EFFECT OF 8-AZAGUANINE AND 2, 4-DICHLOROPHENOXY ACETIC ACID ON INOSITOL METABOLISM IN GERMINATING *PHASEOLUS RADIATUS* SEEDLINGS

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ABSTRACT

The distribution of different forms of myoinositol, like free or total, in different parts of normal germinating seedlings of *Phaseolus radiatus* has been compared with that of seeds germinated in the presence of antimetabolites like 8-azaguanine and 2, 4-dichlorophenoxy acetic acid (2, 4-D). Both these agents produced a deformed, stunted growth, which was found to reflect in a general inhibitory effect on the enzymes like myoinositol dehydrogenase, myoinositol-l phosphatase and phytase, directly involved in the metabolism of myoinositol. 8-Azaguanine was found to be more inhibitory than 2, 4-D in all respects.

THE relationship between auxin induced cell expansion and nucleic acid metabolism has been studied with the help of inhibitors of protein and nucleic acid biosynthesis. A number of reports have discussed the inhibitory effects of these agents in micro-organisms[1-4] but comparatively little is known about these effects in plants. Effects of 2, 4-D application on plant growth are variable according to the concentration used. In excised corn mesocotyls, low concentrations of 2, 4-D accelerate growth and RNase activity, whereas high concentrations inhibit both[5]. In excised soybean hypocotyl sections, 8-azaguanine and 6-methylpurine markedly inhibit synthesis of all types of RNA as well as protein and cell elongation[6]. In the present work it has been attempted to study the influence of an antimetabolite like 8-azaguanine and an auxin like 2, 4-dichlorophenoxy acetic acid (2, 4-D) on the metabolism of inositol in the germinating seeds of *Phaseolus radiatus.*

EXPERIMENTAL

Germination.—A known number of healthy mature *Phaseolus radiatus* seeds was soaked for 18–20 hours in distilled water. Control seeds were spread in petri dishes lined with a thin layer of absorbent cotton, kept wet by periodic watering. A batch of 20 seeds, soaked normally, was germinated in similar petri dishes containing 20 ml of 2, 4-D (National Chemical Laboratory, Poona) solution (5 μg/ml), in otherwise similar conditions. The germinated seeds were removed at stated intervals, washed thoroughly and then used in various determinations. Another group of 20 seeds, normally soaked, was germinated similarly in the presence of 20 ml 8-azaguanine (California Foundation for Biochemical Research) solution (50 μg/ml) and used for different determinations after washing thoroughly, at required intervals of time.

At different time intervals, the normal and treated seedlings were separated into different parts. Determination of free, total inositol, assay of inositol dehydrogenase and inositol-1-phosphatase activities were carried out as described earlier[7].

Assay of Phytase Activity.—20 seedlings were extracted with water at 4° C and the extract made to 40 ml. The extract was centrifuged at 3,000 RPM for 30 minutes. The clear supernatant was used as the enzyme extract. The assay system consisted of 2 ml 0·1 M acetate buffer, pH 5·2, 0·5 ml sodium phytate (50 μ mole) (B.D.H. Ltd.) and 1 ml enzyme extract. The system was incubated at 45° C for 3 hours, deproteinised by heating in a boiling water-bath for 5 minutes. The supernatants after centrifugation were tested for inositol content microbiologically[7]. Phytase activity was expressed as phytase units. One phytase unit was defined as that amount of enzyme which brought about liberation of 1 μg inositol under the conditions mentioned above.

RESULTS AND DISCUSSION

The plant hormones can be broadly divided into three groups, *viz.*, auxins, gibberellins and cytokinins. These are known to influence cell extension, cell division, root growth and rate of germination on the whole. Results from many laboratories have claimed that auxins stimulate synthesis of *m*RNA[8]. On the basis of action of an auxin like 2, 4-D, tissues like corn mesocotyl[9,10], cotton cotyledon[11,12] could be grouped together as they require high concentrations of auxins to initiate increases in level of RNA, while tissues like soybean hypocotyl[13,14] form a second group, which needs low concentrations of 2, 4-D to increase the fresh weight but decrease level of RNA. However, in peanut cotyledons, 2, 4-D at 10^{-5}m concentration has been known to inhibit synthesis of all nucleic

TABLE I

Variation in total, free and bound inositol contents of germinating Phaseolus radiatus *seedlings* μ *gm/gm. seeds under the influence of 8 AG and 2, 4-D*

Day[a] of germination	Germinating medium	Cotyledon			Seedcoat		
		Free	Bound	Total	Free	Bound	Total
1	Water ..	498·8	2708·2	3207·0	17·9	5·6	23·5
3	,, ..	485·9	2083·0	2568·9	14·0	3·8	17·8
3	8 AG[b] ..	341·2	2578·8	2920·0	14·3	0·9	15·2
3	2, 4-D[c] ..	390·3	2212·7	2603·0	13·2	1·7	14·9
5	Water ..	281·9	847·1	1129·0	13·5	3·7	17·2
7	,, ..	167·4	187·6	355·0	12·1	3·1	15·2
7	8 AG[b] ..	220·8	1069·2	1290·0	10·2	2·0	12·2
7	2, 4-D[c] ..	237·1	581·7	818·8	9·7	0·5	10·2

Days[a] of germination	Germinating medium	Hypocotyl			Leaf			Root		
		Free	Bound	Total	Free	Bound	Total	Free	Bound	Total
1	Water ..	34·0[e]	62·5[e]	96·5[e]	..	..	..	..	..	..
3	,, ..	117·6	330·4	448·0	17·6	82·1	99·1	..	..	..
3	8 AG[b] ..	14·9[e]	86·8[e]	101·7[e]	..	..	..	..	..	..
3	2, 4-D[c] ..	25·6	136·2	161·8	14·3	74·5	88·8	..	..	..
5	Water ..	368·9	485·3	854·2	34·2	341·7	375·9	76·2	180·4	257·6
7	,, ..	128·7	461·5	590·2	62·3	247·9	310·2	32·3	140·5	172·8
7	8 AG[b] ..	79·2[d]	320·5[d]	399·7[d]	25·2	30·2	55·4	..	..	..
7	2 4-D[c] ..	117·1[d]	569·5[d]	686·6[d]	27·7	78·5	106·2	..	..	..

[a] Day of germination counted from soaked seed stage represented as 1 day of germination, [b] 8 AG represents 8 azaguanine solution added at 1,000 μg/20 seeds, [c] 2, 4-D represents 2, 4-D solution added at 100 μg/20 seeds, [d] Hypocotyl and root were combined together, [e] Hypocotyl, root and leaf were combined together.
Note: Same notations followed in Table II.

acid fraction to the same extent[15] but in soybean hypocotyls it enhances synthesis of ribosomal RNA particularly[14,16,17].

In the present work, germinating 20 *Phaseolus radiatus* seeds in contact with 2, 4-D solution at 100 μg level inhibited growth to an extent of 50%. Browning of the widely open cotyledons, thickening of the shoot, stunted growth, delayed root development were also observed. Treatment with 8-azaguanine also gave a deformed stunted growth and browning of cotyledons comparatively more pronounced than 2, 4-D. As shown in Table I, free and total inositol contents of different parts of the *Phaseolus radiatus* seedlings are markedly affected by both these compounds. The most sensitive part appears to be the hypocotyl region, where there is a drastic reduction of free inositol on the 3rd day. Though other growing regions accumulate lesser amounts of the free or bound inositol, the cotyledon still possesses appreciably greater quantities of these as compared to the normal, even at the end of the 7th day. The rates of disappearance of free inositol from cotyledons appear to be very much slowed down under the influence of both these treatments thus resulting in higher values of free inositol in treated cotyledons on the 7th day. There appears to be a blockage in transport of the stored inositol of the cotyledons to the growing regions and this effect seems to fade away gradually, so that if the germination be continued further, transport of the cotyledonary stores would be complete. The hampered disappearance may be as a result of blockage of translocation of the inositol free or bound by hampering one or more of the enzymes involved in different pathways of inositol metabolism[7]. The latter possibility seems to hold good in this case as is apparent from the observations on the enzymes studied. Thus as seen from Table II inositol-dehydrogenase, inositol-1-phosphatase as well as phytase are markedly affected under the influence of 2, 4-D and 8-azaguanine at

TABLE II

Effect of 8 azaguanine and 2, 4-D on some enzymes involved in inositol metabolism, at various stages of germination

Day of germination	Germinating medium	Inositol dehydrogenase units/cotyledon	Inositol-1-phosphatase units/cotyledon	Phytase units/g seed
1	Water ..	1·4	15·4	320·3
3	,, ..	2·0	21·7	445·9
3	8 AG[b] ..	0·87	11·3	268·5
3	2, 4-D[c] ..	0·71	8·1	310·5
5	Water ..	2·9	28·3	578·1
5	8 AG[b] ..	1·3	14·87	339·2
5	2, 4-D[c] ..	1·01	10·83	448·1
7	Water ..	2·1	20·5	299·6

the concentrations used here. Though the extents to which depression of the activities takes place are different with the two agents tried here, on the whole both the agents reveal the role of cotyledons and hypocotyls as the most important site of inositol metabolism. The cotyledon itself being a store of all nutrients is actively involved in both breakdown as well as synthesis of different constituents simultaneously. As far as the possible inhibitory action for enzyme synthesis by 8-azaguanine is concerned, it could be due to reduced RNA synthesis as in soybean hypocotyls[6] or also due to its incorporation into RNA at the expense of guanine as in micro-organisms[1,18] or due to formation of structurally altered RNA as reported elsewhere[19]. Both these agents cause inhibition of growth of the germ into well-differentiated plant organs, *viz.*, root and shoot. It is possible that the primary action of these agents, is on the germ growth, so that the inhibited germ activities cause an interference in the translocation of the catabolic products of the storage material in the cotyledons. In this case, it is indicated that the enzymes acting in the cotyledons originate in the germ. Thus the low values for free inositol in cotyledons in treated seedlings on the 3rd day of germination would mean low synthesis of the enzymes possibly in the stunted form. However, it has already been observed with *P. radiatus* seedlings that cotyledons are completely independent of the germ as far as enzyme synthesis during germination is concerned[20]. This rules out the possibility that the enzymes acting in the cotyledons originate in the germ. It is therefore clear that both these agents act as inhibitors of the enzyme synthesis, 8-azaguanine probably due to alteration in the nature of RNA and RNase synthesised[19] and 2, 4-D due to inhibition of RNA synthesis, as is observed with peanut cotyledons[15].

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