman No. 1 filter paper and 1 ml of treatment solution.

After 12 hr in the dark at 20°, the buds were fixed in formalin-acetic acid, dehydrated with ethanol, embedded in Tissuemat, sectioned, and subjected to emulsion autoradiography according to Rappaport and Wolf (22, 24). After the emulsion was developed and the sections were stained, the number of labeled nuclei in the buds was counted. The grains at two randomly selected loci in the median section of the subapical region of the ³H-uridine-treated buds were counted. The density of the grains was measured spectrophotometrically with a microscope. The length of the buds was measured with an ocular micrometer.

RESULTS

During the first 12 hr after excision, synthesis of DNA and RNA in buds treated only with precursors occurred mainly in

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³ Abbreviations: ABA: abscisic acid; GA₃: gibberellin A₃.

Table I. Incorporation of ^3H -Thymidine ($^3\text{H-T}$) by Apical Buds Treated with GA_3 and ABA, Separately and Together

Control buds were not treated with hormones. Some buds were either pretreated (Pre) for 3 days with 10^{-4} M GA_3 or with 10^{-4} M ABA before excision and then treated in petri dishes an additional 12 hr. Others were only treated for 12 hr after excision. They were then fixed in formalin-acetic acid, embedded in Tissuemat, sectioned, and subjected to histoautoradiography. Data are the means of 30 buds.

Treatment	Labeled Nuclei per Bud		
	Apical cells	Vascular tissue	Leaf primordia
$^3\text{H-T} + \text{H}_2\text{O}$	1.5 ± 0.19	12.6 ± 1.05	7.0 ± 1.87
$^3\text{H-T} + \text{GA (Pre)}$	1.8 ± 0.28	17.6 ± 1.58	8.0 ± 1.09
$^3\text{H-T} + \text{GA}$	1.2 ± 0.11	15.4 ± 1.26	6.8 ± 0.68
$^3\text{H-T} + \text{ABA (Pre)}$	0.1 ± 0.05	3.8 ± 0.37	3.4 ± 0.13
$^3\text{H-T} + \text{ABA}$	0.1 ± 0.05	3.7 ± 0.29	2.9 ± 0.54
$^3\text{H-T} + \text{GA} + \text{ABA}$	0.1 ± 0.09	3.9 ± 0.29	2.6 ± 0.33

Table II. Incorporation of ^3H -uridine ($^3\text{H-U}$) by apical potato buds treated with GA_3 , or ABA, or both

Control buds were treated with precursors prepared in water. The buds were either pretreated for 3 days with $5 \mu\text{l}$ per bud three times daily and treated for 12 hr after excision, or they were excised and treated only for 12 hr. They were then prepared histologically as in Table I. Percentage transmission through the sections was determined spectrophotometrically, and the number of grains per $100 \mu^2$ was determined by actual count of grains in two randomly selected loci in the subapical region of median sections.

Treatment	Avg No Grains $100 \mu^2$	Percentage Transmission
$^3\text{H-U} + \text{H}_2\text{O}$	35.5 ± 5.05	87.0
$^3\text{H-U} + \text{GA (Pre)}$	50.8 ± 4.32	85.5
$^3\text{H-U} + \text{GA}$	44.8 ± 3.29	84.5
$^3\text{H-U} + \text{ABA (Pre)}$	20.7 ± 2.11	94.0
$^3\text{H-U} + \text{ABA}$	20.5 ± 3.70	90.0
$^3\text{H-U} + \text{GA} + \text{ABA}$	23.8 ± 4.60	93.0
Control (H_2O)	10.8 ± 2.45	100.0

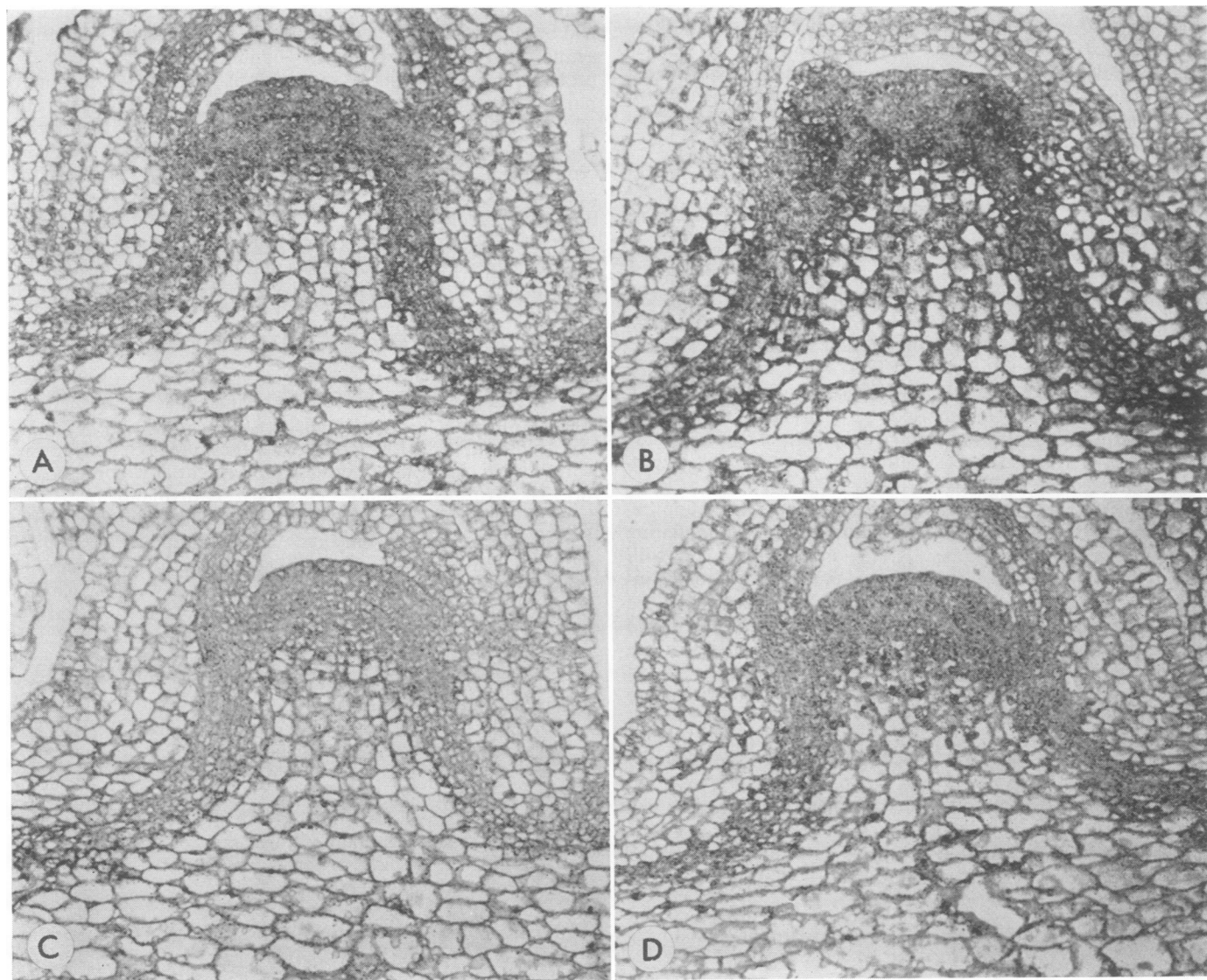


FIG. 1. ^3H -Uridine incorporation in excised potato buds 12 hr after treatment with H_2O (control) (A), GA_3 (B), ABA (C), or ABA + GA_3 (D).

cells of the vascular and provascular tissues (Table I). Leaf primordia and apical cells had few labeled nuclei.

Gibberellin A₃ promoted synthesis of RNA and DNA, but less so than previously reported (22, 24). ABA markedly reduced incorporation of both ³H-thymidine and ³H-uridine (Fig. 1, Table II). The effect was particularly dramatic as incorporation of both precursors by the control buds was already evident after 12 hr. When ABA and GA₃ were applied together in equal concentration (10⁻⁴ M), ABA overcame almost completely the capacity of GA₃ to promote synthesis of RNA and DNA (Tables I and II, Fig. 1).

Treatment of apical buds in intact tubers for 3 days prior to excision did not significantly alter incorporation of precursors (Table I and II, Fig. 1). Control buds incorporated more of the precursors than did buds treated with ABA, but less than buds treated with GA₃. Bud length did not differ significantly among treatments. The results were reproducible over three separate experiments, each involving 10 buds.

DISCUSSION

The pattern of tritium labeling of nuclei indicated that a progression of mitotic reactivation, beginning in the procambium, had occurred in the buds. Tepper and Hollis (28) observed the same phenomenon in white ash (*Fraxinus americana* L.). They considered such mitotic activity to be the result of translocation of growth substances in the cambial tissue. Lovell and Booth (17) obtained similar results with potato shoots. They suggested that GA₃ affected the subapical meristem, an effect analogous to that of this hormone in inducing bolting in rosette plants. Direct evidence for the presence of ABA in the translocation stream was provided by Hoad (13) and Lenton, Bowen, and Saunders (16). However, translocation is not limited to phloem tissues, since Edelman, Jefford, and Singh (8) observed, in sprouting potato tubers, that transport of soluble reserves can occur over the entire tuber, and is not restricted to the vascular shell. In our translocation studies with ³H-GA₁ (unpublished), the labeled material was not confined to any particular tissue of the excised potato plug. Thus, control of mitotic reactivation may be related to the concentrations of growth substances that are present in, or that move to, the meristematic region.

Wounding influences the production of gibberellin-like substances in potato tissues (18, 21). The amount of such substances is essentially nil in freshly wounded, cortical tissue slices; but after 12 hr the amount is sufficient to account for the acceleration of sprouting observed in excised plugs (21). Additional evidence indicating production of GAs in the wounded periphery of plugs has been presented by Madison and Rappaport (18). They showed that treatment of plugs with AMO-1618, an inhibitor of gibberellin synthesis, blocked sprouting, and that a very low dosage of GA₃ quickly reversed the inhibition. Rappaport and Wolf (22), therefore, proposed that gibberellin-like substances produced in the wounded cells along the periphery of the plug move into the bud, thus enhancing nucleic acid synthesis. Bradshaw and Edelman (4) also showed that wounding results in an increase in gibberellin-like substances in the cut surface layers of Jerusalem artichoke (*Cynara scolymus* L.).

Autoradiographic evidence indicating promotion of nucleic acid synthesis by both GA₃ and wounding has been presented by Rappaport and Wolf (22-24). Our results on synthesis of DNA and RNA in both control and GA₃-treated buds are in accord with those of Rappaport and Wolf (22), although the latter found less mitotic activity in the controls. Alternative explanations for induction of nucleic acid synthesis in buds involve loss of a volatile inhibitor (5), which in intact tubers is thought to repress such synthesis (23), or the destruction of ABA or related substances as a result of wounding.

In the present studies only the role of ABA as an inhibitor of synthesis was considered. Two mechanisms might account for the inhibition of nucleic acid synthesis by ABA in potato buds. ABA might block the production of gibberellins (33) or directly interfere with the synthesis of RNA and DNA (31, 33). Our results favor the second alternative, since the inhibition of incorporation of precursors by ABA persists even if GA₃ is present at the same concentration. Further study will be needed to determine whether inhibition of ABA can be completely reversed by GA₃ at physiological concentrations.

No significant alteration of incorporation was observed as a result of the 3-day pretreatment of buds in intact tubers. This was surprising since promotion of nucleic acid synthesis by GA₃ was expected. The 12-hr treatment after excision may have induced the maximum rate of nucleic acid synthesis in the buds, thus masking the effect of the pretreatment. Alternatively, the precursors may not have penetrated under the conditions of the experiment. It should be noted that the buds did not increase significantly in length with any treatment and mitosis and cell elongation were clearly preceded by DNA and RNA synthesis. In this connection Haber *et al.* (10) reported that GA₃ promoted and ABA inhibited lettuce seed germination in the absence of DNA synthesis. While we did not study the effect of inhibitors of DNA synthesis in the present work, it is significant that in another report (17) deoxyadenosine, an inhibitor of DNA synthesis, was a potent inhibitor of sprouting.

Thus, although we cannot conclude definitely that the observed DNA synthesis was essential for termination of rest, such synthesis, which was enhanced by GA₃, clearly preceded the onset of those events that characterize resumption of growth, namely, increased mitotic activity accompanied by cell elongation.

It is not clear at which step in nucleic acid synthesis gibberellin and ABA are functional. Chrispeels and Varner (7) proposed that the expression of the gibberellin effect in the barley endosperm system requires the synthesis of messenger RNA and that ABA inhibits this synthesis or prevents incorporation of the RNA into an active enzyme-synthesizing unit. Jarvis, Frankland, and Cherry (14) further suggested that GA₃ reduces dormancy in hazel (*Corylus avellana* L.) seeds by overcoming gene repression. This is in line with a suggestion by Tuan and Bonner (30) on the role of ethylene chlorhydrin in shortening rest in potato tubers. Histoautoradiographic evidence indicating inhibition of incorporation of uridine and thymidine by ABA has also been reported in the embryo of *Fraxinus* (32).

Thus there is mounting evidence indicating the involvement of nucleic acid synthesis, influenced by hormones, in the regulation of bud rest in a number of plant species. In potato buds, ABA and gibberellin appear to play primary roles.

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