

TOXIC EFFECT OF BARIUM ON GERMINATION AND EARLY GROWTH OF MAIZE SEEDLINGS AND ITS REVERSAL BY NUTRITION AND GIBBERELIC ACID

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ABSTRACT: Maize seeds were soaked in 0-100 mM BaCl₂ for 24h followed by soaking of one batch of seeds in 100 mM GA₃ (Gibberellic acid) for 8h. Subsequently seeds were germinated and grown in four batches: Ba-treated in distilled water; Ba-treated in half strength Hoagland nutrient solution; Ba-GA₃ treated in distilled water; Ba-treated in half strength Hoagland nutrient solution; Ba-GA₃ treated in distilled water and Ba-GA₃ treated in half strength Hoagland nutrient solution. There was a general decline in germination and inhibition of growth in seedlings raised from Ba-treated seeds, at all doses except at 0.1 mM, where a significant improvement in all growth parameters were observed. Both nutrient solution and GA₃ treatment reduced the toxic effects of Ba on germination and growth. The effects of GA₃+ nutrient solution were synergistic as maximum restoration to the effects of Ba were observed when GA₃ treated seeds were grown in nutrient medium. It is concluded that both nutrition solution and GA₃ were effective in reversing the Ba induced suppression of germination and inhibition in root -shoot length, and dry weights.

Key Words: Maize; Barium; Germination; Root-shoot Length; Dry Weight; Gibberellic Acid; Pakistan.

INTRODUCTION

Barium (Ba) toxicity for plant and animal growth has become increasingly important because of its presence in the environment. It finds its way in plants directly by foliage and via water and soil and eventually into animals when they consume Ba enriched plants. The main source of barium accumulation in plants is from the soil. Of the harmful effects, the most pronounced is on plant growth. The critical threshold level of BaCl₂ for plant growth inhibition is quite variable. For example, in mungbean complete growth inhibition is reported at 40 mM Ba₂ (Minton and Wilson, 1973), in rice at 100 mM (Debnath and Mukherji, 1982a), in maize 50% germination is inhibited and 90% shoot growth is observed at 100 mM (Iqbal and Rafique, 1987), in wheat 50% shoot inhibition occurred at 40 mM (Iqbal and Naz, 1989). Contrary to this, Ba has been reported to accumulate to very high levels of upto 4000 ppm in Brazil nut (Bollard and Butler, 1966). The inhibition of growth is an outcome of the damage to

the physiological, cytological and biochemical processes (Minton and Wilson, 1973; Debnath and Mukherji, 1982 a, b; Iqbal and Rafique, 1987; Iqbal and Naz, 1989; Moya et al., 1993; Ali et al., 1999; Prasad et al., 1999).

Compared to the magnitude of the problem, the work on bio-regulants which can revert the toxic effects of Ba is almost non-existent (Debnath and Mukherji, 1982 a and b). Gibberellic acid is so well known for its growth promoting effect under radiation stress (Iqbal and Mahmood, 1980; Iqbal et al., 1984) that it was considered worthwhile to study the effects of GA₃ on restorative processes of plants affected by Ba toxicity. The present work documents the toxic action of Ba on early growth of maize seedlings and reversal of these effects by GA₃.

MATERIALS AND METHODS

Certified seeds of maize (*Zea mays* L. cv Akbar) were obtained from Millet and Maize Research Institute, Yousafwala (Sahiwal). Healthy and uniform sized seeds

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were surface sterilised in 1% chloramin T solution for 30 min, washed thoroughly with running tap water followed by distilled water. The concentrations of BaCl₂ were 0.0, 0.1, 1.0, 5.0, 15.0, 50.0 and 100 mM. In a preliminary experiment, seeds were sown in the above concentrations of BaCl₂ for 8, 16, 24, 32 and 40hr. Ba treatment for 8, and 16 hr produced no pronounced toxic effect on germination and growth, while a treatment of 32 and 40 hr proved extremely toxic, inhibiting germination. With 24hr treatment of Ba, toxic effects at different concentrations were well expressed, hence in all subsequent experiments 24hr soaking was done. The control seeds were soaked in distilled water for the same period. Likewise 0.5 strength of Hoagland nutrient medium (NM) (Devlin, 1969) was selected for optimal growth after a pilot experiment where control seeds were grown in full, 0.75, 0.5 and 0.25 strength of nutrient solution. The Ba soaked seeds of all concentrations were divided into two batches. One batch was post-treated for 8 h with 0, 50 and 100 ppm of GA₃. Subsequent to GA₃ treatment, seeds were germinated and grown in four batches; Ba-treated seeds in distilled water; Ba-treated seeds grown in nutrient medium; Ba-GA₃ treated seeds grown in distilled water, and Ba-GA₃ treated seeds grown in nutrient medium. Hundred seeds of each treatment were placed uniformly in 15 cm sterile glass petri dishes lined with two layers of Whatman filter paper No. 1. Initially 10 ml of each treatment was supplied to every petri dish. Petri dishes were placed in dark at 30°C ± 1.5 in programmed refrigerated incubator (Hotpack, U.S.A). After germination (3 days of sowing), seedlings were given a 16h photoperiod of 10 klux at 30°C ± 1.5. The dishes were regularly watered with equal amounts of respective treatments. The petri dishes were arranged daily in a completely randomised block design. Seedlings were grown for 7 days. Growth measurements included length of shoot and root (cm) and dry weight of shoot and root (mg/seedling). Data were statistically analysed and means were separated by Duncan's new multiple range

test at 5% probability (Steel and Torrie, 1980).

Of the two GA₃ treatments, only the effects of 100 ppm GA₃ are reported in present study as the pattern of restoration of both, i.e., 50 and 100 ppm GA₃ was similar, but the restoration was more emphasized with 100 ppm GA₃.

RESULTS AND DISCUSSION

Effects on Germination

All treatments of Ba proved detrimental for germination (except of 0.1 mM where an increase occurred at all treatments). Reduction in germination initiated at 1 mM Ba (-4.76%) culminated at 100 mM (-45.28%). At Ba-GA₃ treatment small, non-significant increases were observed over all Ba-treatments (Table 1). A remarkable reversal effect was observed at Ba+NM and Ba+GA₃+NM treatments. At these treatments significant increase in germination was observed over Ba-treated control. At 1 mM, increase was 3.75%, 20.0% and 22.50% in Ba+GA₃, Ba+NM and Ba+NM+GA₃ treatments, respectively. At highest Ba-treatment of 100 mM, the increases at the above mentioned combinations were 10.86%, 30.43% and 34.78%, respectively.

Effects on Shoot and Root Length

The reversal effect against Ba-toxicity are fully manifested by NM and GA₃ treatment on shoot length (Table 1). A significant increase in shoot length from 0.1 mm (51.25%) to 75mM (29.03%) was observed with Ba+GA₃ treatment over the Ba treatment. At all Ba+NM treatments the shoot length showed a further significant increase both on Ba and Ba+GA₃ treatment. The maximum reversal affect at all Ba-treatments (0.1-100 mM) was observed with Ba+NM+GA₃ treatment. With this treatment an increase of 97.94% was observed at 0.1 mM and 91.32% at 100 mM, respectively. This treatment besides reversing the toxic effect of Ba, proved rather stimulatory as upto 100 mM the shoot lengths were even more than the untreated shoot.

The data on root length is in confor-

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Table 1. Effect of Barium Chloride treatment and its reversal by gibberellic acid (GA₃) and nutrient medium (NM) treatment on germination and growth characteristics of maize (*Zea mays*).

Parameter	Treat- Ment	Ba Cl ₂ (mM)					
		0.0	0.1	1	5	50	100
%Germination	T ₁	84 ^b	92 ^a	80 ^b	72 ^c	54 ^{de}	46 ^f
	T ₂	86 ^b	95 ^a	83 ^b	73 ^c	56 ^{de}	51 ^c
	T ₃	98 ^a	100 ^a	96 ^a	95 ^a	72 ^c	60 ^d
	T ₄	100 ^a	100 ^a	98 ^a	97 ^a	74 ^b	62 ^c
Shoot length(cm)	T ₁	9.86 ^b	11.20 ^a	10.05 ^b	9.02 ^{bc}	6.91 ^{cd}	5.88 ^d
	T ₂	14.80 ^b	16.94 ^a	15.50 ^b	14.55 ^b	9.75 ^c	6.25 ^c
	T ₃	17.83 ^b	19.10 ^a	17.80 ^b	16.77 ^{bc}	13.09 ^c	9.50 ^c
	T ₄	20.20 ^b	22.17 ^a	20.17 ^b	20.00 ^b	15.32 ^c	11.25 ^c
Root length (cm)	T ₁	9.00 ^a	9.71 ^a	8.20 ^{ab}	7.04 ^b	5.82 ^c	4.40 ^d
	T ₂	10.17 ^a	10.52 ^a	10.30 ^a	9.20 ^b	8.40 ^{bc}	6.07 ^d
	T ₃	11.20 ^c	14.37 ^a	13.20 ^b	9.88 ^b	7.88 ^c	6.55 ^f
	T ₄	11.96 ^a	12.80 ^a	12.30 ^a	11.53 ^{ab}	10.00 ^b	7.31 ^d
Shoot dry wt. (mg/seedling)	T ₁	83.1 ^b	98.1 ^a	85.4 ^b	82.5 ^b	74.10 ^c	62.4 ^d
	T ₂	92.3 ^c	113.5 ^a	102.1 ^b	86.3 ^c	80.8 ^{cd}	71.3 ^e
	T ₃	110.4 ^c	155.1 ^a	120.4 ^b	100.8 ^d	98.2 ^d	87.2 ^e
	T ₄	173.3 ^{ab}	184.6 ^a	182.4 ^a	164.3 ^{ab}	103.5 ^{cd}	94.7 ^d
Root dry wt. (mg/seedling)	T ₁	82.4 ^a	84.1 ^a	78.3 ^a	77.5 ^a	68.1 ^b	58.6 ^c
	T ₂	92.1 ^b	104.5 ^a	82.1 ^c	82.9 ^c	76.5 ^d	65.2 ^e
	T ₃	122.4 ^b	132.5 ^a	98.6 ^c	98.1 ^c	91.1 ^c	84.3 ^d
	T ₄	153.1 ^b	171.7 ^a	133.5 ^c	101.4 ^d	95.4 ^{de}	90.2 ^{de}

T₁ = Ba; T₂ = Ba + GA₃; T₃ = Ba + NM; T₄ = Ba + GA₃ + NM.

Means followed by the same letter do not differ significantly at 5% probability level.

mity with that of shoot. The toxic effects of Ba were reversed by GA₃, NM and GA₃+NM treatments (Table 1). The pattern of reversal was similar to that of shoot at all treatments, except for the fact that relative increases were small as compared to the shoot, e.g., at 0.1 mM the increase with Ba+NM+GA₃ was 31.82% in root compared to 97.94% in shoot.

Effects on Shoot and Root Dry Weights

The data on shoot and root dry weight (Table 1) is in accordance with the data on shoot and root length. As envisaged the dry weight also showed a similar pattern of Ba-

toxicity reversal by GA₃, NM and GA₃+NM treatments as for shoot and root lengths. For example, at 0.1 mM the increases in dry weight of shoot with Ba GA₃, Ba+NM and Ba+NM+GA₃ were 15.69%, 58.10% and 88.17% over Ba-control. Likewise the values for root increase were 24.25%, 57.55% and 104.16%, respectively. At the highest Ba treatments of 100 mM, increases in shoot dry wt were 14.26%, 39.74%, and 51.76%, against 11.26%, 43.85% and 53.92% of root.

The major effects caused by Barium on plants are morphogenetic, physiological and biochemical. Of the morphological changes

resulting from Barium toxicity, delay and reduction in germination and stunted growth are the most common (Iqbal and Naz, 1989; Iqbal and Rafique, 1987; Debnath and Mukheriji, 1982 a,b; Chaudhry and Wallace, 1977). Of the physiological and biochemical changes, inhibition in respiration, increase/decrease in enzyme activity, decrease in sugars, nitrogen and proteins are the commonly observed aspects (Iqbal and Naz, 1989; Iqbal and Rafique, 1987; Debnath and Mukheriji, 1982a; Minton and Wilson, 1973; Ali et al. (1999).

In the present study as well, inhibition in germination and early seedling growth is observed. Stimulation in germination and root-shoot length at 0.1 mM is not only seen in the present study, but is also reported for earlier work (Iqbal and Rafique, 1987). There is no plausible explanation for this stimulation phenomenon, except for the fact that concentration of Ba in plant growth media are thought to partially replace the requirement of Ca⁺⁺ in plants (Minton and Wilson, 1973). This may be a factor in stimulation on priority. As evident from the present work, degree of inhibition of seedling growth is dependent on the concentration of Ba ions accumulated in the plant, greater the dose the greater is the inhibition. Little is known as to the precise factors and specific metabolic lesions which retard growth. However, factors influencing cell division rate, cell expansion and energy turn over must be hampered to inhibit and retard growth. Earlier, studies on *Phaseolus*, *Cephalandra*, *Beta*, *Triticum*, *Lactuca*, mung bean and maize amply support this assumption (Iqbal and Naz, 1989; Iqbal and Rafique, 1987; Debnath and Mukheriji, 1982 a, b; Minton and Wilson, 1973).

Reversal of Barium mediated growth inhibition by NM and GA₃, as amply shown by the present investigation, manifests that the block is at physiological level. The increase in % germination and stimulation of root-shoot length in Ba-NM treatment over Ba⁺⁺ control is attributed to the micronutrients present in the growth me-

dium as advocated by Bollard and Buttler (1966), in contrast to a much earlier statement of Scharrer and Schropp (1937) who reported stimulation of plant growth by inclusion of Ba-compounds in solution cultures. The present study supports the view of Bollard and Buttler (1966) as the effects of micronutrients in promoting growth are dramatic.

The effects of GA₃ on growth, development and metabolism appear to involve the action of GA₃ at several biochemical levels. It has been argued that GA₃ can promote growth of plants by either cell expansion or cell division or both (Green, 1976; Loy, 1977). It is well established that cell expansion is rapidly inhibited by respiratory poisons (Russel and MacMillan, 1984). Of the numerous inhibitory effects caused by Ba treatment suppression in cell division and of expansion of wall is reported by Debnath and Mukheriji (1982a), inhibition of respiration and oxidative phosphorylation are reported by Minton and Wilson (1973) and Moya et al (1993). The idea is gaining ground that since GA₃ and auxin promote growth in a similar fashion, GA₃ may also promote changes in RNA synthesis during elongation. The action of GA₃ in all target tissues may, therefore, be via its effect on the transcriptional process. This could be a quantitative effect where GA₃ could accelerate the synthesis of all classes of RNA, as has been reported for castor bean (Gonzalez and Delsol, 1981) or its effect could be qualitative by the induction of specific enzymes, as in cereal aleurone (Higgins et al., 1982).

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