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Dormancy and Impotency of Cocklebur Seeds

IV. EFFECTS OF GIBBERELLIC ACID, BENZYLADENINE, THIOUREA, AND POTASSIUM NITRATE ON THE GROWTH OF EMBRYONIC AXIS AND COTYLEDON SEGMENTS

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ABSTRACT

Germination of nondormant but impotent small cocklebur seeds (*Xanthium pennsylvanicum* Wallr.) was promoted profoundly with thiourea or benzyladenine, and slightly with gibberellic acid. Gibberellic acid was ineffective in causing the germination of dormant cocklebur seeds, although thiourea and benzyladenine were effective. Experiments with excised seed pieces showed that the promotive effects of thiourea, benzyladenine, and gibberellic acid on cocklebur seed germination were associated with the enhancement of growth of seed parts; thiourea stimulated predominantly the axial growth, whereas benzyladenine stimulated predominantly the cotyledonary growth.

Potassium nitrate or indoleacetic acid had little effect on the initial growth of either axes or cotyledons. Except for gibberellic acid, all of the compounds employed enhanced ethylene production, but in general, the ethylene production seemed more likely to be a consequence of growth rather than a cause of it. We concluded that the chemical regulation of seed germination may be a consequence of the alteration of growth capabilities in either the axes or cotyledons, or both.

In a previous paper (10), we observed that cocklebur seeds respond in different manners to various germination stimulators: seeds treated with TU[1] or CO_2 exhibited normal germination, but O_2 enrichment caused germination in which the seed coat was mostly broken at the cotyledonary side rather than at the axial end. On the other hand, the seeds germinated with BA, C_2H_4, and GA_3 exhibited an intermediate pattern in which approximately half of the germinations were normal. Granting that seed germination in dicot plants is a phenomenon occurring when the axis and the cotyledons generate enough thrust to overcome the restraint by the seed coat (8), these diverse response patterns might result from the differential growth responsiveness of the axis and cotyledon: for example, TU could preferentially enhance growth of the embryonic axis proper, whereas BA could more greatly enhance growth of the cotyledons and hence the abnormal lateral breaking of the seed coat. We have previously presented some evidence that C_2H_4, unlike CO_2, strongly stimulates both the axial and cotyledonary growth (7).

These findings caused us to examine the relative effectiveness of various known germination stimulators (15), such as TU, KNO_3, BA, GA_3, and IAA, in stimulating the growth of axial and cotyledonary seed pieces. Experiments were also directed toward a comparison between the dormant and nondormant seeds in response to these stimulators.

[1] Abbreviation: TU: thiourea.

MATERIALS AND METHODS

Seeds of cocklebur (*Xanthium pennsylvanicum* Wallr.) were used in this experiment. For germination tests, dormant small seeds newly harvested in 1973, and the nondormant but impotent small seeds fully after-ripened since 1972 were exposed to various drugs on two filter paper discs in 5-cm Petri dishes with 2 ml water. For growth tests, the embryonic axis and cotyledon segments were excised from large seeds according to the procedures previously described (7). After being weighed for initial fresh weight, 12 to 15 4-hr imbibed axial or 12 to 25 6-hr imbibed cotyledonary segments were each arranged in a 22-ml vial or 125-ml Erlenmeyer flask containing a filter paper piece or two filter paper discs to which were given 1 or 3 ml of distilled H_2O or test solutions, respectively, and allowed to grow in a darkened growth chamber. The vial and flask were sealed with a rubber stopper, to which a side vessel with 1.5 ml of 2.5 N NaOH or 0.25 M $Hg(ClO_4)_2$ for trapping of endogenously evolved CO_2 or C_2H_4, respectively, was fitted as necessary. All operations were carried out under room light. All results were the average of at least three replicates and were expressed as relative increases (%) in fresh weight or as per cent increase over control with no treatment. C_2H_4 production was measured with a Hitachi gas chromatograph equipped with an activated aluminum column and a flame ionization detector.

RESULTS

Altering the Germination of Dormant and Impotent Cocklebur Seeds. A comparison was made of the germination responses to TU, BA, GA_3, KNO_3, and IAA of the dormant and the nondormant but impotent seeds of cocklebur as shown in Table I. The dormant seeds were less responsive than the impotent seeds. Thus, the slightly promotive effect of GA_3 in the impotent seeds was not detected in the dormant seeds. TU and BA each stimulated germination very effectively. On the other hand, IAA, which is classically known to promote seed germination in some cases (15), was not promotive at any of the concentrations used in this experiment. However, a brief exposure to IAA had a promotive effect on germination, as shown in Table II.

Responses of Axial and Cotyledonary Segments to TU. Regardless of whether the embryonic axis segments were excised from the dormant seeds or from nondormant ones, extension growth of the segments was markedly stimulated by TU, accompanied by an increased C_2H_4 production (Table III). Maximal growth of the nondormant axes was obtained at 10 mM TU, but the dormant axes showed continuing gains with higher concentrations.

Extension growth in cotyledonary segments was also enhanced by TU in a manner similar to that of the axes, but to a lesser

extent. TU also stimulated C_2H_4 production in the cotyledons, particularly the dormant ones (Table III). Thus, TU expanded the ability to grow and to produce C_2H_4 in both the dormant axes and cotyledons.

Table I. Effects of Various Drugs on the Germination of Small Cocklebur Seeds in the Dormant or Nondormant State.
Data are shown for germination after 5 days at 23° C.

Drug	Germination (%)	
	Dormant	Impotent
H_2O	0	3.7
TU 100 mM	100.0	100.0
TU 30 mM	78.2	96.0
BA 300 μM	45.3	96.4
BA 100 μM	24.2	80.8
GA_3 100 μg/ml	0	11.5
GA_3 30 μg/ml	0	7.8
KNO_3 10 mM	0	4.6
KNO_3 3 mM	0	5.3
IAA 100 μM	0	0
IAA 10 μM	0	0
IAA 1 μM	0	0

Table II. Germination of Dormant Cocklebur Seeds as Affected by Various Durations of Treatment with Indoleacetic acid (10^{-3}M).
Germination values after 10 days in the dark.

Treatment	Germination (%)
Water control	13
IAA for first 8 hrs	33
IAA for first day	60
IAA for first 2 days	67
IAA for first 3 days	87
IAA continuous for 10 days	26

Responses of Axial and Cotyledonary Segments to KNO_3. As shown in Table III, no concentration of KNO_3 increased the extension growth of axis segments, although KNO_3 at greater than 1 mM stimulated C_2H_4 production, particularly in the pieces from nondormant seeds. In cotyledons, however, there was a clear stimulation of both growth and C_2H_4 production in the dormant segments by KNO_3. The nondormant cotyledons showed lesser responses.

Responses of Axial and Cotyledonary Segments to BA. As with TU, BA was effective in stimulating the extension growth in both the axial and cotyledonary segments, in conjunction with an increased C_2H_4 production regardless of their dormancy status (Table III). The BA stimulation of growth was particularly profound in the cotyledons, such a BA-stimulated cotyledon expansion having been reported previously by Esashi and Leopold (9). Also, the BA-stimulated C_2H_4 production was previously reported (1).

Responses of Axial and Cotyledonary Segments to GA_3. Unlike TU and BA, GA_3 promoted the extension growth of the axial and cotyledonary segments, except for the dormant axes, without stimulating C_2H_4 production (Table III). The promotion by GA_3 of the axial and cotyledonary growth was far less than that by TU or BA.

Responses of Axial and Cotyledonary Segments to IAA. Unlike the other reagents employed, IAA, strongly stimulated C_2H_4 production in both the axial and cotyledonary segments, particularly the nondormant organs, as in other systems (1), but was without effect or had inhibitory effects on growth (Table III). The C_2H_4 production was stimulated in each of the tissues except in the dormant cotyledons.

Time Courses of Growth as Affected by TU, BA, GA_3 and KNO_3. As illustrated in Fig 1, dormant axial segments failed to grow, and nondormant ones grew at a steady but slow pace. With the addition of optimal concentrations of 10 mM TU, 30

Table III. Effects of Various Drugs on the Growth of Axial and Cotyledonary Segments from Dormant and Nondormant Cocklebur Seeds.
The cotyledons were incubated for 72 hr, but the incubation times in the axes were 14 hr for BA, 15 hr for GA_3, 20 hr for thiourea and IAA, and 22 hr for KNO_3, respectively. Incubation temperatures were 23° C for thiourea and BA, and 24° C for KNO_3, GA_3 and IAA, respectively. Values for C_2H_4 are $nl \cdot hr^{-1} \cdot g^{-1}$ fr.wt.

Drug	Concentration	Axis				Cotyledon			
		Dormant		Nondormant		Dormant		Nondormant	
		fr.wt.	C_2H_4	fr.wt.	C_2H_4	fr.wt.	C_2H_4	fr.wt.	C_2H_4
Thiourea	H_2O	100.0 %	0.019	100.0 %	0.543	100.0 %	0.010	100.0 %	0.187
	0.1 mM	98.9	0.021	102.7	0.608	99.4	0.015	101.2	0.208
	1	101.1	0.022	124.4	0.651	102.8	0.022	111.7	0.286
	10	111.0	0.320	158.9	1.399	114.6	0.136	123.9	0.272
	100	143.5	1.762	135.2	1.552	121.0	0.340	94.0	0.106
KNO_3	H_2O	100.0	0.043	100.0	0.698	100.0	0.024	100.0	0.182
	0.1 mM	101.7	0.050	98.3	0.702	106.8	0.035	97.6	0.198
	1	99.8	0.072	105.1	0.853	112.9	0.052	101.2	0.203
	10	105.5	0.245	101.0	2.055	130.6	0.126	109.0	0.218
	100	91.4	0.826	84.1	3.506	174.0	0.240	127.3	0.233
BA	H_2O	100.0	0.022	100.0	0.252	100.0	0.038	100.0	0.499
	0.1 μM	101.6	0.019	103.6	0.277	106.5	0.034	102.7	0.522
	1	113.2	0.028	111.2	0.321	118.8	0.029	114.2	0.561
	10	122.9	0.192	111.9	0.492	153.9	0.072	143.1	0.661
	100	134.4	0.277	116.1	0.565	218.3	0.607	203.7	0.873
GA_3	H_2O	100.0	0.118	100.0	0.314	100.0	0.021	100.0	0.172
	0.03 μg/ml	100.8	0.123	106.2	0.318	100.2	0.024	102.0	0.157
	0.3	100.0	0.129	110.7	0.304	106.6	0.015	114.5	0.106
	3	98.8	0.123	118.8	0.316	116.9	0.010	129.2	0.105
	30	101.2	0.127	120.6	0.341	120.3	0.018	130.9	0.090
IAA	H_2O	100.0	0.058	100.0	0.564	100.0	0.049	100.0	0.582
	1 μM	99.6	0.049	100.9	0.596	100.2	0.052	99.4	0.585
	10	100.8	0.065	99.2	0.735	100.8	0.050	99.5	0.641
	100	100.6	0.152	94.0	1.414	101.3	0.073	98.2	0.834
	1 mM	97.5	1.573	88.6	2.603	99.0	0.351	89.3	2.830

μM BA, or 30 μg/ml GA_3, growth was accelerated, but not with 30 mM KNO_3.

In Figure 2, a similar experiment is reported for cotyledons. Dormant cotyledons failed to grow and nondormant ones grew at a slow rate as reported previously (7). Each of the drugs had promotive effects on the cotyledon expansion in decreasing order of BA, GA_3, TU, and KNO_3. This order was different from that in the axial growth stimulation in Figure 1. The promotive effect of KNO_3 began on the 2nd day of incubation and then gradually increased with time. In contrast, C_2H_4 increased the cotyledon expansion in the first 24 hr after which its promotive effect ceased.

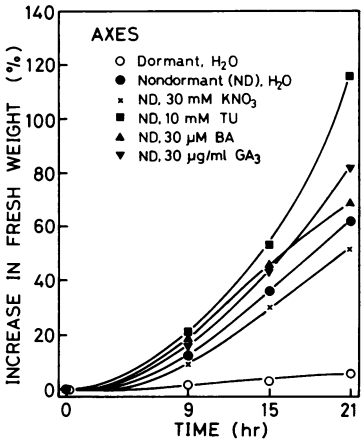

FIG. 1. Time courses of growth of dormant axes and nondormant axes treated with various drugs at 23 C.

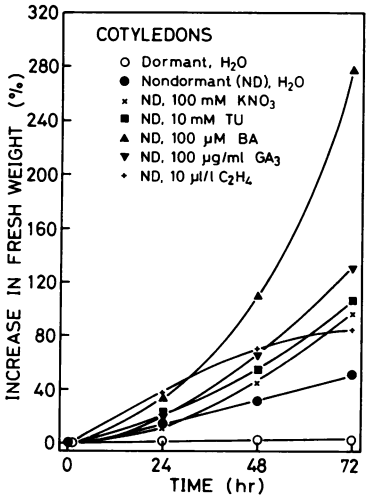

FIG. 2. Time courses of growth of dormant cotyledons and nondormant cotyledons treated with various drugs at 23 C.

Although in Figure 2, the cotyledonary growth as affected by various drugs was followed for a 72-h period, the cotyledon expansion which is possibly significant in supplying a thrust force on seed germination seems to be restricted to the early expansion which occurs within 24 hr of incubation, for the germination of drug-treated cocklebur seeds normally begins at about 24 hr. Therefore, a comparison of effects of various gases and drugs was made of the cotyledon growth at 24 hr (Fig. 3). C_2H_4 was most effective, BA, TU, GA_3, and CO_2 following in the order of a decreasing effectiveness. As indicated in Figure 2, KNO_3 was completely ineffective during this initial time period.

Role of CO_2 and C_2H_4 on Drug-Stimulated Growth. In agreement with the results obtained previously (10), growth was sometimes retarded by inclusion of traps for either C_2H_4 or CO_2 (Table IV). The growth responses of the axes to added drugs were generally altered by the C_2H_4 or CO_2 traps, but the growth responses of the cotyledons to the added drugs are generally unaltered by the traps.

DISCUSSION

From the earlier findings (10, 13) and the present results, it was possible to rank the germination stimulators for their effects on cocklebur seeds: TU, BA, and C_2H_4 were most effective in both the dormant seeds and nondormant, impotent seeds; CO_2 was somewhat promotive in both seeds; GA_3 was slightly promo-

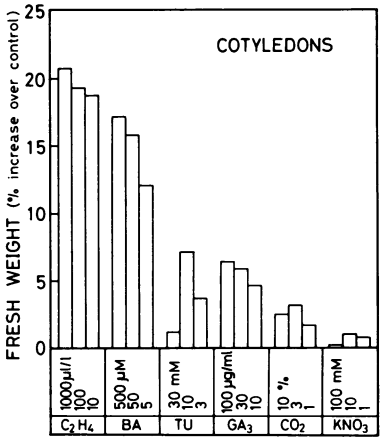

FIG. 3. Comparative activities of various drugs on the initial expansion of nondormant cotyledons. Segments were incubated at 23 C for 24 hr.

Table IV. Effects of the Removal of Endogenously Evolved C_2H_4 or CO_2 on the Axial and Cotyledonary Growth Affected with Various Drugs.

Nondormant axial and cotyledonary segments were incubated at 26° C for indicated times as described in "Materials and Methods". * Significant at p=0.05.

Organ	Drug	No addenda	$Hg(ClO_4)_2$		NaOH	
		fr.wt.	fr.wt.	% of cont.	fr.wt.	% of cont.
Axis 16 h	H_2O	23.72	22.19	93.6*	21.81	92.0*
	10 mM TU	35.45	38.31	108.1	33.02	93.2*
	100 µM BA	25.42	27.37	107.7	27.85	109.6
	100 µg/ml GA_3	25.05	24.88	99.3	21.59	86.2*
	30 mM KNO_3	23.14	21.94	94.8*	21.84	94.4*
Cotyledon 24 h	H_2O	15.81	13.41	84.8*	14.03	90.6*
	10 mM TU	22.84	23.18	101.5	21.87	95.8
	100 µM BA	32.82	33.05	100.7	32.79	99.9
	100 µg/ml GA_3	21.88	22.03	100.7	22.38	102.3
	100 mM KNO_3	10.66	10.70	100.4	10.50	98.5

Table V. Interrelationships between Germination Responses in Cocklebur Seeds and Growth Responses of their Axes and Cotyledons.
-, inhibition; ±, +, ++, increasing intensity of promotion. a) data after 24 hr, b) data 72 hr, c) data cited from a previous paper (10).

Drug	Germination Response		Growth Response					% of Normality of Germinated seeds
			Axis		Nondormant			
	Dormant	Impotent	Dormant	Nondormant	Dormant	Nondormant		
Auxin	±	-	±	-	±	±a)	-b)	c)
Cytokinin	+++	++++	++	++	+++++	++++	+++++	55
Gibberellin	±	+	±	++	+	++	+++	57
Ethylene	++++	++++	++++	+++	++++++	++++	+	48
Carbon Dioxide	+	++	+	++	+	+	±	76
Thiourea	+++++	+++++	+++++	+++++	++	++	++	80
Potassium Nitrate	±	±	±	-	+++	±	++	

tive in the impotent ones; and IAA and KNO_3 were ineffective in either. The different extents of action of these stimulators on the initial growth of cocklebur axes and cotyledons are summarized in Table V, representing results from this study and the preceding one (7).

There have been conflicting suggestions about the action site of cytokinin and gibberellin in stimulating seed germination. For example, Ikuma and Thimann (12) have argued that the main action site of GA_3 may be on the embryonic axis, although it may also be promotive to cotyledon expansion, whereas the main action of kinetin may be on the cotyledons. In contrast, Bradbeer and Pinfield (3) and Pinfield and Stobart (19) have implied that kinetin may be a primary factor for radicle initiation, whereas GA_3 may be significant in stimulating the cotyledon expansion, although acting also on the axial growth. From the data in Table V, however, this conflict can be resolved. Since gibberellins and cytokinins could stimulate the initial extension growth of both the axis and cotyledons, for some seeds the axis may be mainly responsible for rupturing the seed coat, and in other seeds the cotyledons may be mainly responsible.

It is also apparent that the normality of TU-induced germination (10) is due to much greater stimulation of the initial axial growth than of the cotyledonary expansion, resulting in a preferential rupture of the testa at the apical end. On the other hand, BA and C_2H_4 each caused a greater frequency of lateral rupture of the testa (10); the similarity of BA and C_2H_4 effects reminds us of the suggestion of Ketring and Morgan (14) that kinetin might act on seed germination partially through the enhancement of C_2H_4 production. Since the time courses of cotyledon growth differed between BA- and C_2H_4-treated cotyledons (Fig. 2), and the removal of endogenous C_2H_4 produced from BA-treated axes or cotyledons did not have any influence on either the BA-enhanced axis or cotyledon growth (Table IV), BA and C_2H_4 seem to have independent effects in regulating seed germination. Also, the TU-stimulated growth of isolated axes and cotyledons was not affected by trapping the endogenously evolved C_2H_4 (Table IV). Thus, the higher C_2H_4 productivities in the BA- or TU-treated seed organs seem to be associated merely with the enhanced growth activity of the axial and cotyledonary organs in the presence of BA or TU, unlike the cases of enhanced C_2H_4 production in KNO_3- or IAA-treated organs.

The response of cocklebur seeds to GA_3 was very small compared to TU and BA, and was restricted to the nondormant, impotent seeds (Table I). This seems to be due to its weak promotive effect on both the axial and cotyledonary growth as shown in Table III. Nevertheless, it is apparent that GA_3 was able to cause seed germination by stimulating both the axial and cotyledonary growth, resulting in the slight increase of its abnormality (Table V). These data would support the recent view (2, 4–6) that GA_3-induced biogenesis at hydrolytic enzyme may not be the primary action of GA_3 in the stimulation of seed germination.

Musgrave *et al.* (17) and Musgrave and Walters (16) have suggested that a natural level of gibberellin seems essential for the C_2H_4-responding growth in aquatic plants. On the other hand, Esashi *et al.* (10) have suggested that the inability of seeds to respond to GA_3 may be due to their low C_2H_4 productivity, for GA_3-insensitive cocklebur seeds became responsive to GA_3 in the presence of exogenous C_2H_4. Additional support for this view was provided by the result in Table I that GA_3 was completely incapable of stimulating germination in the dormant cocklebur seeds, which possessed little C_2H_4-generating ability (13). This lack of GA_3 response in the dormant seeds, and the lack of a GA_3 growth response (Table III), plus the fact that GA_3 was quite unable to be functional in the axes without a higher C_2H_4 productivity, would support the hypothesis that dormancy of cocklebur seeds is a repressed state of C_2H_4 biogenesis (13).

Regardless of the dormancy status, cocklebur seeds did not respond to KNO_3 for germination (10, Table I), although some other seeds did (15). As shown in Table III, KNO_3 had no effect at concentrations below 10 mM on axial growth or was inhibitory at 100 mM, but accelerated the extension growth of cotyledon segments with increasing concentrations after the first 24 hr. Moreover, KNO_3 enhanced the C_2H_4 production particularly in the nondormant axial segments. Therefore, if some seed was capable of germinating in response to KNO_3, its germination might depend more largely on a thrust created from the cotyledonary growth than on that from the axial growth, or alternatively, its seed might become capable of germinating in response to C_2H_4, which production was enhanced by KNO_3. However, it is known that the effect of KNO_3 is improved when it was applied in combination with GA_3 by which the C_2H_4 production was not enhanced but the axial growth was promoted (11, 18, 20). Based on these results, the former possibility may be more likely.

It had formerly been reported that IAA is promotive to seed germination (15). In Table III, IAA, like KNO_3, stimulated C_2H_4 production, as in many other cases (1), in the both seed organs of axes and cotyledons, but it did not enhance the cotyledonary growth. It seems likely that the cases of germination stimulation by IAA may be due to the stimulation of C_2H_4 production. In any case, it is of interest to note that not only IAA but also KNO_3 stimulates the C_2H_4 production with no increased axial growth.

Trapping of endogenously evolved CO_2 reduced the TU- and GA_3-enhanced axial growth (Table IV). However, these reductions may not be due to a specific requirement for CO_2, since the TU- and GA_3-enhanced cotyledonary growth were not affected by the trapping (Table IV). Nevertheless, in view of the facts that the effect of TU on the cocklebur seed germination was lowered by CO_2 removal (10), and the cotyledonary growth was not affected materially by CO_2 (7), the CO_2 trapping may be still significant for the TU or GA_3 action on the axial growth regulation.

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