BIOLOGIA PLANTARUM (PRAHA)

22 (2) : 86—90, 1980

Effect of Indoleacetic Acid on the Metabolic Activity of *Taraxacum* Root Segments*

M. I. KHAN

Department of Botany, University of Karachi, Karachi-32, Pakistan

Abstract. Incubation of thin slices of *Taraxacum* root segments in 3-indoleacetic acid solutions enhanced the level of total proteins, RNA, DNA and a number of enzymes reaching an optimum at 0.01 mg l^{-1}. It has been shown that the auxin promotes the synthesis of DNA, again with an optimum promotion at 0.01 mg l^{-1} IAA.

It has been known for over half a century that cutting storage tissue results in enhanced metabolic rate, but very little is known concerning the mechanism of this enhancement. There are two possible explanations of the enhanced metabolic rate in excised storage tissues. LATIES (1957) suggested that washing the slices of storage tissue might reduce the level of the endogenous inhibitor of respiration resulting in an increase in oxidative phosphorylation. An alternative explanation comes from the work of CLICK and HACKETT (1963), LEAVER and EDELMAN (1965) and MASUDA (1966). They thought that a change in the rate of the pattern of nucleic acid synthesis might lead to a change in the rate and pattern of protein synthesis, resulting in the high rate of respiration and phosphorylation.

Studies on the effect of growth substances on regulating the levels of metabolic activity in thin slices of dormant storage tissues have been carried out by a number of workers. EDELMAN and HALL (1965) reported that the development of invertase activity in thin slices of Jerusalem artichoke is stimulated by gibberellic acid (GA_3) and inhibited by indoleacetic acid (IAA). On the contrary, GLASZIOU *et al.* (1966) found that the auxin, α-naphthaleneacetic acid, stimulated the development of invertase in sugar cane slices.

This paper describes the effect of the auxin, indoleacetic acid, on the metabolic activity of *Taraxacum* root slices. Attempts have also been made to correlate the activity of the auxin with its biochemical effect.

MATERIAL AND METHODS

In an earlier communication (KHAN 1970) it was reported that the minimum thickness of *Taraxacum* root segments for the regeneration of shoots was 1.5 mm and for roots 2 mm and minimum diameter 5 mm. It was also

Received July 12, 1976; accepted July 6, 1979

* This work was carried out at the Department of Botany, University of Sheffield, Sheffield, England. The author wishes to thank Dr. A. Booth for taking a keen interest in this work.

found (KHAN 1973) that cell division began approximately 200 μm beneath the proximal and distal cut ends of the root segments after 24 h of regeneration. Therefore root slices of *Taraxacum officinale* WEBER 2.1 mm thick and 7—10 mm in diameter were excised and grown as described earlier (KHAN 1972a).

Twenty slices were placed on a filter paper in separate Petri dishes containing 10 ml of either distilled water or 0.0001—10 mg l^{-1} IAA solution. Each treatment was replicated three times and the mean value plotted. After 24 h of incubation at 25 °C in the dark, the slices were rinsed with water, blotted dry and used for the extraction and estimation of proteins, nucleic acids and a number of enzymes.

Protein and nucleic acids were extracted and estimated as described earlier (KHAN 1972b). Enzymes were extracted by crushing the root slices in an ice-jacketed mortar together with 5 ml per 25 slices of cold (5 °C) 0.02 M Tris-HCl buffer (pH 7.4) using acid washed sand. The slurry so obtained was filtered through eight layers of muslin and the filtrate centrifuged at $4000 \times g$ for 20 min at 5 °C. The supernatant solution was decanted and kept surrounded by ice chips and used for the estimation of enzymes.

Acid phosphatase, malic dehydrogenase and invertase were estimated as mentioned earlier (KHAN 1972c). IAA-oxidase was measured by the method of GALSTON and DALBERG (1954). 1 ml of enzyme was mixed with 7 ml of 0.2 M phosphate buffer (pH 6.7), 1 ml of 500 ppm IAA solution and 1 ml of 3.26 ppm 2,4 dichlorophenol (DCP) solution. In addition to the normal control, a further control of enzyme containing the reagents, with no IAA in it, was used to allow the presence of IAA in the enzyme extract to be measured. The flasks containing the reaction mixture were covered with black polythene to prevent light-induced inactivation of IAA and shaken on a rotary shaker for 2 h at 25 °C. 2 ml aliquots (duplicates) were pipetted out and added to the flask containing 8 ml of Salkowski's reagent prepared by mixing 300 ml of conc. H_2SO_4 (spec. gr. 1.84) with 15 ml of 0.5 M $FeCl_3$ and 500 ml of distilled water. The flasks were shaken for 30 min and the optical density recorded at 530 nm.

The effect of IAA on the synthesis of DNA in *Taraxacum* root slices was studied by incubating the slices in 20 ml of 1.5 μCi per ml solution of thymidine-^{3}H (specific activity = 13.6 mCi mg^{-1}, obtained from Radiochemical Centre, Amersham, England) with or without 0.001—0.1 ppm IAA. The slices were kept at 25 °C in the dark for 24 h after which the roots were washed with distilled water and extracted with 0.5 N NaOH and the DNA precipitated with 1 N perchloric acid, and finally digested with 0.5 N NaOH. 0.2 ml samples of the digested DNA were used for analyzing the radioactivity employing the liquid scintillation counter.

RESULTS AND DISCUSSION

The effect of varying concentrations of IAA on the level of protein, RNA and DNA of *Taraxacum* root slices after 24 h of treatment is presented in Fig. 1. Increasing concentration of IAA resulted in an increased protein, RNA and DNA contents with maximum promotion at 0.01 ppm IAA treatment. To understand the mechanism of IAA-induced promotion of protein and nucleic acids, the effects of varying IAA concentrations on the

synthesis of DNA in root slices was also studied. The results presented in Fig. 2 indicate that IAA at all concentrations tested promoted the synthesis of DNA as compared to the untreated control. At 0.01 ppm, synthesis of DNA was about twice as high as that of the control. It is interesting to note

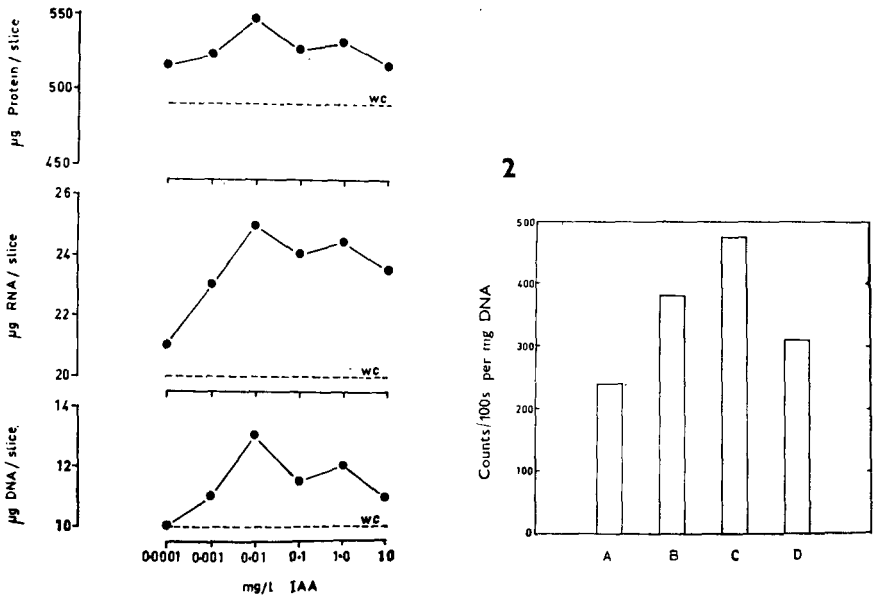

Fig. 1. Changes in the levels of total proteins, RNA and DNA in thin slices of *Taraxacum* roots after treatment with indoleacetic acid. Values are means of three replicates of 20 root slices. WC - slices treated with water only.

Fig. 2. The effect of indoleacetic acid on the incorporation of thymidine-^{3}H into DNA of *Taraxacum* root slices. A: Water control; B: 0.001 ppm IAA; C: 0.01 ppm IAA; D: 0.1 ppm IAA. Values are means of three replicates.

that at 0.01 ppm IAA treatment, the synthesis of DNA was at its optimum, confirming the result presented in Fig. 1. However, the incorporation of IAA-2-^{14}C into DNA fraction of regenerating *Taraxacum* root slices (details to be published elsewhere) has indicated that IAA is not incorporated into DNA at all. This suggests that IAA-induced synthesis of DNA (Fig. 2) is, perhaps, through its direct participation. MASUDA (1966), while studying the effect of actinomycin-D in ageing slices of Jerusalem artichoke tuber found that the synthesis of heavier type RNA was stimulated by auxin, probably through DNA since actinomycin-D inhibited the auxin-stimulated RNA synthesis as well as the auxin-induced growth expansion. He thought that this heavier type RNA may be responsible for the synthesis of special protein(s) which in turn is responsible for the auxin-induced growth, as also suggested by NOODEN and THIMANN (1963, 1965) and KEY and INGLE (1964).

Specific reactions involved in metabolic changes within a living organism are controlled by individual enzymes or an enzyme system. Since IAA treatment of *Taraxacum* root slices led to the increased production of proteins and nucleic acids, it is reasonable to expect that IAA may induce the specific activities of a number of enzymes. The effect of IAA on the specific activities

of acid phosphatase, IAA-oxidase, malic dehydrogenase, peroxidase and invertase of regenerating root slices was therefore studied. The result presented in Fig. 3 indicates that IAA has a promotory effect on these enzymes. The optimum auxin-induced increase in specific activities of acid phosphatase,

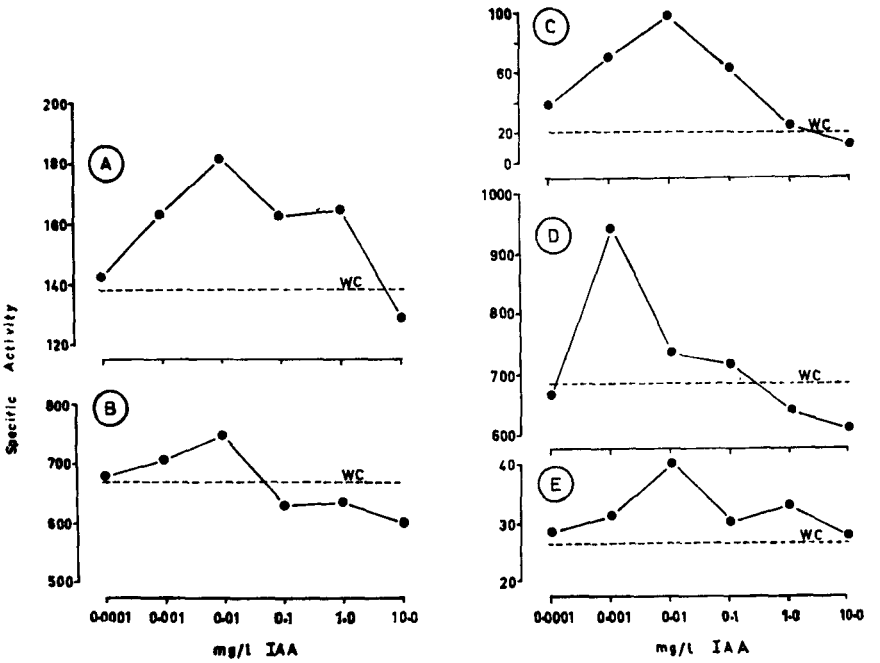

Fig. 3. Changes in the specific activities of acid phosphatase (A), peroxidase (B), IAA-oxidase (C), malic dehydrogenase (D) and invertase (E) in *Taraxacum* root slices treated with IAA solution. WC - slices treated with water only. Values are means of three replicates.

IAA-oxidase, peroxidase and invertase was found at 0.01 ppm IAA treatment and for malic dehydrogenase 0.001 ppm. At a higher concentration of IAA (10 ppm), the auxin was found to suppress the activities of all the enzymes with the exception of invertase where no change over control was observed. However, under *in vitro* conditions, as reported earlier (KHAN 1972c), IAA stimulated the activities of acid phosphatase and invertase with an optimum promotion at 1.0 ppm IAA whereas malic dehydrogenase activity was not affected. CLELAND (1961) reported that the specific activity of plant enzymes *in vivo* is usually increased by auxin concentrations while, with few exceptions, the *in vitro* activities are not affected.

EDELMAN and HALL (1965), LEAVER (1966), CHERRY (1968) and PALMER (1968) have reported that IAA treatment decreased the activity of invertase in different plants while GLAZIOU *et al.* (1966) found that the auxin, α-naphthaleneacetic acid, stimulated the development of invertase in sugar cane. In agreement with GLAZIOU *et al.* (1966) and PALMER (1968), the present study has revealed that the auxin, indoleacetic acid, promotes the development of the enzyme invertase and acid phosphatase during 24 h of treatment.

It may be concluded from the present study that indoleacetic acid could have a profound effect on the metabolic activity of *Taraxacum* root slices during 24 h of treatment. The maximum promotion in the levels of proteins, nucleic acids and enzyme activities after treatment with the auxin (IAA) was always at a similar concentration, suggesting that perhaps all these systems may be controlled by some key reaction such as the synthesis of m-RNA, and that the auxin may have some controlling influence on this reaction.

REFERENCES

CHERRY, J. H.: Regulation of invertase in washed sugar beet tissue. — In: WIGHTMAN, F., SETTERFIELD, G. (ed.): Biochemistry and Physiology of Plant Growth Substances. Pp. 417 to 433. Runge Press, Ottawa 1968.

CLELAND, R.: In: RUHLAND, W. (ed.): Handbuch der Pflanzenphysiologie. Pp. 754—783. Springer Verlag, Berlin—Göttingen—Heidelberg 1961.

CLICK, R. E., HACKETT, D. P.: Rapid stimulation of RNA synthesis by a plant hormone. — Proc. nat. Acad. Sci. USA **50** : 243—250, 1963.

EDELMAN, J., HALL, M. A.: Enzyme formation in higher plant tissues. Development of invertase and ascorbate oxidase activities in mature storage tissue of *Helianthus tuberosus* L. — Biochem. J. **95** : 403—410, 1965.

GALSTON, A. W., DALBERG, L. Y.: The adaptive formation and physiological significance of IAA-oxidase. — Amer. J. Bot. **41** : 373—380, 1954.

GLASZIOU, K. T., WALDRON, J. C., BULL, T. A.: Control of invertase synthesis in sugar cane. Loci of auxin and glucose effects. — Plant Physiol. **41** : 282—288, 1966.

KEY, J. L., INGLE, J.: Requirement for the synthesis of DNA-like RNA for growth of excised tissue. — Proc. nat. Acad. Sci. USA **52** : 1382—1388, 1964.

KHAN, M. I.: Regeneration in relation to root size in *Taraxacum officinale*. — Pak. J. sci. industr. Res. **12** : 310—311, 1970.

KHAN, M. I.: Localization of indoleacetic acid and kinetin in *Taraxacum* roots. — Biochemica (Karachi) **7** : 25—29, 1972a.

KHAN, M. I.: Metabolic studies of the regeneration of the roots of *Taraxacum officinale*. — Pak. J. Biochem. **5** : 61—65, 1972b.

KHAN, M. I.: Effect of indoleacetic acid and kinetin on the *in vitro* enzyme activity of *Taraxacum* roots. — J. Sci. Univ. Karachi **1** : 85—88, 1972c.

KHAN, M. I.: Anatomy of regenerating root segments of *Taraxacum officinale* WEB. — Pak. J. Bot. **5** : 71—78, 1973.

LATIES, G. G.: Survey of Biological Programmes **3** : 215—299, 1957.

LEAVER, C. J.: The Correlation between Nucleic Acid Synthesis and Induced Enzyme Activity in Plant Tissue Slices. — Ph. D. Thesis Univ. London 1966.

LEAVER, C. J., EDELMAN, J.: Antibiotics as a means of control of bacterial contamination of storage tissue disks. — Nature **207** : 1000—1001, 1965.

MASUDA, Y.: Auxin-induced growth of tissue of Jerusalem artichoke. III. Effect of actinomycin-D on auxin-induced expansion growth and RNA synthesis. — Plant Cell Physiol. **7** : 573—581, 1966.

NOODEN, L. D., THIMANN, K. V.: Evidence for a requirement for protein synthesis for auxin-induced cell enlargement. — Proc. nat. Acad. Sci. USA **50** : 194—200, 1963.

NOODEN, L. D., THIMANN, K. V.: Inhibition of protein synthesis and of auxin-induced growth by chloramphenicol. — Plant Physiol. **40** : 193—201, 1965.

PALMER, J. M.: The effect of some plant growth substances on the induction of enzymatic activities in thin slices of plant tubers. — In: WIGHTMAN, F., SETTERFIELD, G. (ed.): Biochemistry and Physiology of Plant Growth Substances. Pp. 401—417. Runge Press, Ottawa 1968.