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SHORT COMMUNICATION

THE EFFECTS OF GIBBERELLIC ACID AND LIGHT ON RNA, DNA AND GROWTH OF THE THREE BASAL INTERNODES OF DWARF AND TALL PEAS

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Abstract—After 14 days growth the lengths of the first three internodes of dwarf (Meteor) and tall (Alderman) peas are proportional to the widely differing RNA, but not to the DNA contents of seedlings grown in dark or light and in dark with gibberellic acid. In the presence of both light and gibberellic acid the relationship between growth and RNA content is altered.

INTRODUCTION

IN THE course of studies on the photomorphogenetic effects of light, large correlated changes in RNA and DNA[1] and protein[2] were found in the hypocotyl of *Lupinus albus*, which during germination grows solely by cell enlargement. Light effects on epicotyl tissue are much more complex; Butler[3,4] working on internodes of *Vicia faba* showed that both cell division and enlargement were involved and that the successive internodes reacted differentially to light. *Pisium sativum* shows analogous morphological reactions to light but has added interest because of the occurrence of dwarf mutants which not only show accentuated light effects but also striking responses to gibberellic acid.

In this investigation of nucleic acid contents of pea internodes we have attempted to separate light and gibberellic acid (G.A.) effects by growing both dwarf (Meteor) and tall (Alderman) varieties of pea in the absence and in the presence of G.A. with and without light, giving eight combinations of treatments. As an internode is a clearly defined population of cells it is possible to compare treatments.

RESULTS

The lengths, DNA and RNA contents were measured after 14 days germination, only the basal three internodes being analysed because in the dark these are the only ones which elongate sufficiently for comparison with those in the light. The results for the individual internodes are presented in Table 1. The relative reponses in each plant to the various treatments may differ in quantitative detail in the individual internodes but not qualitatively so that for ease of discussion the values for the three internodes have been totalled in Table 2.

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[1] K. W. GILES and A. MYERS, *Biochim. Biophys. Acta.* **87**, 460 (1964).
[2] R. J. LEWINGTON, Ph.D. Thesis, University of Southampton (1965).
[3] R. D. BUTLER and G. R. LANE, *J. Linnean Soc. London* **56**, 170 (1958).
[4] R. D. BUTLER, *J. Exp. Bot.* **14**, 142 (1963).

Table 1. The lengths, RNA and DNA contents of the first three internodes of Meteor (M) and Alderman (A) peas grown under continuous light (L) or dark (D), and in presence (+) and absence (−) of gibberellic acid

Parameter	Variety	G.A.	1st Internode		2nd Internode		3rd Internode	
			L	D	L	D	L	D
Length (mm)	M	−	5·5	37·1	3·9	49·3	8·4	79·6
		−	24·0	71·5	42·1	87·4	94·0	118·1
	A	−	8·5	63·9	21·7	103·9	49·2	104·8
		−	26·5	69·6	33·8	113·3	75·5	117·3
RNA (μg/int.)	M	−	2·2	21·7	6·8	35·3	10·3	87·0
		−	8·4	32·7	22·7	53·9	55·9	136·8
	A	−	12·3	47·7	21·7	81·3	52·7	154·7
		−	16·6	40·9	17·1	116·2	52·1	144·6
DNA (μg int.)	M		9·5	21·5	6·4	19·1	9·3	29·4
		−	11·8	24·2	14·4	20·9	27·3	33·6
	A	−	12·1	25·6	12·2	21·1	21·0	37·3
		−	16·9	19·8	14·9	24·8	25·3	34·0

Table 2. The total lengths, RNA and DNA contents of the first three internodes of Metfor (M) and Aldfrman (A) peas grown under continuous light (L) or dark (D), and in the prfsence (+) and absence (−) of gibberellic acid

Variety	G.A.	Length (mm)		RNA (μg)		DNA (μg)	
		L	D	L	D	L	D
M	−	18	166	19	144	25	70
	−	160	277	87	223	54	79
A	−	79	273	87	284	45	84
	−	136	300	86	302	57	79

DISCUSSION

The results show remarkable correlated changes in length and RNA content. Under the extreme conditions of dark plus G.A. and light in the absence of G.A., it can be seen from the totals of the three internodes (Table 2) that in the dwarf Meteor, changes of 15 × in length, 12 × in RNA and 2 × in DNA are found. In the tall Alderman these changes are less marked but still considerable being 4 × in length, 3 × in RNA and 2 × in DNA content.

The obvious control conditions are the absence of both stimuli, i.e. plants grown in the dark in water only. The situation is however complicated by the presence of physiologically effective endogenous gibberellin-like substances,[5] the amount of which may well differ in the two varieties. It can be deduced from Butler's results that light has at least two distinctive effects both of which can be stored: (*a*) the induction of new internodes at the apical meristem and (*b*) the regulation of growth of those internodes already light induced in the maturing seed. It follows that the controls are of necessity only relative.

If the two varieties are both given G.A. the parameters of length, RNA and DNA are virtually identical under the given conditions of light or dark, and furthermore, Alderman when grown in the dark is unaffected by G.A. In the light however G.A. has a pronounced

[5] M. Radley, *Ann. Botany (London)* **22**, 297 (1958).

effect on the growth of Alderman but only slight on its DNA and none on its RNA contents, which demonstrates that endogenous G.A. cannot be optimal, suggesting that light in this case inhibits or destroys G.A. or that endogenous and exogenous G.A. are not functionally identical.

Meteor as expected shows marked growth stimulation together with nucleic acid changes on G.A. treatment in both the light and dark. Thus in Meteor it is possible to examine separately the effect of exogenous G.A., the effect of light and of both treatments together. Without light, G.A. has little effect on DNA but increases RNA by 55 per cent and length by 67 per cent. In the absence of G.A. light has drastic inhibitory effects resulting in decreases of 66 per cent in DNA, 87 per cent in RNA and 89 per cent in length. The result of giving both stimuli together is that DNA is reduced by 23 per cent, RNA by 40 per cent, but the length is unaffected. If the effects were independent the expected changes would be decreases of 52 per cent DNA, 25 per cent RNA and 21 per cent in length. Therefore the stimuli interact.

TABLE 3. THE RATIOS OF LENGTH/RNA FOR THE TOTALS OF THE FIRST THREE INTERNODES OF METEOR (M) AND ALDERMAN (A) PEAS GROWN UNDER CONTINUOUS LIGHT (L) OR DARK (D), AND IN PRESENCE (+) AND ABSENCE (−) OF GIBBERELLIC ACID

		Length (mm)/RNA (μg)	
Variety	G.A.	L	D
M	−	0·95	1·15
	+	1·84	1·24
A	−	0·91	0·96
	+	1·58	0·99

When the results are expressed as growth per unit of RNA (mm/μg)—Table 3—it can immediately be seen that the combined effects, in both varieties, of G.A. and light give ratios which are much higher than with the individual stimuli by a factor of 1·6.

Since 80–90 per cent of the total RNA is ribosomal the ratio expresses a relationship between the number of ribosomes and the final length of the tissue. This relationship is constant except with both stimuli together, so that although the ribosomes appear to be limiting in all cases, the actual mechanism linking number of ribosomes with final length is altered in the presence of both stimuli. The most likely explanation is that some factor is present only with light and G.A. which either increases the relative rate of protein synthesis or delays the senescence of the (poly) ribosomes.[6]

EXPERIMENTAL

The seeds were planted on the surface of John Innes Compost No. 1 in pots, in order to expose the seedlings to light from the start of germination. The light plants were grown under a bank of fluorescent tubes (Phillips Reflectorized Daylight) giving a surface light intensity of about 200 ft-candles. The G.A. treated seedlings were watered with a solution of 100 ppm commercial G.A. per ml.

[6] E. R. GLOWWACKI and R. L. MILLETTE, *J. Mol. Biol.* **11**, 116 (1965).

Batches of ten seedlings were harvested after 14 days at 25° C. the respective internodes were excised, measured and fixed in boiling alcohol. The internodes were cut into 2–3 mm pieces and extracted with alcohol in a soxhlet apparatus and then washed with a large excess of 0·2% PCA (perchloric acid) at 0°. The tissue was transferred to graduated tubes made up to a known volume with water, an equal volume of 20% PCA added and the nucleic acids extracted at 30° overnight. Total nucleic acids were estimated by u.v. absorption at 260 mμ, a correction applied[7] for the small amount of optically interfering substances present. An aliquot was taken for DNA estimation by an improved diphenylamine method[8] and the RNA determined by difference.

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