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Short Communication

Regulation of the Onset of Dormancy in Tubers of Begonia evansiana[1]

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The concept of dormancy as a condition of repressed genetic information was first proposed by Tuan and Bonner (7), from experiments which showed an increased template activity of chromatin isolated from potato tubers upon emergence from dormancy. The repression concept has since received general acceptance (1). It would seem possible that the entry into the dormant state may be a consequence of synthetic events programmed in the genome. If this is true, the instructions for the onset of dormancy may require transcription into RNA and translation into proteins. Our experiments with *Begonia* reveal that the entry into dormancy can be prevented by a wide range of inhibitors of nucleic acid and protein synthesis.

Tubers of *Begonia evansiana* Andr. are especially convenient for this type of experiment since they are induced to become dormant by exposure to relatively brief periods of low temperature and red light (5). Aerial tubers were obtained from plants grown in the greenhouse under short photoperiods (9 hr). Tubers were harvested at about the half-grown condition (stage 5 to 7 of Esashi, 4) at which stage they are not dormant. The standard treatment used to induce dormancy was to place the tubers on moist filter paper in Petri dishes for 20 days in a chamber at 15° under a red light (60 w tungsten lamp behind ruby glass filter transmitting above 560 mμ), after which germinability was determined over an additional 50 days in a growth chamber (1000 ft-c, 16 hr photoperiod, 25°). The tubers (25 per treatment) were supplied with the various inhibitors in the solution used to moisten the filter paper. After the 20 days of dormancy induction, the tubers were rinsed and placed on paper moistened with water.

The effectiveness of the dormancy inducing treatments is illustrated in Fig. 1, showing the progress of germination of tubers after 20 days at 15 or 25°, in red light or in darkness. As reported previously by Esashi (5), the 20 day exposure to 15° plus red light resulted in fairly complete dormancy as evidenced by the small percentage of tubers sprouting over the subsequent 50 days in the growth chamber.

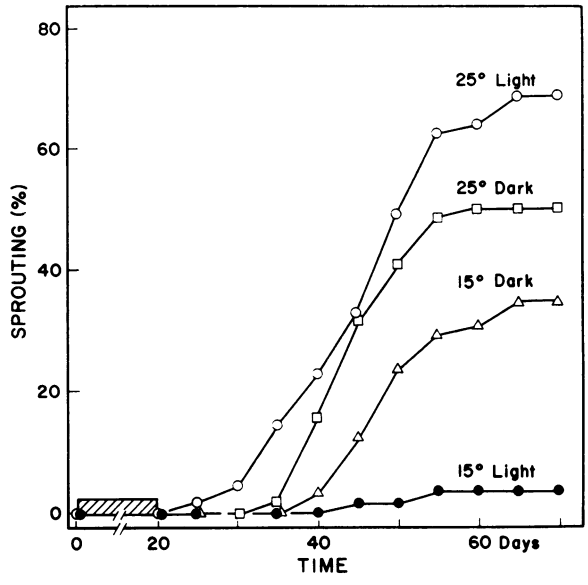

FIG. 1. Induction of dormancy in tubers of *Begonia* by light and temperature. Moistened tubers were held for 20 days under red light or darkness at the temperatures indicated, and subsequently sprouting in a growth chamber was recorded.

Application of various inhibitors of nucleic acid or of protein synthesis during the 20 day induction of dormancy resulted in strikingly higher subsequent sprouting percentages, as shown in table I. The inhibitors were applied at concentrations between 10^{-4} and 10^{-3} M as indicated. Inhibitors of nucleic acid synthesis (5-fluorouracil, 2-thiouracil, 8-azaguanine, 8-azaadenine, 5-fluorodeoxyuridine) were effective in preventing the induction of dormancy, as also were inhibitors of protein synthesis (canavanine, ethionine, puromycin, cycloheximide). The only

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Table I. *Prevention of the Onset of Dormancy in* Begonia *Tubers by Inhibitors of Nucleic Acid and Protein Synthesis*

Inhibitors were applied during a 20-day period of red light at 15° to induce dormancy.

Inhibitor	Concn.	Sprouting after 70 days	
		Treated	Controls
	$M \times 10^{-4}$	%	%
5-Fluorouracil	10	87	26
2-Thiouracil	10	83	11
8-Azaguanine	3	86	4
8-Azaadenine	5	91	4
5-Fluorodeoxyuridine	3	87	4
Canavanine	1	90	26
Ethionine	1	69	26
Puromycin	1	77	26
p-Fluorophenylalanine	2	67	26
Cycloheximide	3	87	4
Actinomycin D, 0.2 μg/ml	...	37	35

nucleic acid inhibitor which did not prevent the induction of dormancy was Actinomycin D. Each of the inhibitors which were effective in preventing the induction of dormancy resulted also in depressions of the protein content of the tubers, ranging from 10 to 60 %.

In these dormancy induction experiments there is some variation in dormancy reactions of tubers at various stages of development. For example, in Fig. 2 a comparison is made between the extent of dormancy induction in tubers at relatively early and late stages of development (stage 4–10 of Esashi, 4).

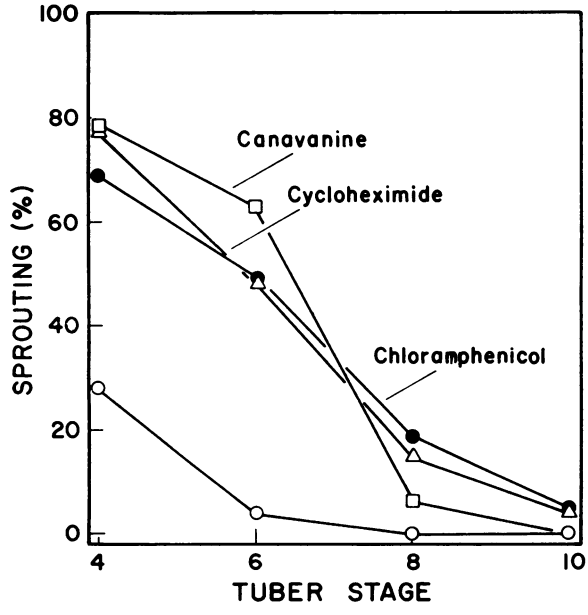

FIG. 2. Changes in dormancy responses of *Begonia* tubers at different stages of maturity. All treatments given 20 days at 15° in red light to induce dormancy; inhibitor treatments as in table I. Sprouting recorded at 70 days.

The standard 20 day period at 15° under red light does not bring all of the stage 4 tubers into dormancy. Applications of canavanine, cycloheximide or chloramphenicol during the induction period were nevertheless effective in preventing the onset of dormancy, as indicated by the high percentages of subsequent sprouting. More mature tubers, stages 8 or 10, showed little or no response to the applied inhibitors; in these tubers dormancy had apparently been entered before the 20 day treatment.

The dormant condition is considered to be one in which the synthetic machinery of the cells lies idle because of a repressed condition of the nucleic acid system. The onset of the dormant condition can be most readily imagined, then, to be A) the failure of synthetic processes because of inadequate replenishment of some critical segments of the synthetic apparatus, or B) the programmed synthesis of materials which bring about the shutdown of the synthetic apparatus. If the former were the case, synthesis inhibitors might be expected to enhance the onset of dormancy; if the latter, such inhibitors might block the onset of dormancy. The evidence reported here for *Begonia* is strongly suggestive that in this material, a programmed synthesis controls the entry into dormancy.

Some related observations of the effects of inhibitors of nucleic acid and protein synthesis as stimulants of emergence from dormancy are suggestive of the interpretation we are suggesting. Black and Richardson (2) found that inhibitors of nucleic acid synthesis could promote the emergence of lettuce seeds from dormancy; they suggested that the inhibitors were preferentially acting against some suppressive reaction in the seeds. A similar interpretation has been made by Frankland and Smith (6). An earlier observation by Walton (8) that *p*-fluorophenylalanine could enhance growth of embryos taken from bean seeds might reflect a somewhat similar situation, possibly through the suppression of phenolic syntheses. There are also evidences that the active synthesis of growth inhibitors may be an integral part of the dormant condition (3, 9).

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