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EFFECT OF POTASSIUM NAPHTHENATE ON RIBONUCLEIC ACID IN THE LEAF OF *PHASEOLUS VULGARIS* L.

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Abstract

The RNA of leaf tissue of *Phaseolus vulgaris* L. var. Top crop treated with potassium naphthenate and subsequently incubated with ^{32}P, was extracted and fractionated by sucrose gradient method. A comparison of results i.e., radioactivity and O.D. showed that there was more RNA synthesis in potassium naphthenate-treated plants than in those of control. An unidentified fraction of RNA showed highest incorporation of ^{32}P in treated plants. In addition, there was more ^{32}P incorporation in 28 S—, 18 S—, and 4S RNAs of treated plants than in those of control.

Introduction

Naphthenic growth substances have been extensively used by Russian scientists as a stimulator of growth and yield of a number of plants (Agakishev and Bazanova 1966, Ladygina 1965, Marshaniya *et al.* 1965). It has been found that Bush bean plants treated with 0.5% potassium naphthenate (KNap) give more increase in vegetative and reproductive growth, increase in pod yield (Wort 1969, Fattah and Wort 1970 b) ; increase in the rate of photosynthesis and respiration, increase in specific activity of some enzymes (Fattah and Wort 1970 a) and also Vitamin C contents of pods (Fattah unpublished). Pakhomova (1965) reported that following treatment with naphthenic growth substance the content of α-nucleoprotein complex of tomato plants increased and that of β-nucleoprotein complex decreased. To date hardly anything has been reported about the effect of KNap on RNA contents of plants. In order to know the effect of this growth substance on RNA contents the present study was undertaken and the material chosen was Bush bean with a view to understanding the mechanism of KNap action.

Materials and Methods

a. *Treatment*

Bush bean plants, *Phaseolus vulgaris* L. var. Top crop were grown in 15-cm pots of composted soil in a growth room provided with a day and night constant temperature of 26.6 ± 1C and the plants received 16.1 Klux (1500 Ft-C) of light

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on the top during the light period from a source of cool white fluorescent tubes and tungsten bulbs. The relative humidity of the growth room was 60 to 70% during the light period and 70 to 80% during the dark period. Fourteen days after the seed had been sown, the foliage of 16 plants, growing one in a pot, was thoroughly sprayed with a 0.5% aqueous KNap solution. Seven days after treatments four plants of uniform size were randomly selected from the treatment group and four plants from the control group. All the plants were removed from the pots and their roots were carefully washed with tap water.

b. *RNA extraction*

Four treated plants were placed in a water-culture jar with their roots immersed in 400 ml $\frac{1}{4}$ conc of Hoagland solution and 40 μc of carrier-free $H_3{}^{32}PO_4$. Control plants were also treated in the same way. Plants were provided with ^{32}P solution for 17 hrs, out of which for seven hrs they were supplied with light.

At the end of ^{32}P treatment 29 g of leaves were collected from both treated and control plants. RNA was extracted by the method of Ralph and Bellamy (1964) with slight modifications. Leaves were deribbed and were homogenized in a mixture of equal vols of 0.5% aqueous sodium naphthalene disulphonate solution and 90% aqueous phenol containing 0.1% 8-hydroxyquinoline in a Waring blendor for four mins at full speed. 0.2% sodium dodecyl sulphate (SDS) was added to the homogenates and were stirred with a magnetic stirrer for ten mins. The homogenates were filtered through four layers of cheese cloth and were then centrifuged at 5000 g for ten mins at 0—4C. The clear supernatant solution was removed and kept in a flask. Thirty ml of napthalene disulphonate and 0.1% SDS were added to the phenol and interphase and was stirred for ten mins. The clear supernatant was removed as before. Twenty-five ml of phenol and SDS to 0.1% were added to the combined supernatant and was stirred for ten mins, centrifuged at 5000 g for ten mins. The clear supernatant was collected and added to two vols of 95% chilled ethanol and was kept in cold, 0-4 C, for three hrs for the precipitate to form. The precipitate was collected by centrifugation of the ethanol mixture at 12,000 g for 15 mins. Fresh 40 ml 95% ethanol was added to the precipitate and was thoroughly stirred with a glass rod for two to three mins and it was then centrifuged at 12,000 g for five mins. The alcohol was poured out and fresh 20 ml of 95% ethanol was added, stirred and centrifuged as before. The precipitate was added to 20 ml 70% alcohol, stirred with a glass rod for two to three mins and was kept at 0-4 C for 12 hrs and then centrifuged at 12000 g for seven mins and the precipitate was collected, which contained crude preparation of RNA. RNA solutoin was made by disolving the precipitate in two ml Tris-HCl buffer solution, pH 8.1.

c. *Sucrose gradient*

Linear gradients from 20 to 5% sucrose made up in 0.01% M Na-acetate, pH 5, 0.1 M KCl and 0.001 M EDTA was made and 0.2 ml of stock solution of RNA was layered on top of the sucrose gradient and was spun in a Spinco SW 39 rotor at 37,000 rpm for five hrs. After the run the bottom of the centrifuge tube was pierced with a hypodermic needle and five drop fractions were collected in separate tubes having 2.8 ml Tris-HCl buffer, pH 8.1. OD and radioactivity of the sucrose gradient fractions were measured. Moreover, 0.1 ml of the stock RNA solution was diluted to ten ml with Tris-HCl buffer solution and the O.D. and radioactivity was measured.

O.D. were recorded with a SP 500 spectrophotometer and a SP-800 Ultraviolet spectrophotometer. Radioactivity of solutions was measured with a Nuclear Chicago model 151 A G.M. radiation counter. Necessary background count correction and decay correction were made.

Results and Discussion

When the plants were incubated in culture solution containing ^{32}P it was found that at the end of incubation period there was more ^{32}P uptake by control plants (Table I). It could be due to the fact that KNap treatment decreased the uptake of ^{32}P. Wort and Loughman (1961) observed that treatment with amitrole diminished ^{32}P uptake by barley plants.

Table I

^{32}P uptake by control and treated plants

	*Solution at start	*Solution at the end	^{32}P uptake	% uptake
Control	26781 cpm**	5242 cpm	21539 cpm	80 42
KNap-treated	25645 ,,	11137 ,,	14508 ,,	56.57

* Readings of 1 ml culture solution + 24 ml dist H_2O.
** Counts per minute.

The RNA isolated from KNap treated plants gave higher O.D. at 260 mμ and higher radioactivity in comparison to that of control plants. The specific activity (radioactivity per unit O.D.) of the RNA from treated plants was also higher (Table 2). These results indicated that there was more RNA synthesis following KNap treatment in Bush bean leaves.

Table 2

O.D. radioactivity and specific activity of RNA solutions

	O.D. at 260 mμ*	O.D. at 280 mμ of RNA soln*	260 mμ / 280 mμ	Radioactivity of RNA soln
Control	1.0	0.61	1.64	2190 cpm
KNap-treated	1.4	0.88	1.59	3821 ,,

		Specific activity **
(Control)	...	2190
(KNap-treated)	...	2729.28

* 0.1 ml stock RNA soln. + 99.9 ml Tris-HCl buffer
** radioactivity per unit O.D.

A comparison between O.D. at 260 mμ and 280 mμ showed a slightly higher value for control plants and this indicated that there was slightly more impurities in the extracts obtained from KNap-treated plants (Table 2 and Fig. 1).

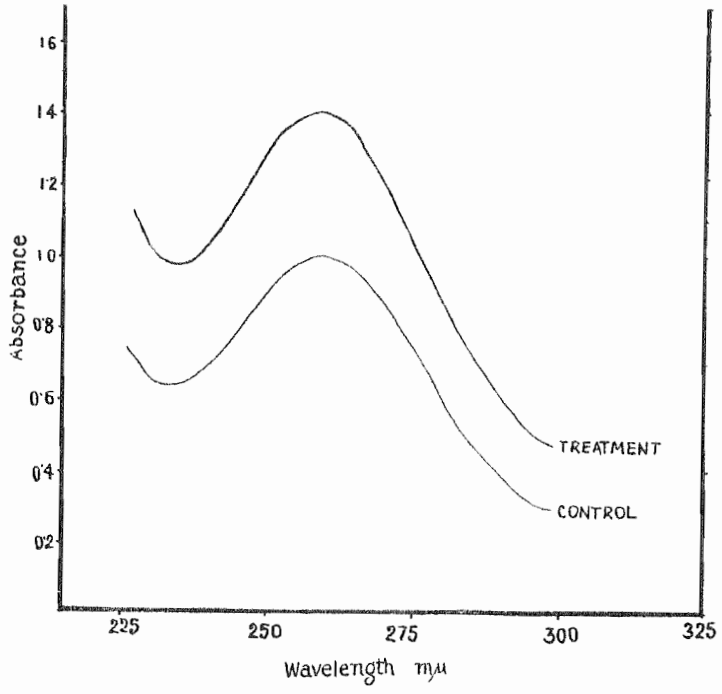

FIG 1.

The radioactivity and O.D. of sucrose gradient fractions showed three distinct peaks in RNA of both control and treated plants. The peak at 3, 6 and 11 were

thought to be of 28S-, 18S -, and 4S RNA respectively. In case of treated plants there was an additional peak at fraction number 9 (Fig. 2).

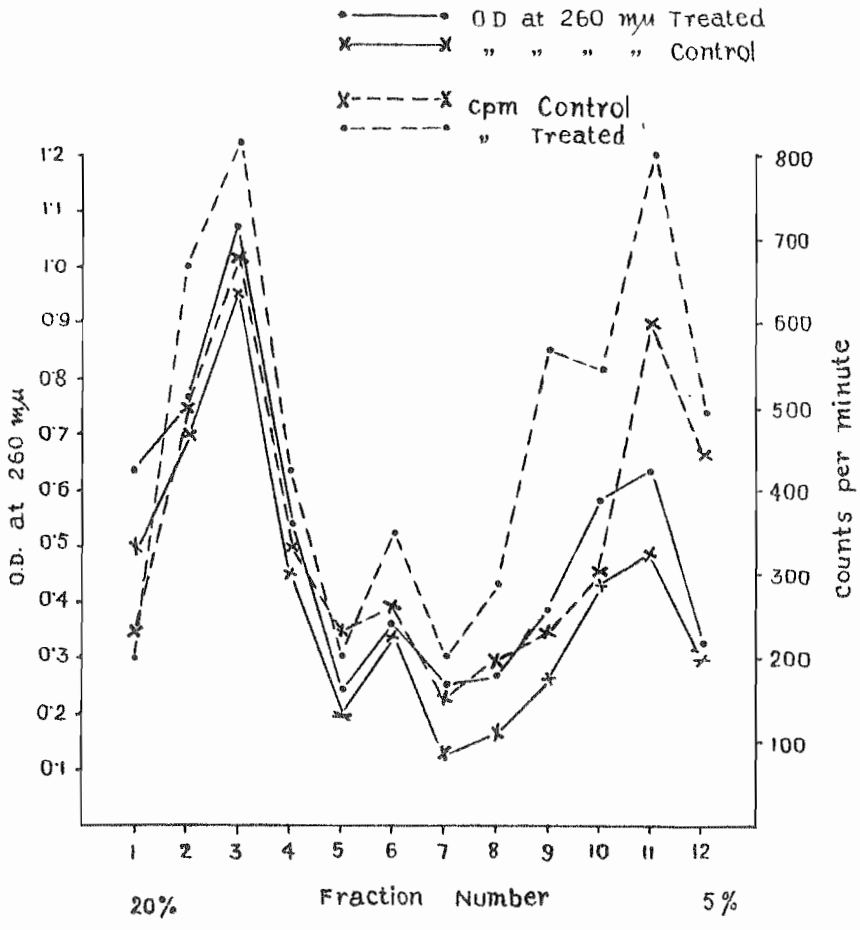

FIG. 2.

The specific activity of RNA of different fractions showed that there was more radioactivity incorporated in RNA of treated plants in 28S-, 28S - and 4S fractions (Table 3). However, highest specific activity was found in fraction number 9 of treated plants in comparison to that of control. The nature of this fraction of RNA could not be ascertained from the present study. It is being suggested, however, that this fraction could be of messenger RNA (mRNA).

The above results showed that KNap treatment increased RNA synthesis of Bush bean plants.

Table 3

Specific activity of RNA at different sucrose gradient fractions

Fraction number	Specific activity		Treated / control
	Control	Treated	
1	465.07	313.48	0.67
2	729.93	888.29	1.21
3	714.73	759.81	1.06
4	738.46	792.59	1.07
5	1182.74	714.89	0.60
6	840.58	980.55	1.17
7	1224.00	796.81	0.65
8	1178.57	1051.47	0.89
9	888.46	1468.91	1.65
10	702.32	930.03	1.32
11	1224.48	1253.92	1.02
12	1503.33	1518.51	1.01

Although, hardly anything is known about the effect of KNap on RNA synthesis, there are, however, reports that due to the action of some hormones there is an increase in nucleic acid synthesis. Extensive work has been done on the effects of IAA, gibberellic acid, kinetin and 2, 4-D on RNA metabolism of plants (Biswas and Sen 1959, Basler and Nakazawa 1961, Key and Hanson 1961, Roy Chowdhury and Sen 1964, Saccher 1967). The work of Silberger and Skoog (1953) showed that auxin enhanced nucleic acid synthesis in tobacco pith cultures at growth promoting concentrations. As with several other hormones the enhancement of RNA synthesis by auxin resulted from an increase in all major RNA species, with a preferential increase in ribosomal RNA. Wort (1964) mentioned that one of the most marked chemical changes induced by 2, 4-D application to a number of plant species was the increase in their RNA content. He also found that the treatment of barley plants with a 2, 4-D mineral dust resulted in a greater production of acid soluble phosphorus in the leaves in the form of nucleotides and sugar phosphates.

About the primary action of kinetin, Osborne (1965) suggested that it might regulate the synthesis of a particular RNA fraction and put forward evidences that kinetin influenced the synthesis of ribosomal and soluble RNAs. Thus, the present study is in conformity with other works on hormones in being that the growth stimulators increase the synthesis of RNAs. Experiments by several workers including the author showed that naphthenate solutions when applied as foliar spray in suitable concentration could increase several physiological and biochemical processes including increase in the synthesis and specific activity of several enzyme systems (Burachevskii 1965, Kolesnik 1965, Babaev 1966, Bazanova and Akopova 1966, Fattah and Wort 1970a). Whether the increase in specific activity of enzymes is due to an increase in the actual synthesis of enzymes concerned or due to the increase in enzymatic activity is hardly known. An answer to the action of KNap may lie in the control of the mechanism by which enzymes are made in the cell e.g., at the transcription or translation level. The present study showed that KNap increased synthesis of RNAs and thus it has effect at the transcription level.

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