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LACK OF EFFECT OF PLANT GROWTH-REGULATORS ON THE ACTION OF ALPHA-AMYLASE SECRETED BY VIRUS TUMOR TISSUE¹

M. K. BRAKKE AND L. G. NICKELL

Introduction

This report deals with the effect of certain synthetic plant growth-regulators on the activity of alpha-amylase (1) secreted by virus tumor tissue grown *in vitro*. Previous studies have lacked adequately controlled conditions and have been carried out with enzyme preparations obtained through the usual methods of destruction of cells. In this particular study (*a*) the assay employed to measure enzyme activity includes control of pH and temperature and (*b*) this enzyme, being secreted, is not subject to the drastic preparative procedures involving destruction of cellular structure usually necessary to obtain enzymes.

Material and methods

The tumor tissue used was induced by the wound-tumor virus (*Aureogenus magnivena* Black) in the roots of the sorrel plant (*Rumex acetosa* L.) and was the same R₁ isolate used in other studies (2, 7-9). Enzyme activity was assayed (1) by following spectrophotometrically the decrease in optical density of the starch-iodine complex. This method is sensitive to low levels of amylase, but, because of the many factors affecting the color of

the complex, the reproducibility is only about $\pm 5\%$. The growth-regulators tested and the concentrations used are given in table 1.

Three different enzyme preparations consisting of unpurified spent liquid medium no. 24 (2) were used. The medium in each case had been inoculated with approximately one-tenth its weight of tumors 2-10 days earlier. The medium was used immediately after the tumors were removed and after storage overnight in the refrigerator. Enzyme activity was assayed in 1% soluble starch and 0.02 M, pH 4.6, acetate buffer at 40° C. Growth-regulator solutions were adjusted to pH 4.6 before being added to the reaction mixture.

Results

It is apparent that these compounds have no significant effect on the activity of the alpha-amylase (table 1). The greatest effect (10% decrease by beta-naphthoxyacetic acid at 100 p.p.m.) approximates the limit of experimental error for the comparison of two runs. The low levels of activity at all growth-regulator concentrations used in this particular experiment indicate that the control may have been high. For this tumor tissue these same growth-regulators at 100 p.p.m. cause a growth inhibition of between 90% and 100% and an inhibition

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of respiration amounting to 37% for indoleacetic acid, 10% for 2,4-dichlorophenoxyacetic acid, and 70% for beta-naphthoxyacetic acid (7).

Discussion

EYSTER (3) reported that indolepropionic acid retarded the action of malt diastase on soluble starch but accelerated the action of the enzyme in the presence

plant growth-regulators: indoleacetic, indolebutyric, indolepropionic, alpha-naphthaleneacetic, 2,4-dichlorophenoxyacetic, beta-naphthoxyacetic, and 2,3,5-triiodobenzoic acids (11, 12). In these instances the growth-regulators were used at concentrations as low as 100 p.p.m., but no figures were given to indicate what constituted effective inhibition. NEELY *et al.* (6) have reported reduced

TABLE 1
EFFECT OF PLANT GROWTH-REGULATORS ON SECRETED α -AMYLASE

GROWTH-REGULATOR	ACTIVITY OF CONTROL*	ACTIVITY (CONTROL=100%) IN PRESENCE OF GROWTH-REGULATOR† AT		
		1 p.p.m.	10 p.p.m.	100 p.p.m.
2,4-Dichlorophenoxyacetic acid.	{ 0.338	96	99	93
	{ .085	100	97	91
Indoleacetic acid.	{ .090	98	101	104
	{ .130	100	96	98
Beta-naphthoxyacetic acid.	{ .325	93	98	97
	{ 0.135	94	91	90

* Milligrams of starch hydrolyzed per hour per milliliter of enzyme solution.

† Based on activity of control as 100%.

of charcoal, presumably by releasing the adsorbed enzyme from the charcoal. The same author later (4) mentioned similar action by indolebutyric, indoleacetic, and alpha-naphthaleneacetic acids at concentrations of 25 and 50 p.p.m. and reached the conclusion that the effectiveness of these compounds was due entirely to a pH effect (4, 5). However, SMITH *et al.* (10) pointed out the lack of pH control in EYSTER'S experiments. These workers were unable to duplicate EYSTER'S results under controlled conditions. They could find no effect of indoleacetic acid on diastatic activity in the presence or absence of charcoal.

Still other reports indicate effective inhibition of salivary amylase by many

levels of alpha- and beta-amylase activity in the stems and leaves of bean plants treated with 2,4-D.

Summary

1. Plant growth-regulators representing the indole, phenoxy, and naphthoxy types of structure were tested to determine their effect on the activity of an alpha-amylase secreted by virus tumor tissue from the roots of *Rumex acetosa* grown *in vitro*.

2. At concentrations up to and including 100 p.p.m. they had no significant effect on its activity. The greatest effect was within the limit of experimental error.

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A SAND PRESS METHOD FOR PRESERVATION OF NATURAL COLORS IN HERBARIUM SPECIMENS

Collection and preservation of plant specimens have been essential in the studies of plant pathologists and taxonomists. Not only the preservation of plant materials but retention of their natural colors is desired by botanists, especially by plant pathologists. So far, no method has been available for retaining the natural colors of herbarium specimens. The various presses used for preparation of such specimens have been unsatisfactory because: (a) a single worker making large-scale collections finds repeated changes of blotting paper to hasten drying of the pressed specimens laborious and time-consuming; (b) thicker structures, such as the sori and galls of rusts, smuts, etc., are crushed; (c) colors are rarely maintained.

After unsatisfactory experience with this traditional method, the writer has recently successfully evolved a labor-saving, cheap way to prepare herbarium specimens in natural colors. This method uses layers of clean, dry, sand of medium texture to press the specimens and, at the same time, through porosity of the sand mass, provides ample aeration which facilitates rapid removal of moisture from the specimens. A level layer of sand 1 inch thick is spread on a platform, and an absorptive paper is laid over

the sand. The specimens are placed on it without overlapping and then covered by a similar sheet of paper. Over this is spread another layer of sand 1 inch thick. This process is repeated, alternating layers of sand with plant specimens until a stack 10 inches high is made, thus pressing five layers of specimens. The intervening layers of sand are leveled each time.

The stack is kept exposed, preferably under a powerful fan or facing a constant wind current. Specimens dry in about 3-5 days depending upon the factors that affect rapid removal of moisture, such as humidity, wind velocity, breadth and height of the stack, thickness of the sand layers, etc. The specimens may be taken out after this period or may be left indefinitely. To remove dried material the sand layer is carefully pushed aside, the top sheet of paper below it is lifted from one side, and the pressed specimens then lie exposed on the other sheet. They are dry, flat, and retain their natural colors to a remarkable extent. Since the sand mass tends to distribute pressure uniformly, morbid and natural prominences on the plants are not crushed. The unhindered rapid removal of moisture from the fresh specimens through the porous sand apparently aids preservation of the plant pig-