hemolysate was determined by atomic absorption spectrophotometry according to Ludmilla[6]. The data so obtained were statistically analysed according to Steel and Torrie[7].

The specific activity of the metalloenzyme carbonic anhydrase during different weeks of growth is shown in table 1. No significant differences were observed in the specific activity of the enzyme in relation to the age of the animal indicating that the quantity of the enzyme remains constant from birth onwards. No significant differences were observed in the zinc content of the hemolysate (table 1) which further substantiates our findings that the quantity of the enzyme carbonic anhydrase remained constant from birth onwards.

The PCV (packed ceil volume) levels, however, showed significant ($P<0.01$) differences between different weeks of growth (table 1). The levels showed a decline till 11 weeks of age. The highest values were found on the day of birth. There was a decline of 18.72% by the 5th week. Thereafter the decline was less steep (13.85%) till the 11th week. When the ratio of the enzyme levels to PCV was calculated it was seen that the ratio increased to ten-folds from birth till 15th weeks of age. The increase in the enzyme content per unit PCV was erratic from the day of birth till 10 weeks of age but from the 11th week onwards there was a uniform elevation by 10 folds as compared to the previous 10 weeks. PCV is directly related to erythrocyte count and haemoglobin content[8]. Hence from our data it is evident that the amount of carbonic anhydrase in the red blood corpuscles (RBC) increases as the animal matures.

These results clearly indicate that the level of the enzyme per RBC increases from birth onwards and to lower the pCO_2 during the early neonatal period[3], the body compensates by having higher blood volume[9] and PCV thereby increasing the total amount of the enzyme available for catalysing the transport of carbon dioxide and maintaining proper H^+ concentration. During early neonatal period there is increased thyroidal activity[10] and glucose utilization[11] which is necessary for functional and metabolic adaptation of the new born. This leads to excessive production of carbon dioxide. To maintain the body homeostasis the animal responds by increasing the availability of carbonic anhydrase for elimination of CO_2.

24 November 1986; Revised 21 February 1987

1. Lampe, L., *Intenziv Szuloszoba*, Medicina Budapest.
2. Walser, K. and Maurer-Schweizer H., *Tierarztl Praxis*, 1978, **6**, 451.
3. Maurer-Schweizer H. Wilhelmn, U. and Walser, K., *Tierarztl Wschr*, 1977, **90**, 192.
4. Roughton F. J. W. and Booth V. H., *Biochem. J.*, 1946, **40**, 309.
5. Meulemans, O., *Clin. Chem. Acta.* 1960, **5**, 757.
6. Ludmilla, D., *Clinical analysis by atomic absorption spectroscopy*, Varian Techtron Pvt. Ltd., Australia, 1976, p. 37.
7. Steel, R. G. D. and Torrie J. H., *Principles and procedures of statistical analysis a biometrical approach, 2nd edn.* McGraw Hill and Kogakusha, Tokyo, 1980, p. 137.
8. Swenson M. J., *Duke's physiology of domestic animals* , Comstock Publishing Associates, Cornell University Press, Ithaca, London 1970, p. 39, 8th edn.
9. Sodhi, S. P. S., Ph.D. thesis, Punjab Agricultural University, Ludhiana, 1979, p. 169.
10. Setia. M. S. and Verman P. N., *Indian J. Anim. Res.*, 1983, **17**, 17.
11. Setia, M. S. and Verman, P. N., *Z. Tierphysiol. Tierernachr. Futtermittelkd.*, 1980, **44**, 93.

RESPONSE OF PEROXIDASE ISOENZYMES TO CHEMICAL SEX MODIFICATION IN CUCUMBER (*CUCUMIS SATIVUS* L.)

E. A. EL-GHAMRINY, N. SINGH and V. K. VERMA
Division of Vegetable Crops, Indian Agricultural Research Institute, New Delhi 110 012, India.

It has been established that normal plant growth and development is governed by endogenous plant hormones. The exogenous applications of chemicals at 2-true leaf stage of the plants modifies the development and sex ratio in different genera of cucurbits.

The biochemical analyses were performed in monoecious cucumber var. 'Khira Poona' to find out the changes in peroxidase isoenzymes patterns in tips, leaves and flower buds to chemical treatment and to understand, why 2-true leaf stage is so critical for effective sex modification and also why ethrel behaves in different manner when compared to that of silver nitrate. It was also worthwhile to study the

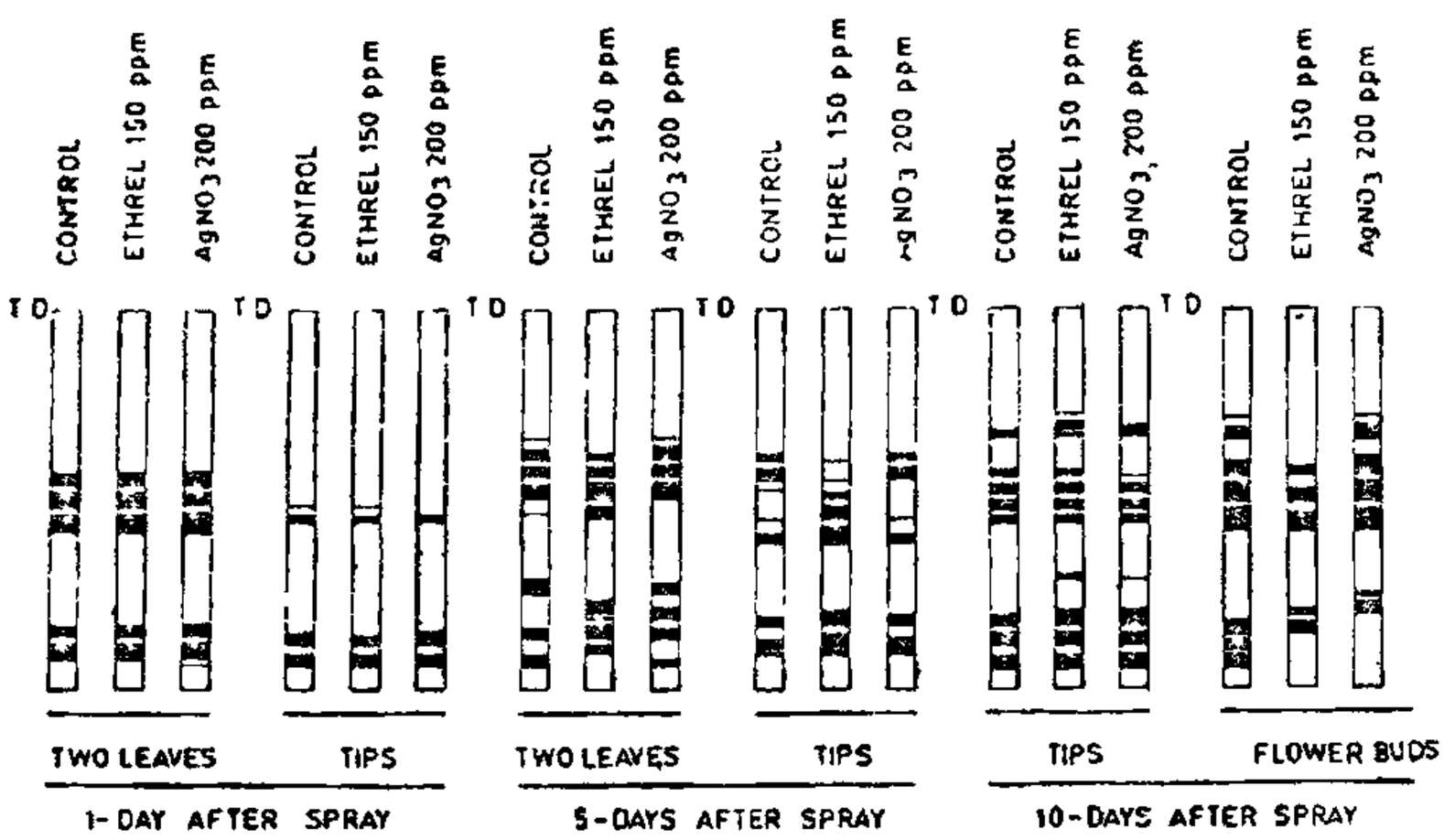


Figure 1. Peroxidase isoenzymes obtained on gel electrophoresis from tips, two leaves and flower buds of *Monoecious cucumber.*

changes in peroxidase isoenzymes patterns at intervals of different days after the application of chemicals which lead to sex modification. It will be helpful in providing an optimum duration of time taken for sex differentiation by chemical applications at 2-leaf stage.

The experiment was conducted during 1983. The chemicals, ethrel (150 ppm) silver nitrate (200 ppm) and control (distilled water) were applied at 2-true leaf stage on the plants grown in pots. The samples of tips, leaves and flower buds were analysed after 24 hr, 5 days and 10 days of spray treatment. Crude extract was prepared by extracting 1 g of the fresh material with 2 ml phosphate buffer (pH 7.4, 1:2 w/v) containing 5 mM B-marcaptoethenol by gel electrophoresis. Discontinuous PAGE was used to separate peroxidase isoenzymes. Anionic system of Davis[1] and Ornstein[2] was followed. Densitograph was obtained by scanning gels in JOYCELOEBLE Chromoscan.

The peroxidase isoenzymes with higher electrophoretic mobility were few with less intensity in tips as compared to that of 2-true leaves in both treated and control plants after 24 hr spray. The patterns of ethrel-treated plant tips were similar to $AgNo_3$ treated and control plant tips (figure 1). The band at R_m 0.49 present in ethrel-treated plant tips was absent in silver nitrate treatment. Perioxidase isoenzyme in 2-true leaves, 5 days after treatment, was similar to the pattern obtained earlier after treatment for one day. The patterns in the control and the treated plants in 2-true leaves were similar except that the relative intensity of the bands with higher electrophoretic mobility was greater in silver nitrate-treated plants than the ethrel-treated plant leaves after 5 days of spray. The band at R_m 0.11 present in ethrel-treated plants was absent in control or $AgNo_3$ treated plant leaves. Isoenzyme number in tips was much greater than at 24 hr after treatment. The isoenzyme at R_m 0.55 and 0.59 had much lower activity with ethrel treatment while at R_m 0.17, 0.11, 0.45 and 0.49 had much greater intensity than the other two treatments. Peroxidase isoenzyme pattern at 10 days after spray in tips was different as compared to the tips at earlier stages. The bands at R_m 0.67 had greater intensity in ethrel treatments.

The perioxidase isoenzyme pattern in flowerbuds of ethrel-treated plants differred considerably. The isoenzymes at R_m 0.63 and 0.67 present in control and silver nitrate-treated plant flowerbuds were absent in ethrel. This appreared to be characteristic of pistillate flowers. The absence of bands with higher electrophoretic mobility(R_m 0.65 and 0.67) in ethrel-treated plant flower buds indicates the importance in sex expression.

Retig and Rudich[4] have correlated isoenzyme activity with auxin level and the consequent effect on sex expression in cucumber; Siegel and Galston[5] have shown that pattern in peroxidase activity is associated with auxin level and tissue enlargements. McCune[3] reported that GA alters the level of

perioxidase isoenzymes. Endogenous hormone levels affect enzyme activity and thereby, may regulate sex expression but such an effect at enzyme level with exogenous application has not been investigated. The effect of $AgNO_3$ or ethrel becomes manifested in plant parts other than 2-true leaves. Even at 5 days after spray, the patterns in 2-true leaves were nearly similar in both treatments. It has been shown that peroxidase with high electrophoretic mobility have higher IAA oxidase activity. Therefore, a decrease in peroxidase activity will result in a decrease in IAA oxidase activity which would result in higher auxin levels in ethrel-treated plants as compared to silver nitrate. Thus, hormone balance in these two treatments would be different, which may result in differential sex expression. The modification of sex, therefore, in cucumber plants occurs due to an interplay in *in vivo* auxin levels as also of differential gene expression for peroxidases and modulation of their activity.

8 October 1986; Revised 2 March 1987

1. Davis, B. J. *Ann. NY. Acad. Sci.*, 1964, **121**, 404.
2. Ornstein, L., *Ann. NY. Acad. Sci.*, 1964, **121**, 321.
3. McCune., *Ann. NY. Acad. Sci.*, 1961, **94**, 723.
4. Retig, N. and Rudich, J., *Physiol. Plant.*, 1972, **27**, 156.
5. Siegel, B. Z. and Galston, A. W., *Plant physiol*, 1967, **42**, 221.

A SERIOUS ROOT DISEASE OF TOMATO CAUSED BY *PYTHIUM INFLATUM* MATTHEWS

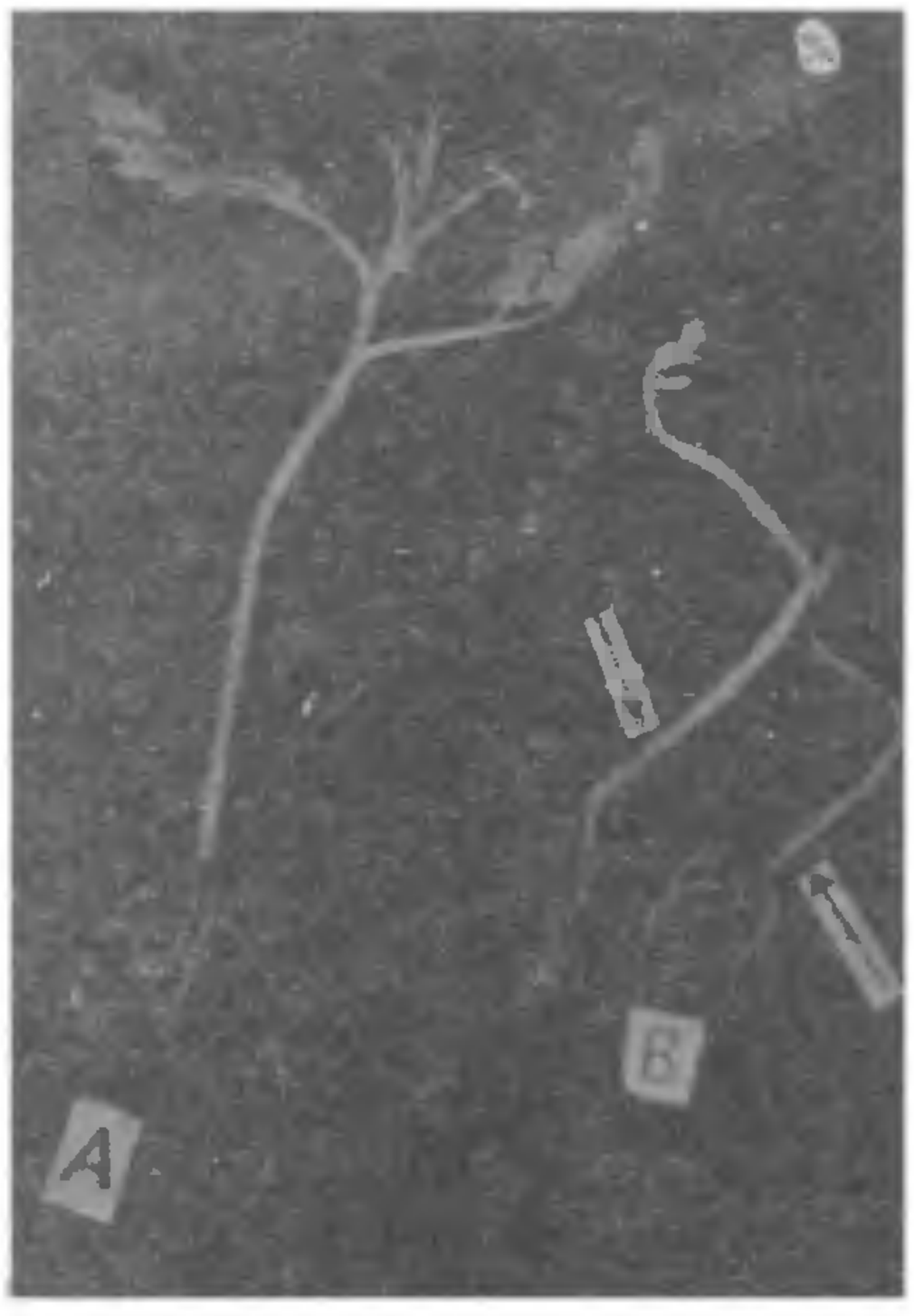
B. L. VERMA
Department of Botany, Kumaun University, Nainital 263 002, India.

PYTHIUM species are common in cultivated soils and generally associated with seedling diseases of various crops[1]. During the course of a study on *Pythium* species in vegetable growing fields of Tarai region of Nainital, a serious root rot of tomato (*Lycopersicon esculentum* Mill.) incited by *Pythium inflatum* Matthews was observed in the fields[2].

Infected roots of tomato collected from fields were washed in vigorously running tapwater for 20 min treated for 30 sec in 0.5% NaOCL solution, rinsed with sterlized water, cut into small pieces and plated out on agar media[3]. After 5 days at room temperature, *Pythium* colonies were transferred to sterilized water and cultured on boiled hempseed halves, identified with the help of monographs by Middleton[4] and Robertson[5].

Pathogenicity tests were carried out on glass house bench in sterilized pot soil. Four-day-old culture of the isolate grown on CMA was used as inoculum. Eight agar discs of 8 mm diameter obtained with the aid of sterile metal cork borer were mixed in the pot soils prepared for the pathogenicity tests. Seeds of tomato were planted in the pot soil after infestation. Ten seeds were sown in each pot (12·5 cm diam.). Five replicates were used to test the isolate. The soil was moistened regularly throughout the test period[6]. In control pot, seeds were sown in the soil without inoculum.

Rotting of seedlings occurred most commonly in pot soil under glasshouse experiments. Pre-emergence rotting of seeds was less common. However, some seeds failed to germinate while some young seedlings were killed at the soil surface after 2 or 3



Figures 1 A and B. A. Controlled seedling with well developed roots: **B.** Infected seedlings show poor development of roots; arrows indicate the water soaking lesions developed in the collar region of the seedlings after infection.