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TOXIC EFFECTS OF $BaCl_2$ ON GERMINATION, EARLY SEEDLING GROWTH, SOLUBLE PROTEINS AND ACID PHOSPHATASES IN *ZEA MAYS* L.

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Abstract

Maize seeds were grown in 0 to 100 mM concentrations of $BaCl_2$. A concentration of 0.1 mM was stimulatory for seed germination, shoot length and shoot-root dry weights. All other treatments (1-100 mM) were inhibitory for seedling growth, as reductions in % germination, root-shoot length and dry weights were observed. The decreases were proportional with the increasing concentrations of Ba^{++}.

The soluble protein content and acid phosphatases activity was estimated from the shoots of 1, 4 and 7 day old seedlings. The protein content of the shoots showed a decline on all days at all Ba^{++} treatments (except at 0.1 mM, where a significant increase in shoots of 1 day and insignificant in 4 day old seedlings was noted). The acid phosphatases activity increased with increasing $BaCl_2$ treatments in shoots of 1 and 4 day old seedlings, while in 7 day old seedlings the activity was inhibited at 20-100 mM. The possible morpho-physiological causes underlying the Ba^{++} mediated growth retardation are discussed.

Introduction

Barium toxicity has become important due to its increased presence in the environment, because of nuclear fall outs, gases emitted from gun discharges and diesal fuel. It is regarded as one of the natural air pollutant (Schroeder, 1970). The effects produced by Ba^{++} correspond to its dosage. Low concentrations in plant growth media partially replace the requirement for Ca^{++} (Wallace & Romney, 1971), while higher concentrations are extremely toxic (Robinson, *et al.*, 1938).

Of the toxic morpho-physiological effects, reduction in germination (Minton & Wilson, 1973; Debnath & Mukherji, 1982a), in shoot length and yield (Debnath & Mukherji, 1982a, b; Chaudhary & Wallace, 1977), decrease in respiration (Minton & Wilson, 1973; Debnath & Mukherji, 1982a), reduction in peroxidases, IAA oxidase, ascorbic acid oxidase (Debnath & Mukherji, 1982a), and in phosphatases are reported (Suzuki *et al.*, 1980).

The present paper describes the effect of different concentrations of $BaCl_2$ on germination, early seedling growth, soluble proteins and acid phosphatases of maize.

Material and Methods

Maize seeds (*Zea mays* L. cv. Akbar) were obtained from Punjab Seed Corporation, Lahore. Healthy seeds were surface sterilised with 1% Chloramine for 15 min, followed by a thorough washing in distilled water (Iqbal, *et al.*, 1974). Eight concentrations of $BaCl_2.2\ H_2O$ (E. Merck, Darmstadt, West Germany), i.e. 0.1, 1.0, 10, 20, 40, 60, 80 and 100 mM were prepared in distilled water. Control seeds were grown in distilled water.

Growth conditions: Washed Petri dishes (ϕ 12 cm) lined with a thin layer of absorbent cotton covered by a Whatman filter paper No. 1 were used for germination. Twenty-five ml of the respective $BaCl_2$ solution was added to each Petri dish containing 25 seeds. The Petri dishes were kept in darkness at 30 ± 2°C in a growth room for germination. Percent germination was scored on 4th day and was based on 200 seeds for control and every Ba-concentration, with three replications for every treatment. Only those seeds were taken as germinated where both the plumule and radicle had emerged.

Germinated seeds were transferred to plastic pots (12 x 10 x 10 cm) with a sieve cover containing 250 ml of a single salt ($BaCl_2$) test solution of different Ba^{++} concentrations outlined above. Five seedlings were grown per pot. The seedlings were grown in a growth room at 30 ± 2°C, light intensity 10 k-Lux, day length 16 h and relative humidity around 70%. The nutrient solutions were aerated, pots randomised and rearranged daily. For growth analysis 7 day old seedlings (10 days after sowing) were used, while estimation of soluble proteins and acid phosphatases were made from 1, 4 and 7 days old seedlings.

Morphological observations: Effects of Ba^{++} toxicity were studied on shoot and root length, and dry weights. Data on these parameters was collected as reported earlier (Iqbal, *et al.*, 1974).

Protein estimation: The extraction of soluble proteins and estimation was carried as reported by Iqbal & Schraudolf (1985).

Assay for acid phosphatases: For acid phosphatases, a weighed amount of fresh shoots were homogenised in a chilled mortar using ice cold Tris-HCl extraction buffer, pH 7.6; 5 mM mercaptoethanol (ratio of buffer to material 5:1). Following homogenisation the tissue fragments were removed from the sample by straining through 4 layers of cheese cloth. The sample was centrifuged at 14000 rpm for 20 min at 4°C in a refrigerated RC-5B Sorval centrifuge. The supernatant was used for the assay of the enzyme.

The standard assay used in all experiments involved the hydrolysis of p-nitrophenyl-phosphate (p-NPP). In a preliminary test, enzyme extract was assayed at pH 4.0, 4.5,

5.0, 5.5 and 6.0 for different time intervals (10-30 min) for optimal range finding. The highest activity was obtained at pH 5.0 for 15 min. at 30°C. In all subsequent estimations these optima were used. All assays were done in triplicate.

For assay 100 µl of enzyme solution was added to a 1 ml reaction mixture consisting of 2.5 mM p-NPP in 0.10 Na-acetate, pH 5.0, containing 1 mM EDTA. After incubation for 15 min at 30°C the reaction was stopped by the addition of 2 ml of 0.2 M NaOH, and the amount of p-nitrophenol released was determined by measuring the absorption of the solution at 405 nm. Blank was prepared in a similar manner, except that the enzyme solution was added after the NaOH. The molar extinction coefficient for p-nitrophenol in 0.02 M NaOH (E^{1M}_{1cm} = 18.8 x 10^3 at 405 nm) was used to calculate the µmoles of the product formed (Clark & Switzer, 1977).

Table 1. Effects of Barium chloride on germination and growth of *Zea mays* L. seedlings (7 days old)

Ba Conc. mM	Percent germination	Mean shoot length (cm)	Mean root length (cm)	Mean shoot dry wt mg/seedling	Mean root dry wt, mg/seedling
0.0	98.66 ± 1.09	10.76 ± 1.34	15.99 ± 1.02	50.92 ± 1.76	29.17 ± 0.82
0.1	100.00 ± 1.09	+ 14.41 ± 0.75	– 14.32 ± 1.31	+ 59.49 ± 2.03	+ 33.70 ± 1.07
1.0	94.66 ± 2.31	– 9.28 ± 0.77	– 7.02 ± 0.92	– 46.95 ± 1.58	– 27.42 ± 0.75
10	– 93.38 ± 2.09	– 5.29 ± 0.40	– 5.43 ± 0.21	– 25.24 ± 0.72	– 24.31 ± 0.69
20	– 89.33 ± 2.56	– 2.34 ± 0.22	– 4.27 ± 0.25	– 14.55 ± 0.41	– 16.63 ± 0.31
40	– 72.00 ± 1.90	– 2.12 ± 0.20	– 2.85 ± 0.18	– 10.48 ± 0.37	– 11.35 ± 0.19
60	– 69.33 ± 1.87	– 1.63 ± 0.13	– 1.89 ± 0.11	– 8.49 ± 0.30	– 6.67 ± 0.25
80	– 60.00 ± 1.79	– 1.15 ± 0.12	– 1.49 ± 0.17	– 6.29 ± 0.19	– 5.15 ± 0.14
100	– 52.00 ± 1.64	– 1.11 ± 0.08	– 1.05 ± 0.06	– 4.67 ± 0.64	– 3.53 ± 0.47

± = Standard Error of the mean.

+/– = Increases/decreases significantly different from control (P = 0.05). Duncan's Multiple range test of composite means.

In all three independent experiments were carried out, with three replications in each experiment. The data presented is a mean of the three experiments.

Results

(a) *Effects on early seedling growth:* The % germination and growth of seedlings grown in Ba^{++} is presented in Table 1. In germination a small stimulation (1.34%) was observed only at 0.1 mM. At all other treatments ranging from 1-100 mM a decrease was, however, observed. Growth of shoot and root, in general was also retarded. In conformity with the data on germination, a significant (P = 0.05) increase in shoot length was observed at 0.1 mM, while significant (P = 0.05) inhibition was observed at 1-100 mM. Decline was sharp from 1-20 mM (78.25% decrease at 20 mM). The root length declined significantly at all treatments, even at 0.1 mM, where a rise in % germination and shoot length was observed. The inhibition pattern of root length was similar to shoots. The root and shoot dry weight also decreased at 1-100 mM. However, at 0.1 mM a significant increase in dry weight of roots and shoots was observed (Table 1).

(b) *Effects on soluble proteins:* The data on soluble protein content of the shoots is shown in Fig. 1. The Ba^{++} treatment was inhibitory for the soluble proteins, except for

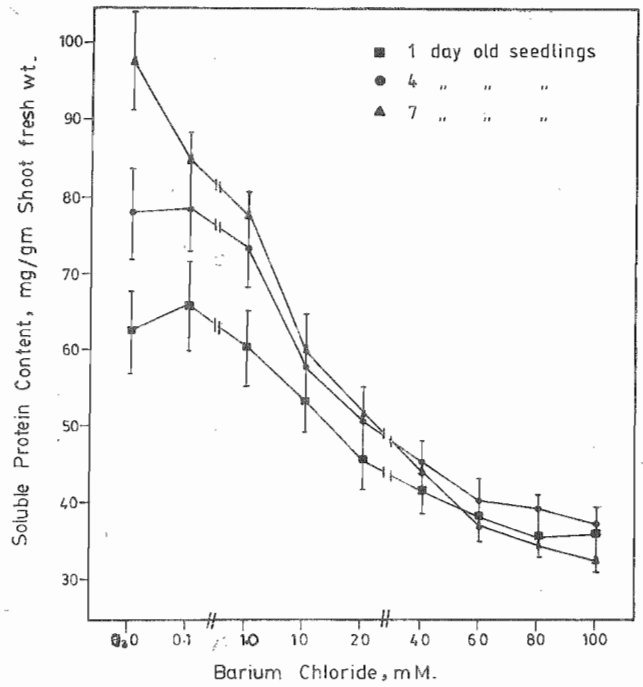

Fig. 1. Effect of Barium Chloride on soluble protein content in shoots of *Zea mays* L. (Standard errors are given as Vertical bars).

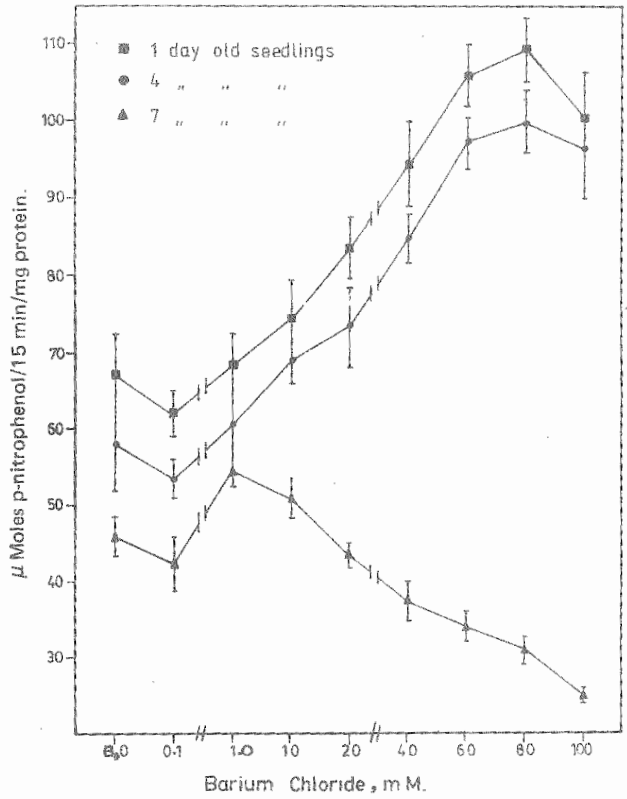

Fig. 2a. Effect of Barium Chloride on total activity of Acid Phosphatases in shoots of *Zea mays* L. (S.E. are given as vertical bars).

0.1 mM, where a small stimulation was observed in 1 and 4 day old seedlings. At all other treatments (1-100 mM) protein content gradually decreased from the shoots of 1 day old seedlings to 7 day old seedlings. Maximum decrease at all concentrations was observed in 7 day old seedlings.

(c) *Effects on acid phosphatases:* The acid phosphatases showed a general trend of stimulation in the total activity with Ba-treatment. The increase in activity was observed at all Ba-treatments except at 0.1 mM, where a decline in activity, 1.37% and 8.03% was found in shoots of 1 and 4 day old seedlings, respectively. The maximum increase in activity was observed at 80 mM, in shoots of both 1 and 4 day old seedlings. The increase was 63.85% and 64.05%, respectively. In shoots of 7 day old seedlings, however, increase was observed only at 1 and 10 mM, whereas at all other concentrations (0.1, 20-100 mM) a fall in activity was observed (Fig. 2a).

Acid phosphatases activity declined with increasing age. Maximum activity in control seedlings was observed in 1 day old seedlings (66.85 μmoles/15 min/mg). The activity was

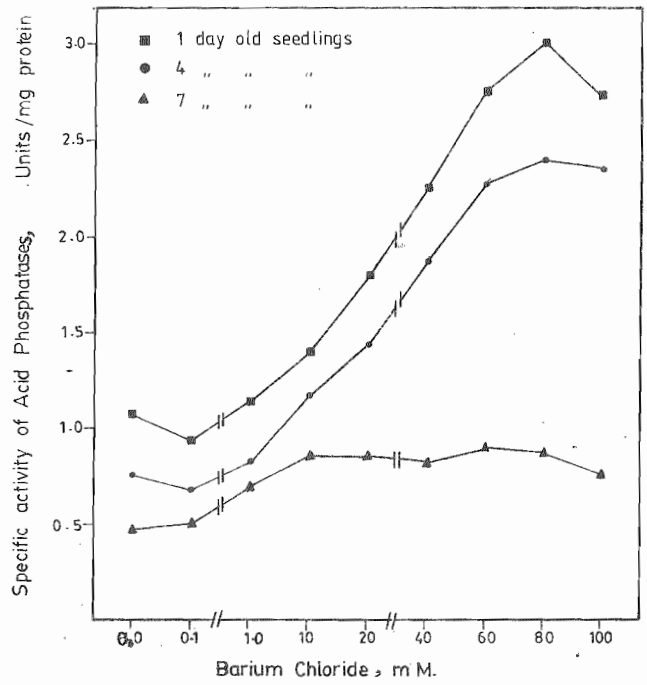

Fig. 2b. Effect of Barium Chloride on specific activity of Acid Phosphatases in shoots of *Zea mays* L.

reduced in 4 day old seedlings and was minimum in shoots of 7 day old seedlings (45.88 μmoles/15 min/mg). The specific activities of the acid phosphatases at different Ba-treatments showed the same general pattern of stimulation and inhibition as the total activities (Fig. 2b).

Discussion

Barium generally produces detrimental effects on growth and metabolism of plants (Minton & Wilson, 1973; Debnath & Mukherji, 1982a, b; Miller *et al.*, 1970). The present study agrees with the earlier reports as a reduction in % germination, root and shoot growth was observed (except at 0.1 mM where a slight stimulatory effect was observed). As reported the low concentrations of Ba^{++} in plant growth media are thought to partially replace the requirement of Ca^{++} in plants (Minton & Wilson, 1973). This may be a factor in stimulation on *a priori* basis.

The causes for stunted growth could be differen[t] [p]hological basis growth can be resolved into two basic components, i.e., increa[se] [n]mber and cell enlargement. An inhibition in any one of these factors coul[d] [g]rowth retardation. The in-

hibition of cell enlargement due to Ba^{++} toxicity has already been reported in *Phaseolus, Cephalandra, Beta, Triticum* and *Lactuca* (Debnath & Mukherji, 1982b). However, the role of proteins and ATP levels are important contributory factors in this process. Both ATP and protein levels have been reported to fall due to Ba^{++} toxicity in so far investigated plants (Minton & Wilson, 1973; Debnath & Mukherji, 1982a). In mung bean it was shown that excessive Ba^{++} level might lead to an imbalance in oxidation energy and the phosphate potential within the whole plant cells, significantly altering the ATP levels in cells (Minton & Wilson, 1973). In rice a decrease in protein content was attributed to slow conversion of total nitrogen into proteins (Debnath & Mukherji, 1982a). A Ba^{++} mediated reduction in soluble proteins is also reported in the present investigation. Thus decreased protein synthesis and fall in ATP can be significant contributory factors affecting cell division. Hence retardation in both cell expansion and division results in stunting and retarding growth.

Acid phosphatases content in shoots of control seedlings registered a continuous decrease from 1 day to 7 day old seedlings. Germinating seeds are rich in acid phosphatases and play a role in early differentiation and maturation (Clark & Switzer, 1977). The greater activity of acid phosphatases in 1 day old seedlings and a gradual decline in 4 and 7 days old seedlings is in conformity with the postulated role of acid phosphatases in those physiological processes that are involved in differentiation and maturation, particularly in early growth and development of the plant (Onofeghara & Koroma, 1974; Van Fleet, 1952).

The acid phosphatase activity showed a stimulatory effect in response to Ba^{++}. The activity, in general, increased with the increasing concentration of the metal ion. The present observation agrees with earlier reports where the enzyme activities of peroxidase, catalase, IAA oxidase and ascorbic acid oxidase are regarded as general biochemical symptoms of metal toxicity (Debnath & Mukherji, 1982a). Hence an increase in the activity of acid phosphatases may also be regarded symptomatic of the metal toxicity in young seedlings of *Zea mays.*

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