J. Orchid Soc. India, 4(1,2): 37-44, 1990

ACTIVITIES OF SOME OXIDATIVE ENZYMES IN AXENIC CULTURES OF PROTOCORMS OF *CYMBIDIUM GIGANTEUM* WALL. AS INFLUENCED BY DIFFERENT GROWTH REGULATORS

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Abstract

The effects of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) ∝ -naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 6-furfurylaminopurine (KN) and 6-benzylaminopurine (BAP) were studied on the activities of some oxidative enzymes viz., peroxidase, polyphenoloxidase, IAA oxidase, and the tissue concentration of soluble protein in protocorms of *Cymbidium giganteum*, 30 days after the treatments. Compared to the untreated controls, the specific activity of both peroxidase and polyphenoloxidase in the auxin and cytokinin treated protocorms was slightly suppressed. However, the treatments had no significant effect on the activity of IAA oxidase in the protocorm tissues.

Introduction

In nature, the orchid seeds show a very low percentage of germination because of the specific nutrient requirements of their embryos. However, *in vitro* seed germination can be manipulated by the addition of various growth factors in the nutrient medium. Incorporation of IAA, IBA, and NAA in the medium enhances germination and seedling growth in many orchids (Arditti and Ernst, 1984). However, the overall inhibitory effects of IAA (above the concentration of 0.1 mgl^{-1}) and 2,4-D have been reported in *Coleogyne punctulata* (Sharma and Tandon, 1986). KN in medium has been reported to stimulate both germination and seedling growth in *Cypripedium reginae* (Harvais, 1982) and *Galeola septentrionalis* (Nakamura, 1982). Van Waes and Debergh (1986) showed that the effects of BAP and benzyladenine depended upon the species and the concentration tested. However, the data on the physiological basis of the diverse requirements of orchid seeds during their germination and the effects of various growth factors on the metabolic pathways underlying the process of seed germination and seedling growth are scanty.

The present investigation was undertaken to study the effects of some growth regulators on growth and activities of some oxidative enzymes in the germinating protocorms of *Cymbidium giganteum*.

Materials and Methods

Four-month old capsules of *Cymbidium giganteum* Wall. (collected from the forests of Meghalaya and grown in the University Botanical Garden) were harvested, sterilized with sodium hypochlorite solution (1.25%

available chlorine) for 15-20 minutes and rinsed with double-distilled sterile water. The seeds were exposed aseptically by cutting open the capsules and inoculated on Vacin and Went's (1949) medium, followed by incubation in the dark for three weeks at 25± 2°C. Subsequent incubation was carried out under 8h L and 16h D photoperiod at 3000 lux at 25± 2°C. Two-month old developing green protocorms were transferred to fresh media supplemented with IAA, NAA, IBA, 2, 4-D, KN, BAP incorporated separately at concentrations ranging from 0.1-1.0 mgl^{-1}. On the 30th day of the treatments, the protocorms were harvested, washed thoroughly with distilled water to remove adhering agar. A suitable weight of the tissue was homogenised in a pre-chilled pestle and mortar with 0.1M phosphate buffer (pH 6.0). The homogenate was filtered through muslin and centrifuged at 2000 x g for 15 minutes at -5°C. The supernatant was collected and used for analytical studies. Soluble protein was estimated by the method of Lowry *et al.* (1951). The activities of peroxidase, polyphenol oxidase and IAA oxidase were assayed following the methods of Mahadevan (1974), Ponting and Joslyn (1948) and Tandon and Arya (1982), respectively. The extract was also subjected to PAGE according to Ornstein (1964) and Davis (1964) and the gels stained for soluble proteins using 0.1% amido black in 7% acetic acid and peroxidase isoenzymes using 1.5% benzidine in 25% glacial acetic acid and 1% H_2O_2. A suitable fresh weight of the harvested tissue was dried in an oven over P_2O_5 for 48 h at 70°C for determining the dry weight. Each treatment and analysis was carried out in triplicate.

Results and Discussion

Irrespective of the treatments given, the amount of dry matter accumulation in the protocorms at harvest showed no significant difference. However, with increase in the concentration of IAA and IBA in the nutrient medium, a progressive decrease in the amount of dry weight at harvest could be observed (Fig. 1). When compared with

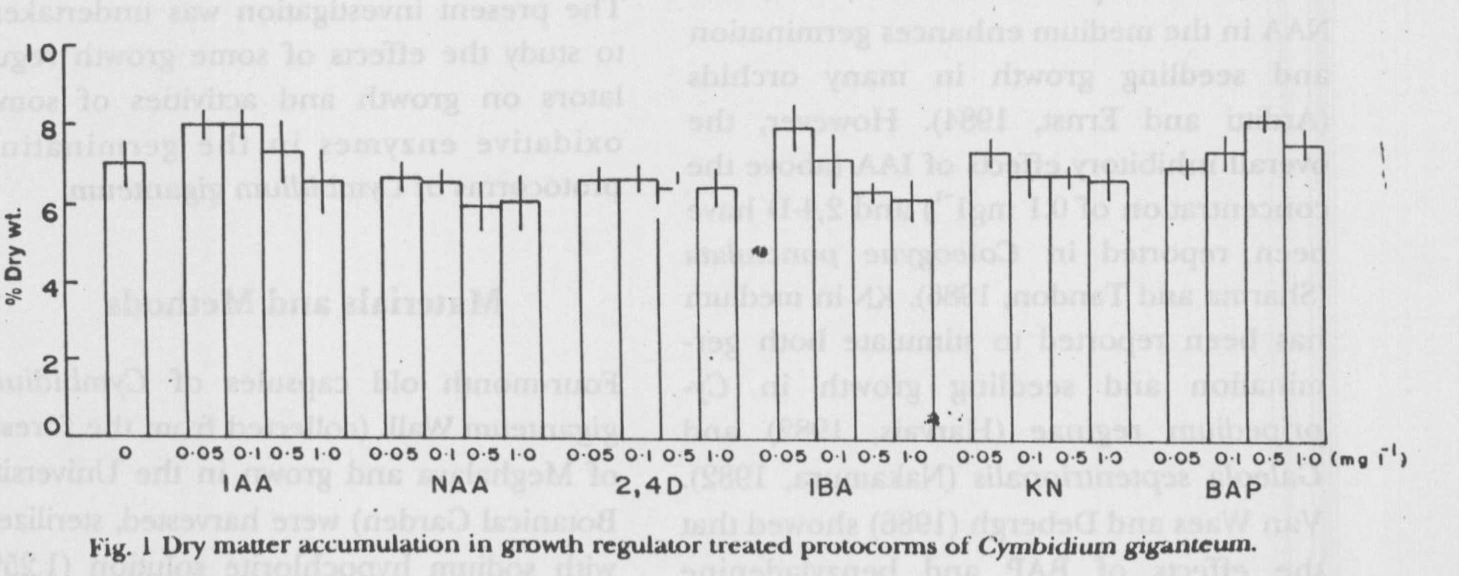

Fig. 1 Dry matter accumulation in growth regulator treated protocorms of *Cymbidium giganteum*.

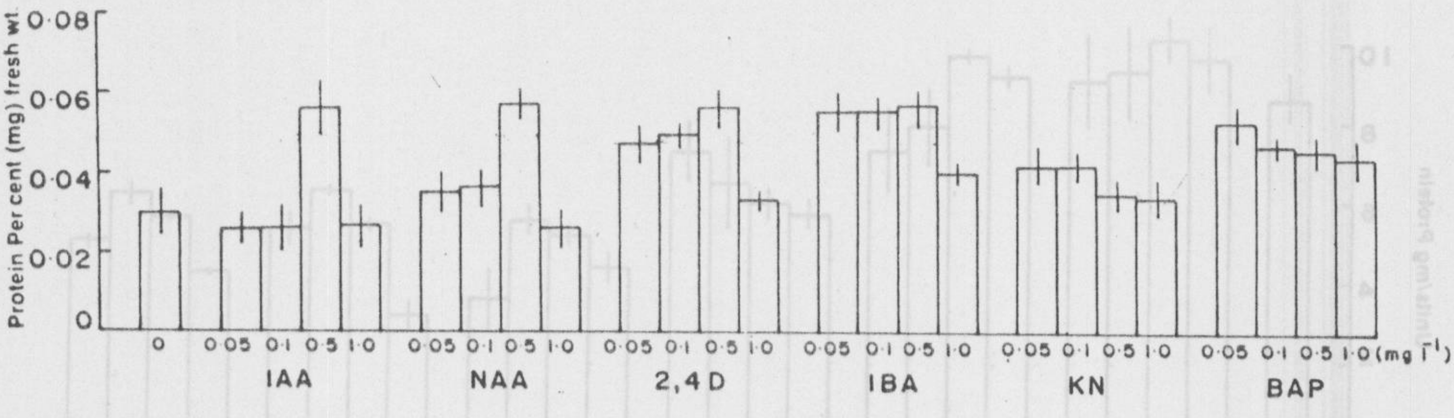

Fig. 2. Effect of growth regulators on the synthesis of soluble proteins in *Cymbidium giganteum* protocorms.

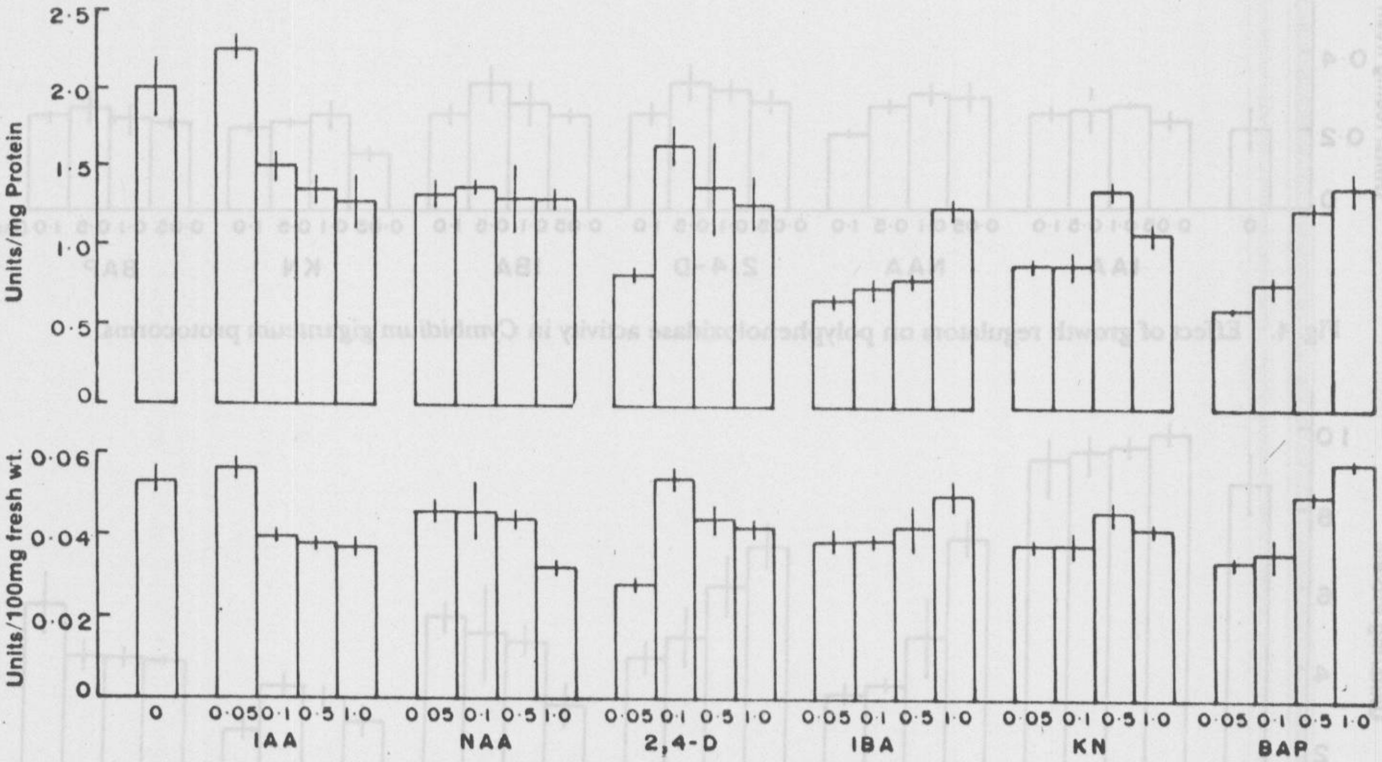

Fig. 3. Effect of growth regulators on peroxidase activity in *Cymbidium giganteum* protocorms.

the untreated control, the tissues of the treated protocorms showed a relatively higher level of soluble protein. The highest soluble protein content was recorded at 0.5 mg l^{-1} in most of the cases (Fig. 2).

By and large, the activities of peroxidase and polyphenol oxidase when expressed on fresh weight basis in the tissues of untreated protocorm was not much different from those supplied with either the auxins or the cytokinins. However, on a unit protein basis, the treated protocorms showed a relatively lower level of peroxidase and polyphenol oxidase activities than the untreated control (Figs. 3, 4). On fresh

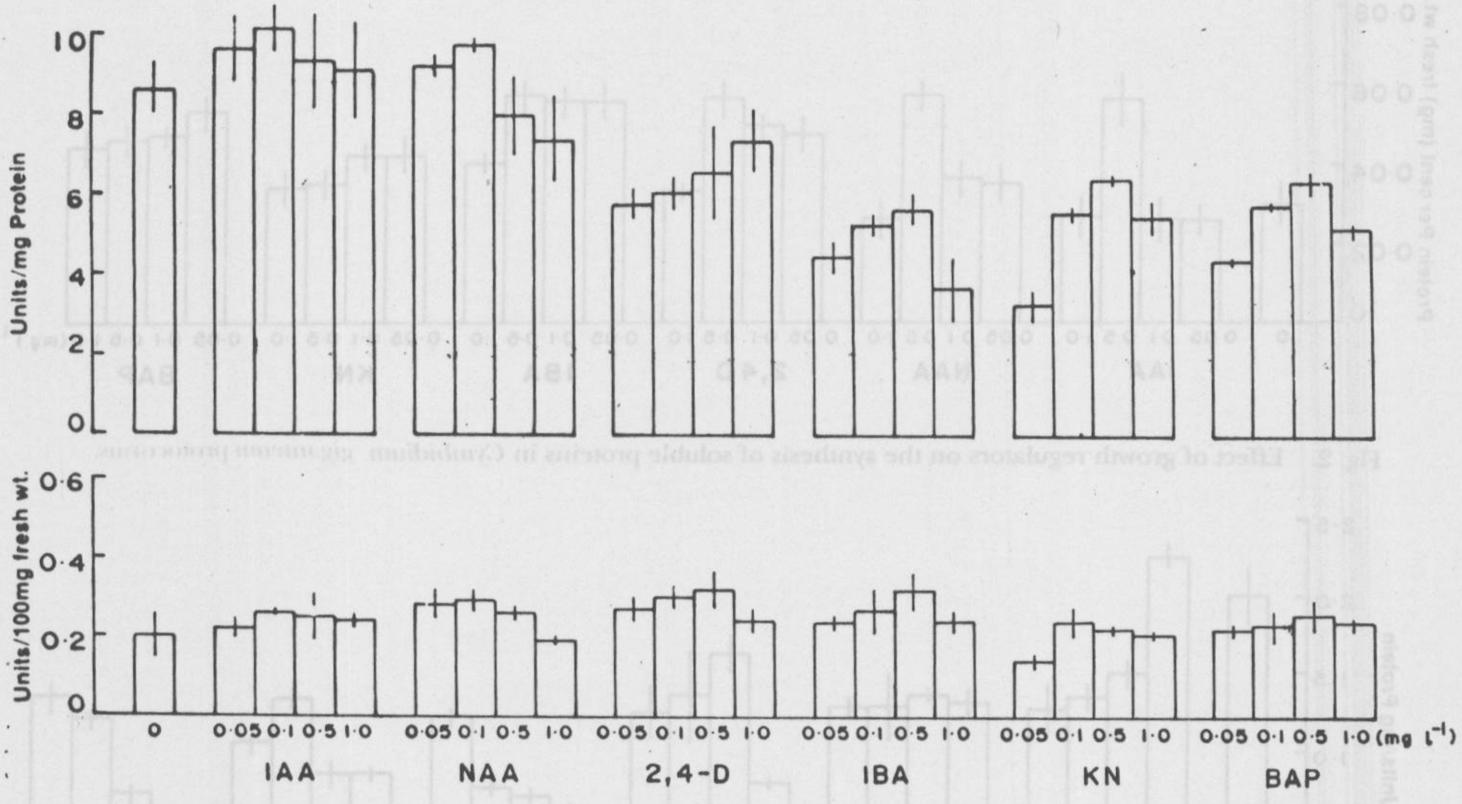

Fig. 4. Effect of growth regulators on polyphenoloxidase activity in *Cymbidium giganteum* protocorms.

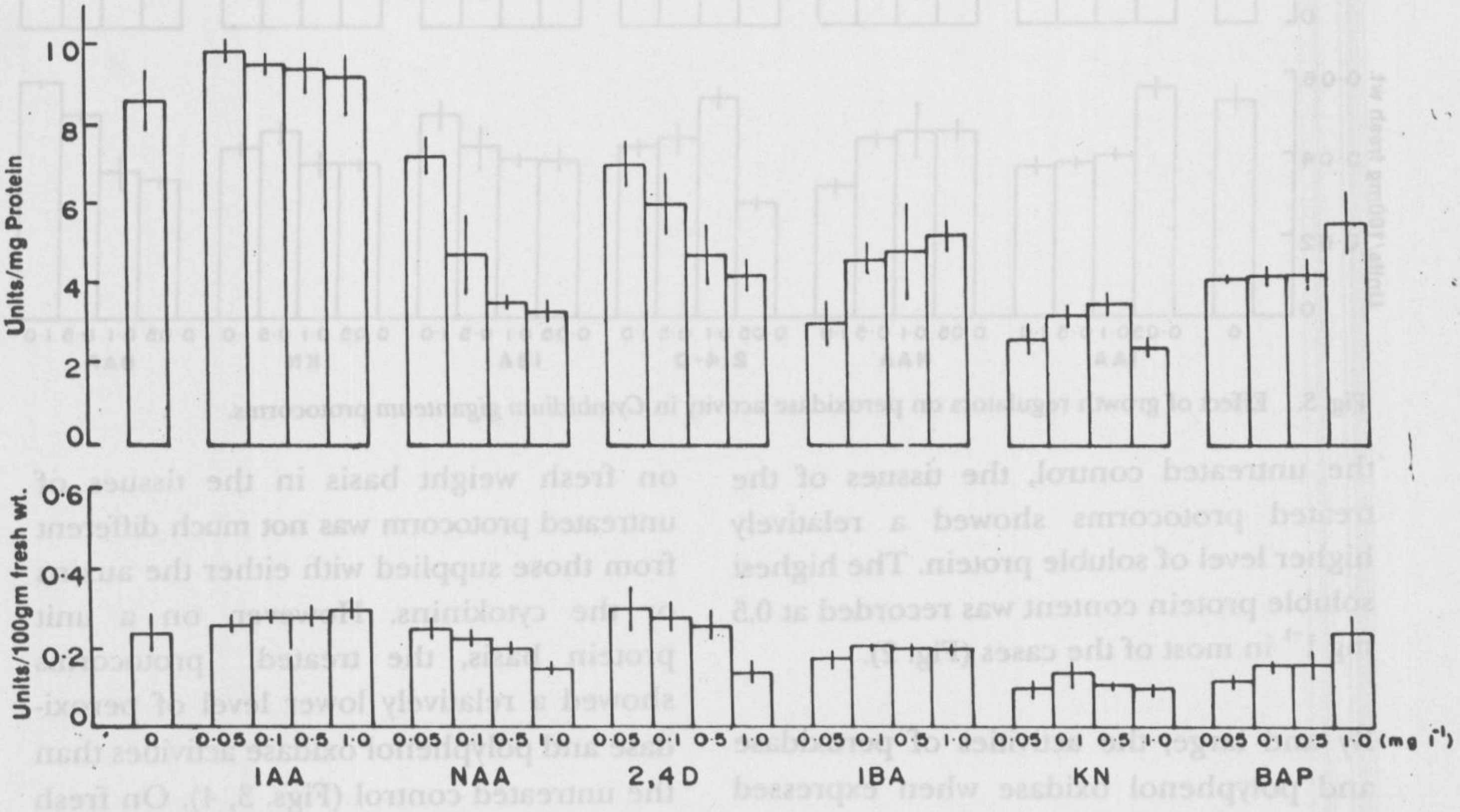

Fig. 5. Effect of growth regulators on IAA oxidase activity in *Cymbidium giganteum* protocorms.

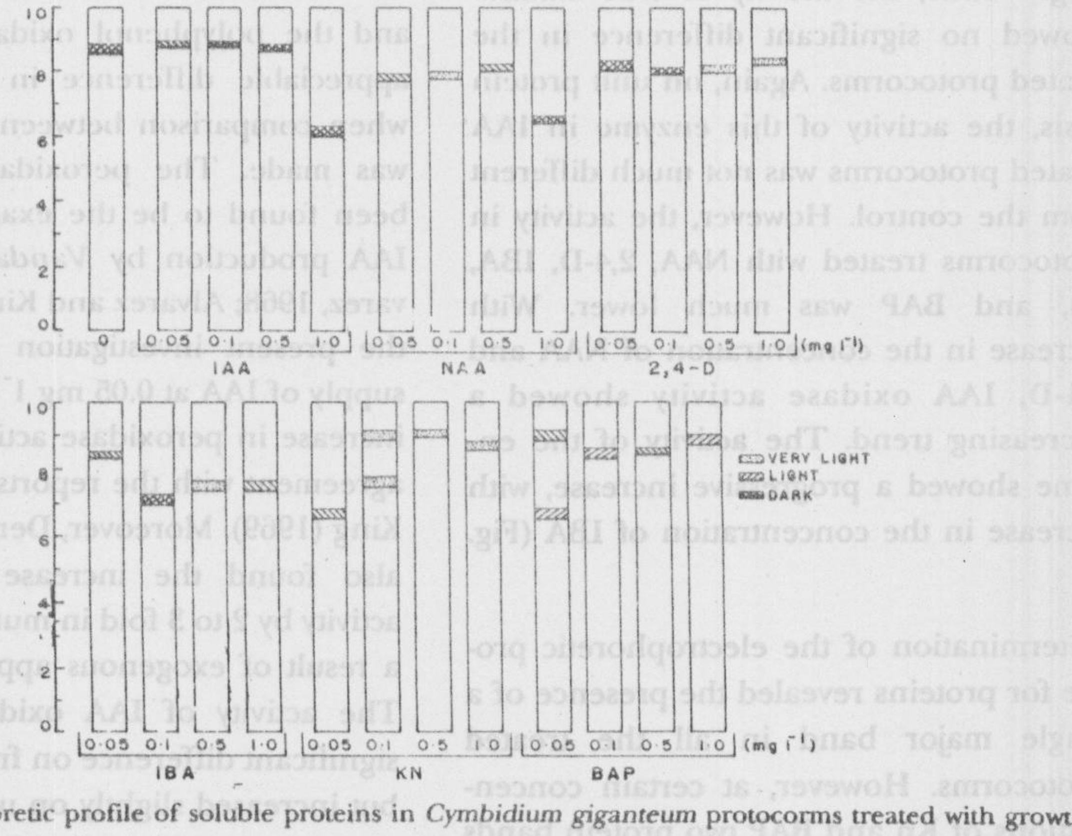

Fig. 6. **Electrophoretic profile of soluble proteins in *Cymbidium giganteum* protocorms treated with growth regulators.**

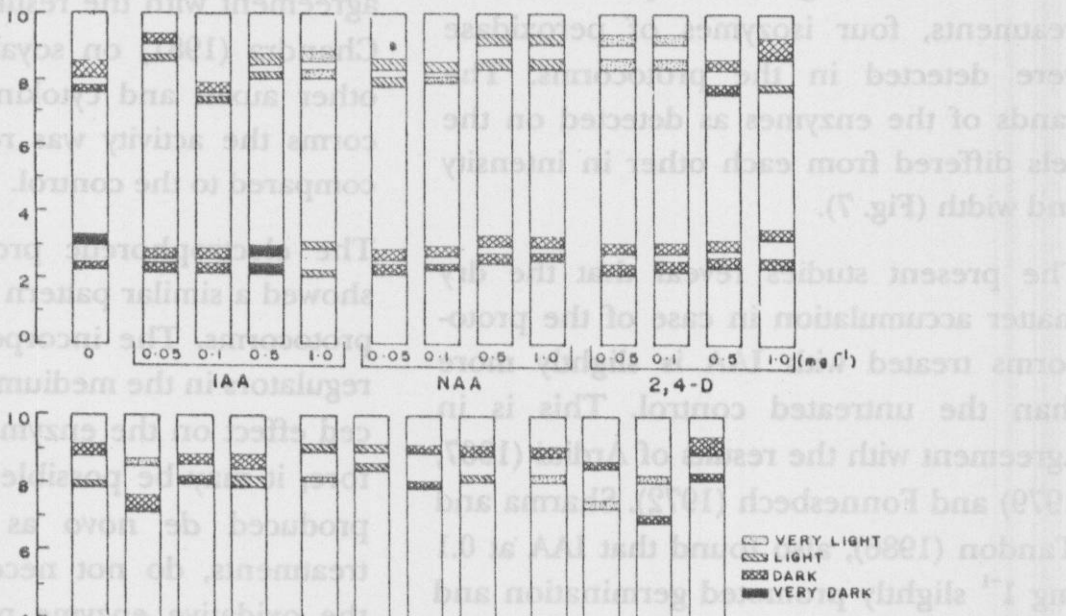

Fig. 7. Isozyme pattern of peroxidase in *Cymbidium giganteum* protocorms treated with growth regulators.

weight basis, the activity of IAA oxidase showed no significant difference in the treated protocorms. Again, on unit protein basis, the activity of this enzyme in IAA treated protocorms was not much different from the control. However, the activity in protocorms treated with NAA, 2,4-D, IBA, Kn, and BAP was much lower. With increase in the concentration of NAA and 2,4-D, IAA oxidase activity showed a decreasing trend. The activity of the enzyme showed a progressive increase, with increase in the concentration of IBA (Fig. 5).

Determination of the electrophoretic profile for proteins revealed the presence of a single major band in all the treated protocorms. However, at certain concentrations of Kn and BAP two protein bands were observed (Fig. 6). Irrespective of the treatments, four isozymes of peroxidase were detected in the protocorms. The bands of the enzymes as detected on the gels differed from each other in intensity and width (Fig. 7).

The present studies reveal that the dry matter accumulation in case of the protocorms treated with IAA is slightly more than the untreated control. This is in agreement with the results of Arditti (1967, 1979) and Fonnesbech (1972). Sharma and Tandon (1986), also found that IAA at 0.1 mg l^{-1} slightly promoted germination and seedling development in *Coelogyne punctulata.* The growth factors had a stimulatory effect on the soluble protein content which was seen to be relatively higher in all the treated protocorms as compared to the untreated ones. Both the peroxidase and the polyphenol oxidase showed no appreciable difference in their activities when comparison between the treatments was made. The peroxidase activity has been found to be the exact reciprocal of IAA production by *Vanda* seedlings (Alvarez, 1968; Alvarez and King, 1969). But in the present investigation the exogenous supply of IAA at 0.05 mg l^{-1} caused a slight increase in peroxidase activity which is in agreement with the reports of Alvarez and King (1969). Moreover, Dendsay (1989) has also found the increase in peroxidase activity by 2 to 3 fold in mung cotyledons as a result of exogenous application of IAA. The activity of IAA oxidase showed no significant difference on fresh weight basis but increased slightly on unit protein basis in protocorms treated with IAA, which is in agreement with the results of Purohit and Chandra (1981) on soyabean root. In all other auxin and cytokinin treated protocorms the activity was relatively lower as compared to the control.

The electrophoretic profile for proteins showed a similar pattern for all the treated protocorms. The incorporation of growth regulators in the medium had no pronounced effect on the enzymes studied. Therefore, it may be possible that the proteins produced *de novo* as a result of the treatments, do not necessarily belong to the oxidative enzyme pathway only. The difference in the intensity and width of the isoenzyme bands of peroxidase accounts for the differential effect of the growth regulators on the isozyme patterns.

From the present study it is obvious that the growth regulators have a pronounced effect on the growth and physiology of development of the protocorms of *Cymbidium giganteum*. It appears that besides their effect on the activities of peroxidase, polyphenol oxidase and IAA oxidase, the treatments influence some other enzymes which are involved in some other metabolic pathways.

Acknowledgement

This study was supported by a research grant to PT from North-Eastern Council, Ministry of Home Affairs, Government of India.

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