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EFFECTS OF BARIUM ON GERMINATION, SEEDLING GROWTH, SOLUBLE PROTEIN AND ISOZYMIC FORMS OF PEROXIDASE IN WHEAT

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ABSTRACT: Wheat seeds were grown in 0.0-100 mM $BaCl_2$. The treatments of 60-100 mM Ba^{++} were lethal for germination and subsequent seedling growth. At 5-40 mM, a reduction in percent germination was observed. Inhibitory effect of Ba^{++} was also noticed on seedling growth. There was a gradual decrease in root-shoot length and dry weight upto 40 mM except at 0.1 mM, where a non-significant increase in shoot length and its dry weight was observed. The soluble protein content of shoots of control seedlings showed a gradual increase from three to seven days, while an inhibition was observed at all treatments of Ba^{++} except at 0.1 mM. During this period peroxidase activity in control shoots decreased with the age of seedling, while under the Ba^{++} stress it increased in shoots of three to seven days' old seedlings. The number of isozymic forms of peroxidase in control shoots of three days' old seedlings was 3, which decreased to 2 forms on 5th and 7th days. The Ba^{++} treatments did not induce any qualitative change in the isozymic forms though distinct quantitative differences were observed in control and Ba^{++} grown seedlings.

Key Words: Triticum aestivum; Barium; Protein Content; Peroxidases; Root; Shoot; Length; Pakistan.

INTRODUCTION

Barium is one of the natural air pollutant because of its increased presence in the environment due to nuclear fall out, from gases emitted from gun discharges and diesel fuel (Schroeder, 1970). The effects of Ba^{++} on plant growth are in general extremely toxic, as small concentrations can retard growth (Robinson et al., 1938).

Of the detrimental effects, reduction in germination (Minton and Wilson, 1973; Gopala, 1980; Debnath and Mukerji, 1982a; Iqbal and Rafique, 1987) root-shoot length, grain yield (Boltalico and Antonio, 1973; Chaudhary and Wallace, 1977; Davis and Becklet, 1978; Debnath and Mukerji, 1982a) and changes in the activity of various enzymes are reported (Hock, 1939; Mukerji and Miatra, 1977; Takhashita and Shinosu, 1977; Suzuki et al., 1980; Leblova and Syla, 1981; Debnath and Mukerji, 1982b; Jones and Russel, 1983; Iqbal and Rafique, 1987; Iqbal and Mushtaq, 1987). The present study deals with the effects of Ba^{++} on germination, early growth, soluble protein content, peroxidase activity and its isozymic forms in wheat.

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MATERIALS AND METHODS

Certified seed of wheat (water content: 12.0 $\pm$ 0.3%) was obtained from Punjab Seed Corporation, Lahore. Seed was surface sterilised and sown initially in petridishes (till germination) and then in plastic pots (15cm x 15cm x 10cm) upto seven days.

Ten concentrations of $BaCl_2$, i.e., 0.0, 0.1, 1.0, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, 100.0 mM were used. Seed was grown in petridishes which were kept in dark for 72h at 23 $\pm$ 2°C in the climatic room. After germination, the seedlings were

shifted to plastic pots. Each pot was filled with 200 ml of 3/4 concentration of Hoagland nutrient solution alongwith the respective Ba^{++} concentration. Five seedlings were grown per pot. The seedlings were also grown in the climatic room at 23 ± 2 °C; light intensity, 10 K-lux; day length, 12h. The nutrient solutions were aerated, pots randomised and rearranged daily.

The data on percent germination, root and shoot length (cm) and dry weight (mg/seedling) were recorded on seven days' old seedlings. Soluble protein content of shoots were estimated from three, five and seven days' old seedlings. Procedural details for these parameters are reported by Iqbal and Rafique (1987).

The peroxidase activity of shoots of three, five and seven days' old seedlings was also estimated. The excised shoots were taken from each $BaCl_2$ treatment and weighed. The crude enzyme was extracted as reported by Iqbal and Schraudolf (1984).

Assay of peroxidase activity was carried out as described by David and Murray, (1965). Crude enzyme extract (0.2 ml) was mixed with 2.6 ml of phosphate buffer, pH 7.0 and 0.2 ml of 1% guaicol solution in a 1 cm wide silica cell. In blank, guaicol was not added. Optical density (O.D) was observed at 470 nm and activity calculated as O.D/mg protein/30 sec.

Isozyme Pattern of Peroxidase

Polyacrylamide slab get electrophoresis system (7.5% gels) was used according to Laemmli (1970). Samples containing 10-50 ug of protein were loaded on the gels. Electrophoresis were performed at a constant current of 30 amp for 6h, using a Tris-phosphate buffer, pH 7.0. Peroxidase activity was detected on the gels by flooding with 0.005M guaicol, 0.005M hydrogen peroxide in 0.2M phosphate buffer adjusted to pH 5.8 (Siegel and Galston, 1967).

Four independent experiments were conducted starting from the first week of November, 1986 upto March, 1987 and the results presented are the mean of these experiments.

Standard error of the mean was calculated. The significance between means of treatment and control was calculated by Dunnett's method, while significance among means of treatment was obtained through S-N-K test.

RESULTS AND DISCUSSION

Germination and Early Seedling Growth

Barium seems to have an inhibitory effect on percentage germination at all concentrations (except at 0.1 mM and 1 mM, where a non-significant increase over the control was observed (Table 1). Reduction at 20-40 mM was significant in relation to control. The percent reduction at these two treatments over control was 13.2 and 25.3, respectively. Treatments of 60-100 mM barium were lethal for growth, as no germination was observed at these doses. Shoot length retarded at 1-40 mM, but significantly only at 20-40 mM. All Ba^{++} concentrations significantly reduced root growth. Root was more sensitive than shoot, as relative decrease in length at different Ba^{++} treatments was more in comparison with the shoot. The root and shoot dry weights also decreased at all Ba^{++} treatments. The decrease in shoot dry weight was significant at 10-40 mM, while of roots at 5-40 mM in relation to control.

Table 1. Effect of Barium chloride on germination and growth of 7 days old wheat seedlings

Ba++ Conc. (mM)	Percent germination	Shoot length (cm)	Root length (cm)	Shoot dry weight (mg/seedling)	Root dry weight (mg/seedling)
0.0	91a ± 0.197	6.75ab ± 0.421	3.84a ± 0.348	9.4a* ± 0.414	3.5a ± 0.368
0.1	93a ± 0.207	6.78ab ± 0.408	2.57a* ± 0.153	9.2a ± 0.397	3.4a ± 0.64
1.0	92a ± 0.260	6.71ab ± 0.284	2.50a* ± 0.125	8.6ab ± 0.315	3.09a ± 0.44
5.0	87b ± 0.267	5.81abc ± 0.284	2.47abc* ± 0.128	7.83ab ± 0.178	2.8ab* ± 0.36
10.0	83bc ± 0.450	3.96bcd ± 0.183	1.49cd* ± 0.112	6.0cd* ± 0.113	1.6bc* ± 0.21
20.0	79c* ± 0.535	3.18cd* ± 0.150	1.37cd* ± 0.068	5.4cd* ± 0.134	1.3bc* ± 0.21
40.0	68c* ± 0.595	3.17cd* ± 0.081	1.37cd* ± 0.05	5.0cd* ± 0.076	0.87d* ± 0.081

** = Treatments means significant for a joint confidence coefficient of P = 0.95 (Dunnett's test).*
a,b = Values followed by different letters in the same column differ significantly, alpha = 0.05 (S-N-K test).

Barium showed, in general, an inhibitory effect on percent germination and seedling growth of wheat. Concomitant with the decreased growth, dry weight of root and shoot also decreased. An inhibition in percentage germination and seedling growth by the Ba^{++} treatments has also been reported in *Phaseolus lunatus* (Hegwood, 1972); *Vigna mungo* (Minton and Wilson, 1973); spring barley (Davis and Becklet, 1978); *Cucumis sativus* (Gopala, 1980); *Oryza sativa* (Debnath and Mukerji, 1982a); *Phaseolus aureus, Cephallandra indica, Beta vulgaris, Triticum aestivum* and *Lactuca sativa* (Debnath and Mukerji, 1982b) and *Zea mays* (Iqbal and Rafique, 1987).

Causes of growth inhibition could be many and varied. However, factors affecting cell division and cell expansion play a key role from the morphogenetic view point. Both these phenomena are impaired by Ba^{++} toxicity Debnath and Mukerji (1982) have reported inhibition of cell enlargement due to Ba^{++} toxicity.

Likewise an imbalance in oxidation energy and phosphate potential significantly alters the ATP levels in cells, eventually affecting the divison rate (Minton and Wilson, 1973). Hence retardation in cell division and expansion is a key factor in Ba^{++} mediated growth inhibition.

Soluble Protein

The data on soluble protein content of shoots of three, five and seven days' old seedlings depicted that under Ba^{++} stress, there was a decline in soluble protein content on all days (Figure 1). The inhibitory effect appears to be time dependent as maximum decrease was observed in shoots of seven days' old

seedlings and minimum in shoots of three days' old seedlings. Decreases at 1-40 mM Ba^{++} treatments on all days were significant (P = 0.05).

Decrease in soluble protein content at all Ba^{++} treatments revealed a reflection of lesions in the biochemical process. Decreased protein synthesis will also affect the phenomenon of the growth. The reports on the Ba^{++} effect on proteins are scanty, however, the available work suggests that protein synthesis is, in general, retarded by Ba^{++} (Debnath and Mukerji, 1982a; Iqbal and Rafique, 1987).

Peroxidase Activity

This activity in the shoots of control seedlings decreased with ontogenetic development, i.e., being maximum in three day's and minimum in seven days' old shoots (Figure 2).

An increase in peroxidase activity was observed at all Ba^{++} treatment taken on shoots of three, five and seven days' old seedlings. There was a gradual rise in peroxidase activity from 0.1 to 40 mM of Ba^{++} treatments in all three observations.

Isozyme Pattern of Peroxidase

The effects of Ba^{++} on isozymic forms of peroxidase by polyacrylamide gel

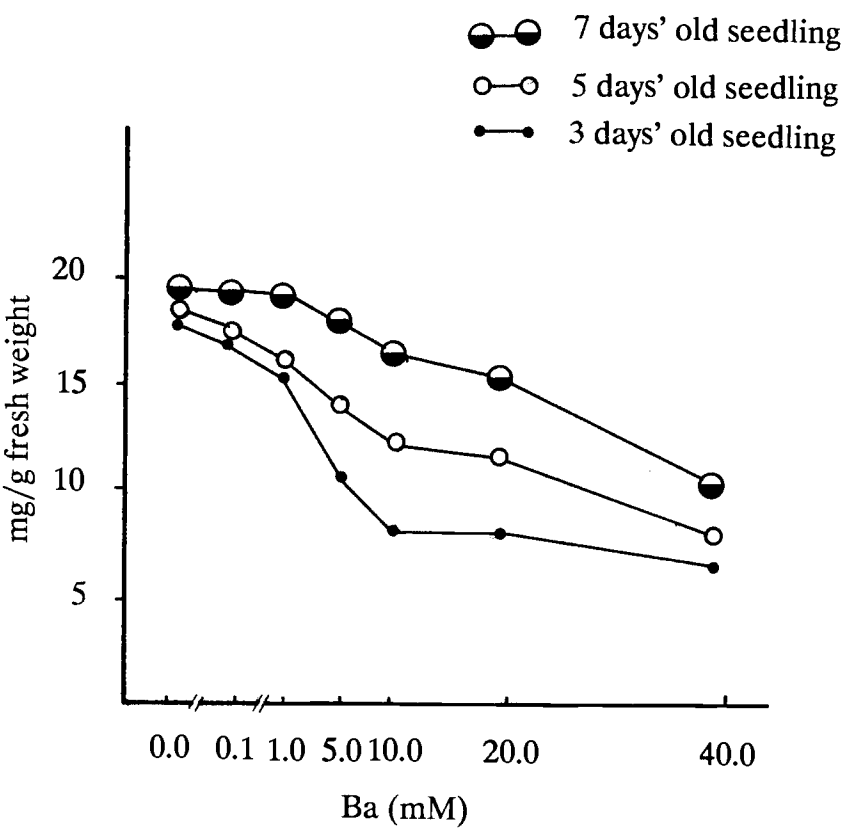

Figure 1. Effect of Barium chloride on soluble protein content in shoots of *Triticum aestivum* L.

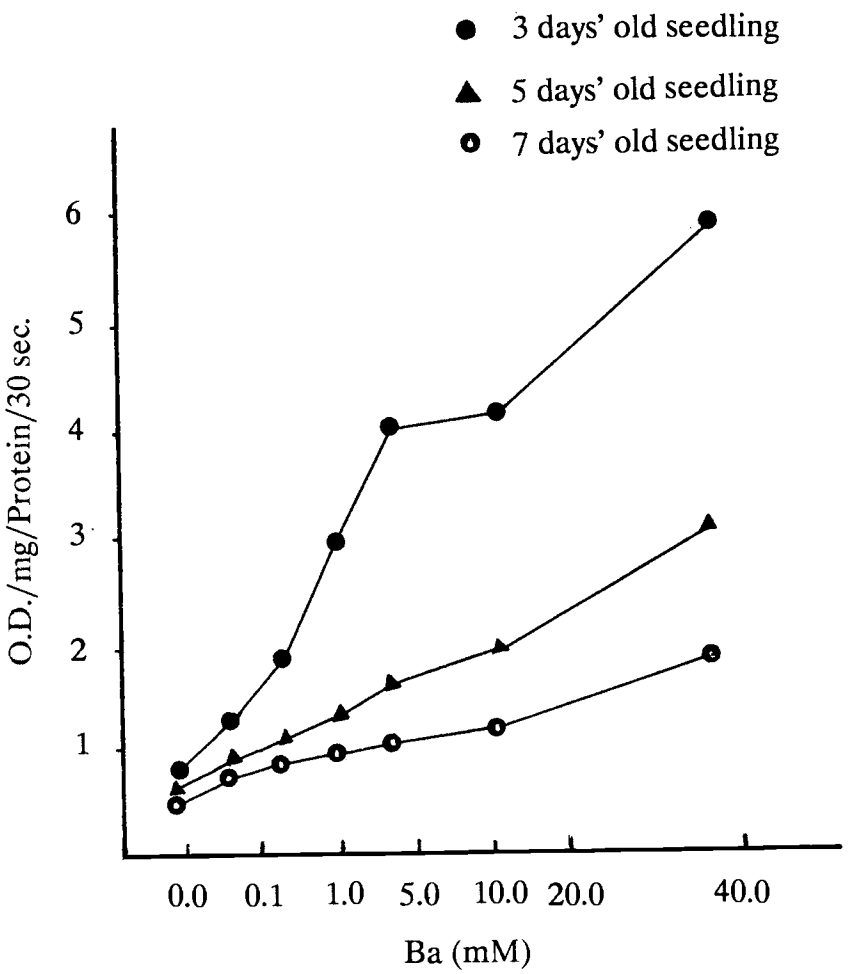

Figure 2. Effect of $BaCl_2$ on specific activity of peroxidases in shoots of *Triticum aestivum* L.

electrophoresis showed that three isozymic forms at Rf 0.11, 0.36 and 0.58 were visible in control shoots of three days' old seedlings. In shoots of five and seven days' old seedlings only two isozymic forms at equal Rf of 0.62 and 0.74 were observed (Figure 3). The Ba^{++} treatments did not induce any qualitative change on the number of isozymic forms in shoots of three, five and seven days' old seedlings. The number of bands remained the same as observed in control for different days. However, quantitative difference, at various concentrations was visible, as indicated by the relative differences in the intensity of respective bands.

Enhancement in peroxidase activity is symptomatic of Ba^{++} toxicity. Increase in enzyme activity resulting with treatments of heavy metals is reported for catalase, ascorbic acid oxidase in rice (Debnath and Mukerji, 1982a), alpha-amylase in barley (Jones and Russel, 1983) and acid phosphatases in maize (Iqbal and Rafique, 1987) and is regarded as typical response to increased toxicity.

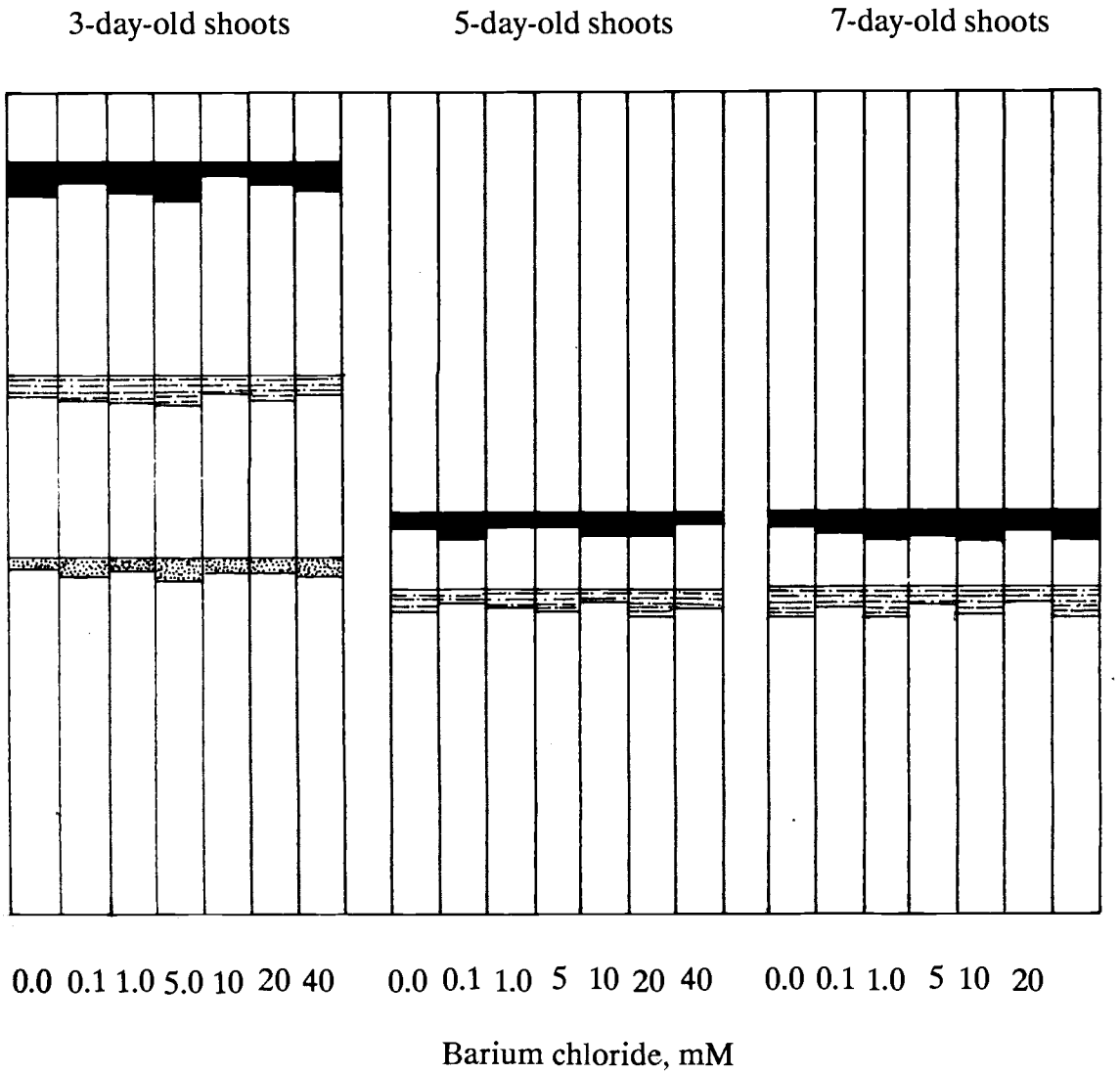

Figure 3. Peroxidase isozymic forms in control and Ba^{++} treated shoots of 3, 5 and 7-days' old seedlings (■ dark band; ▤ light band and ▩ very light band)

Peroxidase is an iron porphyrin enzyme whose precise role in plant metabolism is rather obscure, although it has been implicated in numerous plant functions. In plant tissue, naturally occurring phenols and amines may serve as hydrogen donors and a possible metabolic role for peroxidase would be to oxidise these toxic compounds to less harmful products (Sullivan, 1946). Peroxidase may also serve to eliminate H_2O_2 and may also be important for the destruction of the plant growth hormone IAA (McCune, 1961). All these diverse functions of the enzyme point out, that the role of peroxidase mainly is in catabolism. The present study revealed that the increased activity of peroxidase for combating the increased barium toxicity within the plant body which

hinders and blocks physiological processes and also affects growth.

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