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EFFECT OF GIBBERELLINS ON GROWTH OF PEA SEEDLING INTERNODE

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Key Word Index—*Pisum sativum*; Leguminosae; dwarf pea; gibberellins; GA_1; GA_3; GA_5; cell elongation; interconversion.

Abstract—Elongation of internode segments of dwarf pea seedlings excised 4 mm below the plumular hook was stimulated by GA_3 but not by GA_1 or GA_5. However, all three gibberellins induced cell elongation in the region from which this segment was isolated on application to intact seedlings. It is concluded that GA_1 and GA_5 are converted to a GA_3-like hormone. Measurement of epidermal cell elongation in the epicotyl further indicates that GA_3 or a GA_3-like hormone may be the functional form of the hormone required for cell elongation.

INTRODUCTION

It has been demonstrated by analysis of the time course of pea epicotyl growth that a 6–8 hr lag period was required before GA_1 and GA_5 became effective, while no detectable lag period was associated with GA_3. It was suggested that GA_1 and GA_5 were converted to a GA_3-like gibberellin GA [1]. The conversion of GA_5-[U-^{3}H] to GA_3 has been further demonstrated in the same cultivar by GC–RC [2]. A mutant of *Gibberella fujikuroi* has also been shown to convert GA_1 to GA_3 [3]. There are clearly metabolic relationships between GAs occurring in the same plant; however, there is little information to indicate whether there is functional specificity associated with the various hormones [4]. The present study indicates that in the pea epicotyl GA_3 or a GA_3-like hormone may be the functional form involved in cell elongation.

RESULTS AND DISCUSSION

Figure 1 illustrates the effect of GA_1, GA_3 and GA_5 on growth of the S-4 segment of pea epicotyl. Neither GA_1 nor GA_5 had any significant growth effect at concentrations ranging from 0·003 to 30 ppm. However, the segment responded to GA_3 by exhibiting a dose response with no apparent optimum. It was previously demonstrated that the excised apical 10 mm of the internode, including the plumular hook, bud, and leaves, responded to all three GAs; therefore, the failure of the S-4 segment to respond to GA_1 and GA_5 suggests that the apical region is required for the conversion of GA_1 and GA_5 to a GA_3-like gibberellin.

To determine if GA_1 and GA_5 are simply intermediates in the conversion to GA_3 or whether they represent functional forms of the GA required by cells of different stages of development, a time course study was made by measuring epidermal cells at various levels below the plumular hook. During the initial 10 hr growth period no significant gibberellin response could be demonstrated.

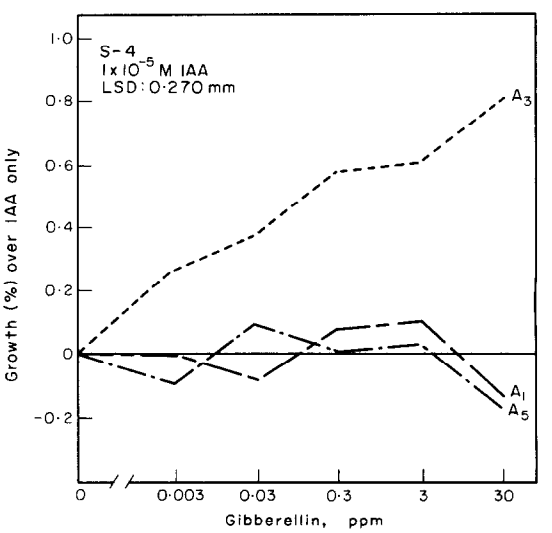

Fig. 1. Effect of gibberellin on 5 mm segments excised 4 mm below the most apical point of the concave side of the plumular hook. Segments were cultured for 18 hr at 28°.

However, measurements after 18 hr revealed significant responses (Fig. 2). In the subapical region (2 mm) there was no significant response to any of the gibberellins. In the mid-region (5 mm) GA_1 and GA_3 induced a significant growth response over GA_5; however, the GA_5 response was not significantly different from that of the control. The cells of the basal region were responsive to both GA_3 and GA_5 but they were unresponsive to GA_1. The growth response of the basal epidermal cells to GA_3 and GA_5, whilst the cells of the S-4 segment were unresponsive to GA_5 (Fig. 1), indicate that GA_5 has to be converted to a GA_3-like hormone. The unresponsiveness of the basal cells to GA_1 while the cells of the mid-region responded to both GA_1 and GA_3 may indicate that cells of this mid-region are able to convert GA_1 and GA_3 but not GA_5, whilst basal cells are unable to make the conversion. It is tempting to suggest that GA_5 was translocated acropetally into the apex where it was converted to a GA_3-like hormone and subsequently transported basipetally to the target cells. However, the failure of the cells of the mid-region to respond to GA_5 while responding to both GA_1 and GA_3 tends to rule out this interpretation. No satisfactory explanation is presently available.

A different growth response of the epidermal cells to different GAs might reflect a slow translocation or distribution to the site of hormone action. To determine the uptake and distribution of GA along the axis of epicotyl, GA_3-[^{14}C] and GA_1-[^{3}H] were supplied to the cut surface of the apical 10 mm segment. Segments were harvested at various time intervals, cut into successive 2 mm segments and extracted with MeOH. Figure 3 shows that cells isolated 2–4 mm below the hook region preferentially accumulated GA_3-[^{3}H]. By 20 min, the accumulation reached a maximum and thereafter declined. The second largest accumulation occurred in the section in direct contact with the agar (8–10 mm). The 0–2 mm section, which included the leaves, apical bud, and a portion of the plumular hook accumulated the least amount of GA_3.

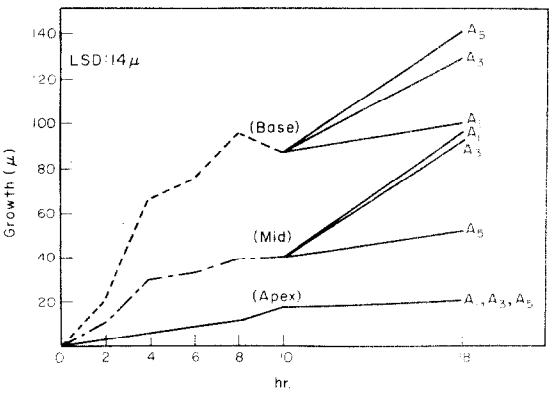

Fig. 2. Effect of gibberellin on growth of epidermal cells at various regions below the most apical point of the concave side of the plumular hook. Apex: 2 mm control growth same as GA_1, GA_3 and GA_5, mid: 5 mm control growth same as GA_5 response, base: 8 mm control growth same as GA_1 response.

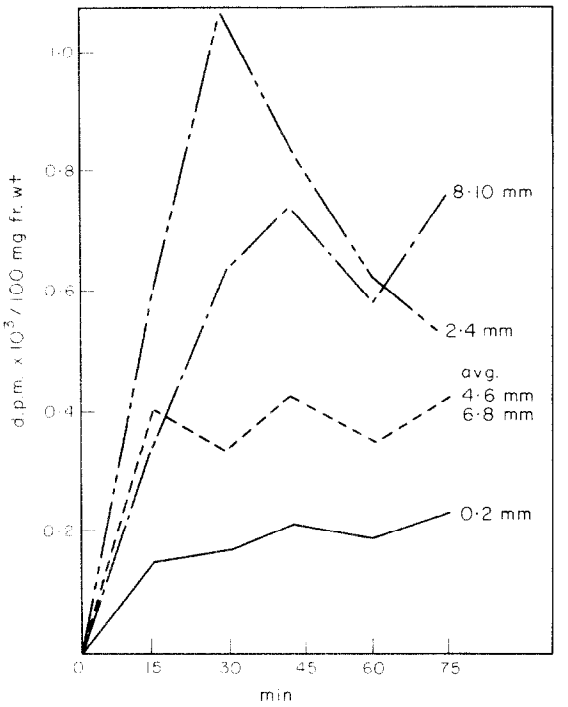

Fig. 3. Distribution of GA_3-[^{14}C] in methanolic extracts from various regions along the axis of etiolated pea epicotyls.

Figure 4 illustrates the distribution of GA_1-[^{3}H]. In this study the leaves and apical bud were removed from the 0–2 mm segment. The 2–4 mm segment accumulated the greatest amount of GA_1-[^{3}H] after *ca* 20 min. Thereafter, GA_1 appeared to move both acropetally and basipetally. As GA_1-[^{3}H] declined in the 2–4 mm segment it rose in the 0–2 and 4–6 mm segments. In the preceding study the large amount of tissue associated with the apical bud and leaves apparently masked the accumulation of GA_3-[^{14}C] in the stem region of the plumular hook. The 6–8 and 8–10 mm segments accumulated about the same amount of labelled GA_1

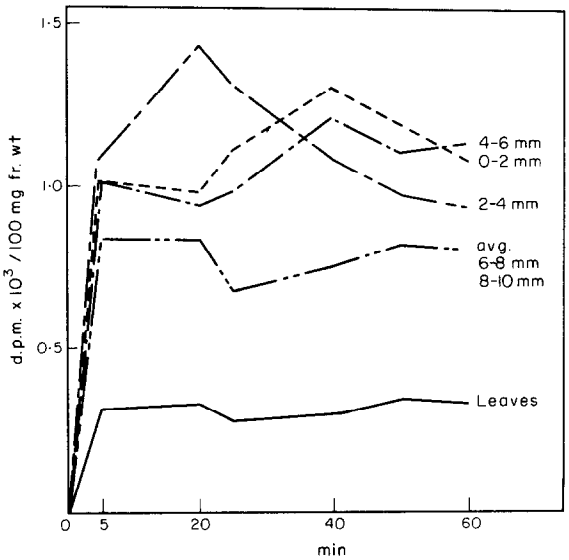

Fig. 4. Distribution of GA_1-[^{3}H] in methanolic extracts from various regions along the axis of etiolated pea epicotyls.

Table 1. Accumulation of GA_1-[^{3}H] extracted from various regions along the axis of pea epicotyls

Section (mm)	0–2	2–4	4–6	6–8	8–10
Time (min)	Bound-GA_1 (dpm/100 mg/fr. wt)				
5	102	127	48	47	—
20	190	109	17	42	93
25	102	162	86	33	130
40	151	288	36	46	—
50	155	171	60	48	123
55	265	139	66	44	169
Average	160	166	52	43	128
	Free acid				
5	66	68	48	47	73
20	83	66	16	42	88
25	96	67	87	33	191
40	64	91	36	46	91
50	74	141	60	48	179
55	104	57	66	44	224
Average	81	82	52	43	141

Free acid is considered to be GA_1 which was freely soluble in EtOAc at pH 2·5. Bound-GA_1 remained in the aq. phase following a 30 vol. extraction with dry EtOAc.

but somewhat less than the more acropetal segments. The rapidity of uptake and distribution along the epicotyl clearly was not responsible for the differential growth response exhibited by the epidermal cells to the different GAs. The rapid uptake suggests that the hormone was carried acropetally in the transpiration stream. This was substantiated by applying tritiated water to the cut surface of the epicotyl. ^{3}H was found in the stem region of the plumular hook within 3 min.

Table 1 illustrates the recovery of free acid and bound GA from the various zones along the epicotyl. The basal segment which was in direct contact with the agar accumulated the greatest amount of free acid, followed by the two most apical segments. The bound GA was concentrated below the plumular hook (0–2 and 2–4 mm segments). Cells that accumulated the greatest amount of bound-GA_1 are the same cells which are rather unresponsive to the exogenous application of GA (Fig. 2). The biological role of bound GAs is unknown [4]; however, it has been suggested that GA-glucosides are storage forms which can be transported to target tissues [5]. Cells immediately below the site of synthesis occupy an ideal position to regulate the flow of GA to the cells requiring the hormone for elongation.

Cells that undergo the greatest elongation appear to accumulate the largest amount of free GA_1. The basal 6 mm accumulated more total free acid than the apical 4 mm. If the cells of the epicotyl are unable to distinguish between the uptake of GA_1 and GA_3 then accumulation by target cells cannot be involved in the growth responses reported in this study. The previously reported lag periods for GA_1 and GA_5 [1], then truly represent periods required for the conversion of GA_1 and GA_5 to a GA_3-like gibberellin.

EXPERIMENTAL

Culture techniques. Seeds of *Pisum sativum* L. cv. Progress No. 9 were soaked 18 hr in H_2O with vigorous aeration. They were then planted in moist vermiculite in a dark room for 6–7 days at 27–28° and watered at least once with Hunter's soln [6]. Plants were harvested when the third internode was *ca.* 20–30 nm long. All operations were carried out under a dim green light (Kodak Wratten Series 3 filter with a 25 W lamp). 5-mm segments were cut 4 mm below the most apical point of the concave side of the plumular hook. Following the terminology of Ref. 7 this segment is termed S-4. After excision the segments were placed in 0·02 M phosphate buffer, pH 6·1, for *ca* 2 hr. The segments were incubated for 17–18 hr at 28° in 5 ml of 0·02 M phosphate buffer, pH 6·1, containing 2% sucrose, 10^{-5} M IAA and the various GAs.

Epidermal growth analysis. Only those seedlings with essentially the same degree of hook opening were selected. The apical 10 mm which included the plumular hook, bud, and leaves was excised. The basal end of the segment was then placed on 0·8% agar containing buffer, 2% sucrose and 0·3 ppm GA A_1, A_3 or

A_5. The segments were cultured in the dark in a moist atmosphere at 28°. Segments were removed every 2 hr over a 10 hr period with a final measurement at 18 hr. The segments were broken in the middle in such a manner that the epidermis could be peeled off the side opposite the plumular leaves. The epidermis was then mounted on a slide and stained with 0·5% aq. methylene blue. Photomicrographs were made at 3 regions along the axis, 2, 5 and 8 mm below the apex of the plumular hook. The last measurement (18 hr) was made 2 mm below the apex, in the middle of the segment and 2 mm up from the base. These adjustments were made to compensate for the increase in the length of the segment. The cell images were measured from the photomicrographs with a calibrated ocular and converted to μm. Usually 50–60 cells were measured at each zone along the axis. Growth was entirely by cell elongation as no mitotic figures were seen either in the epidermal tissue or serial tangential sections of epicotyls. The maintenance of cell division in the apical region has been previously reported to be contingent upon the continued attachment of the plumular hook to the plant [8].

Gibberellin uptake and distribution. GA_3-[1,12,6-carboxyl, 4-methyl-^{14}C] (1·8 μCi/mg) was supplied by Prof. P. W. Brian, Cambridge, England, and GA_1-[1,2-^{3}H] (1 Ci/mmol) was obtained from Prof. L. Rappaport, University of California, Davis. The culture medium contained either GA_3-[^{14}C] in 0·3 ppm GA_3 to give 920 cpm/ml or GA_1-[^{3}H] to give 42900 cpm/ml in 0·3 ppm GA_1. Nine, 10-mm epicotyls were placed with their basal end in contact with 0·8% agar containing the labelled GAs in the dark. At various time intervals, the epicotyls were removed and cut into 2 mm segments. Because of the low sp. act. of GA_3-[^{14}C] only MeOH extracts were analyzed. However, in GA_1-[^{3}H] studies the 2 mm segments were ground in 5 ml MeOH and extracted for 18 hr. Following extraction and counting the MeOH extracts were dried down and taken up in 0·02 M Na phosphate buffer (pH 8·5). No appreciable activity was removed on extraction with 3 vol. of petrol. The buffer extract was then acidified to pH 2·5 with 0·6 N HCl and extracted with 30 vols of dry EtOAc. The GA in the EtOAc fraction was considered to be free acid. The activity remaining in the aq. phase was considered to be bound-GA_1. The bound GA following hydrolysis in 1 N H_2SO_4 at 100° for 18 hr chromatographed as authentic GA_1 on TLC in isopropyl ether : HOAc (95:5). The aq. samples were counted in 10 ml of Aquasol (New England Nuclear) while the organic extracts were counted in Bray's scintillation fluid. The data were corrected for quench and efficiency. Each sample was counted sufficiently long for the same per cent standard deviation (2·5–3·0% or gross count range of 1000–3999).

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