attenuation relations will be extremely useful in estimating strong ground motion parameters for future earthquakes in the Himalayan region. The present relations show the estimate of peak horizontal acceleration and velocity up to 1000 km from the epicentre of the earthquakes. Such relations are also needed for other major geological regions of India, e.g. Indo-gangetic basin and Peninsular regions, which can only be developed after having huge data base of the strong ground motion data from future earthquakes in these regions. Such relations will provide a useful guideline to the professional engineers while planning for major structures in the Himalayan region.

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Involvement of calcium in brassinolide and auxin-induced cell elongation

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Coleoptile segments incubated in brassinolide (BR) or auxin (IAA) along with calmodulin inhibitor, chlorpromazine (CPZ) or calcium chelating agent (EGTA) or calcium surface antagonist, lanthanum chloride ($La^{+3}Cl_3$) resulted in inhibition of BR and IAA-induced elongation growth of coleoptiles. Pretreatment of coleoptile segments for 4 h with EGTA or $La^{+3}Cl_3$ also inhibited both BR and IAA-induced growth. Coleoptile segments with enriched endogenous levels of free calcium showed more elongation in the presence of BR or IAA. However, BR and IAA were ineffective in inducing coleoptile growth in the presence of calcium inhibitors. Results indicated that BR and IAA act through calcium and or calmodulin protein in inducing elongation growth in coleoptile.

BRASSINOSTEROID, a novel plant growth-promoting steroidal lactone, was first isolated and identified from pollen grains of brassica (*Brassica napus*)[1]. Recently, the presence of this new class of plant hormones has been shown in a number of plant species. It has also been shown to be involved in growth responses in many test systems[2–5], especially inducing cell elongation and cell division[1,5–7]. In addition to acting independently, it is found to act synergistically with other growth hormones found in plants[3 8,9]. One such interaction widely reported is that between BR and auxin in inducing cell elongation[3 8,10]. Auxin-induced cell elongation has been shown earlier to be mediated through the release of Ca^{2+} from membrane vesicles, which on complexing with the calcium-modulated protein, calmodulin, results in the response. It is known that calmodulin modulates auxin-induced growth[11]. Though the involvement of calcium in auxin action has been recognized for many years[12], the nature of interaction between auxin and calcium is poorly understood. Auxin-induced elongation in pea epicotyl is blocked by treating the segments with calcium chelators (EGTA and CTC), calcium surface antagonist (Lanthanum) and calcium channel blocker, Verapamil. The existing evidence suggests that auxin causes calcium efflux. In recent years, Ca^{2+} has been regarded as a second messenger and in conjunction with the calcium-binding protein, calmodulin, has been reported to be involved in the regulation of a number of cell processes, both in animals and plants[13–17]. Because of the close mimicking of the action of auxin by BR, we hypothesize that the mechanism underlying BR-induced cell elongation could also be mediated through calcium. We test this hypothesis here by using surface calcium antagonist, calcium chelating agent and finally potent antagonists of calcium calmodulin protein.

Brasinolide (BR) chemical was obtained from Godrej Soaps Ltd, Bombay. Calmodulin antagonist – chlorpromazine (CPZ), calcium chelating agent – ethylene glycol-*bis*(aminoethyl)tetraacetic acid (EGTA) and calcium surface antagonist – lanthanum chloride ($La^{+3}Cl_3$) and indole-3-acetic acid (IAA) were from Sigma Co, USA.

The assay of cell elongation induced by BR and IAA was carried out using wheat coleoptiles. Wheat seeds

were sown in plastic trays filled with vermiculite and kept in the dark for 5 days at 24°C. The dark grown coleoptiles were then harvested under dim red light and 10 mm segments were excised by excluding 3 mm tip using a coleoptile cutter. Excised coleoptile segments were transferred to a beaker containing distilled water and kept floating for an hour prior to the experimental treatments.

A set of 10 presoaked coleoptiles was transferred to petri dishes (5 cm diameter) containing 10 ml of incubating medium consisting of 1 mM phosphate buffer (pH 6), various calcium chelator/calcium surface antagonist solutions (CPZ, EGTA and $La^{+3}Cl_3$) along with 10 µM IAA or 0.2 µM BR; suitable controls without IAA or BR were maintained in all experiments. The coleoptiles were incubated in dark for 20 h at 24°C and the increase in the coleoptile length was measured using a millimeter scale and magnifying lens.

In another set of experiments, coleoptile segments were preincubated in the above-mentioned calcium chelators/calcium surface antagonists solution for 4 h, the coleoptile sections were rinsed in distilled water, surface-dried and then transferred to IAA or BR solutions. The response in terms of coleoptile segment elongation was monitored.

An attempt was also made to examine the effect of IAA or BR on cell elongation of coleoptiles enriched with calcium. Calcium-enriched coleoptiles were obtained by planting wheat seeds previously soaked in 1% $CaCl_2$ solution and sown in vermiculite irrigated with 1% $CaCl_2$ solution. Following germination for 5 days, the coleoptiles were processed as mentioned above and incubated in either water, IAA or BR and the increase in length was monitored.

The presence of calcium calmodulin antagonist-CPZ along with IAA or BR inhibited elongation growth of the coleoptile segments. A concentration-dependent reduction in IAA or BR-induced growth was observed with increasing concentration of CPZ in the medium. At a concentration of 75 µM of CPZ about 50% inhibition of IAA and BR-induced elongation was observed and at a concentration of 140 µM, both IAA as well as BR-induced growth were completely inhibited (Figure 1).

The addition of increasing concentrations of calcium chelating agent (EGTA) or calcium surface antagonist, $La^{+3}Cl_3$ to the incubation media progressively decreased IAA as well as BR-induced elongation. IAA and BR-induced elongation was inhibited to an extent of 50% in the presence of 2–3 mM EGTA (Figure 2) and 1–5 nM of $La^{+3}Cl_3$ (Figure 3).

Coleoptile segments pretreated with CPZ showed reduced growth when transferred to IAA and BR. There was a concentration-dependent reduction in coleoptile growth with increasing concentration of CPZ used for pretreatment. Segments incubated in IAA (10 µM)

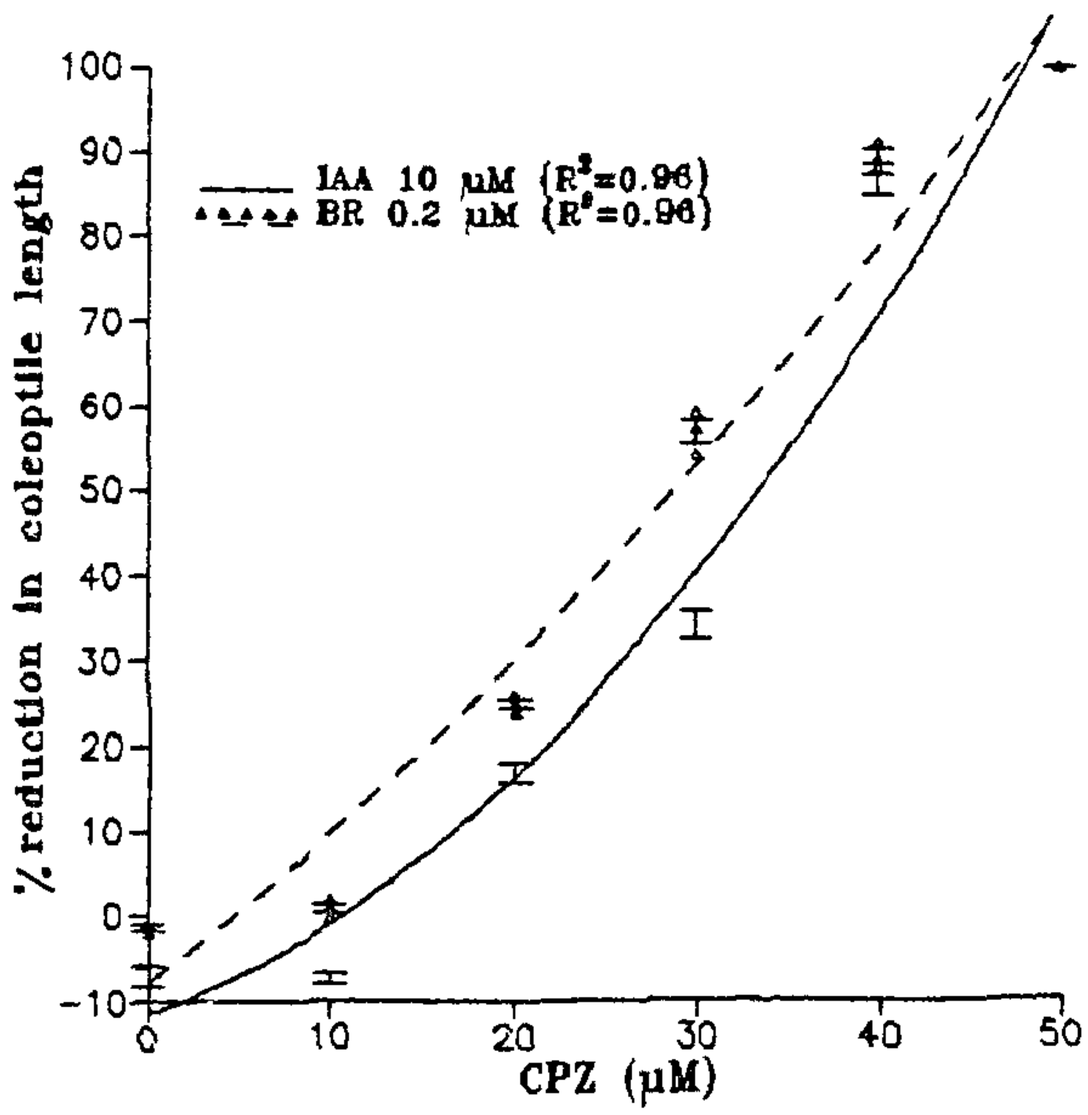

Figure 1. Effect of CPZ on IAA and BR-induced growth of wheat coleoptile segments. (Coleoptile segments were incubated in medium containing IAA (10 µM) or BR (0.2 µM) with different concentrations of CPZ The final lengths of coleoptile segments were measured at the end of 24 h incubation in dark).

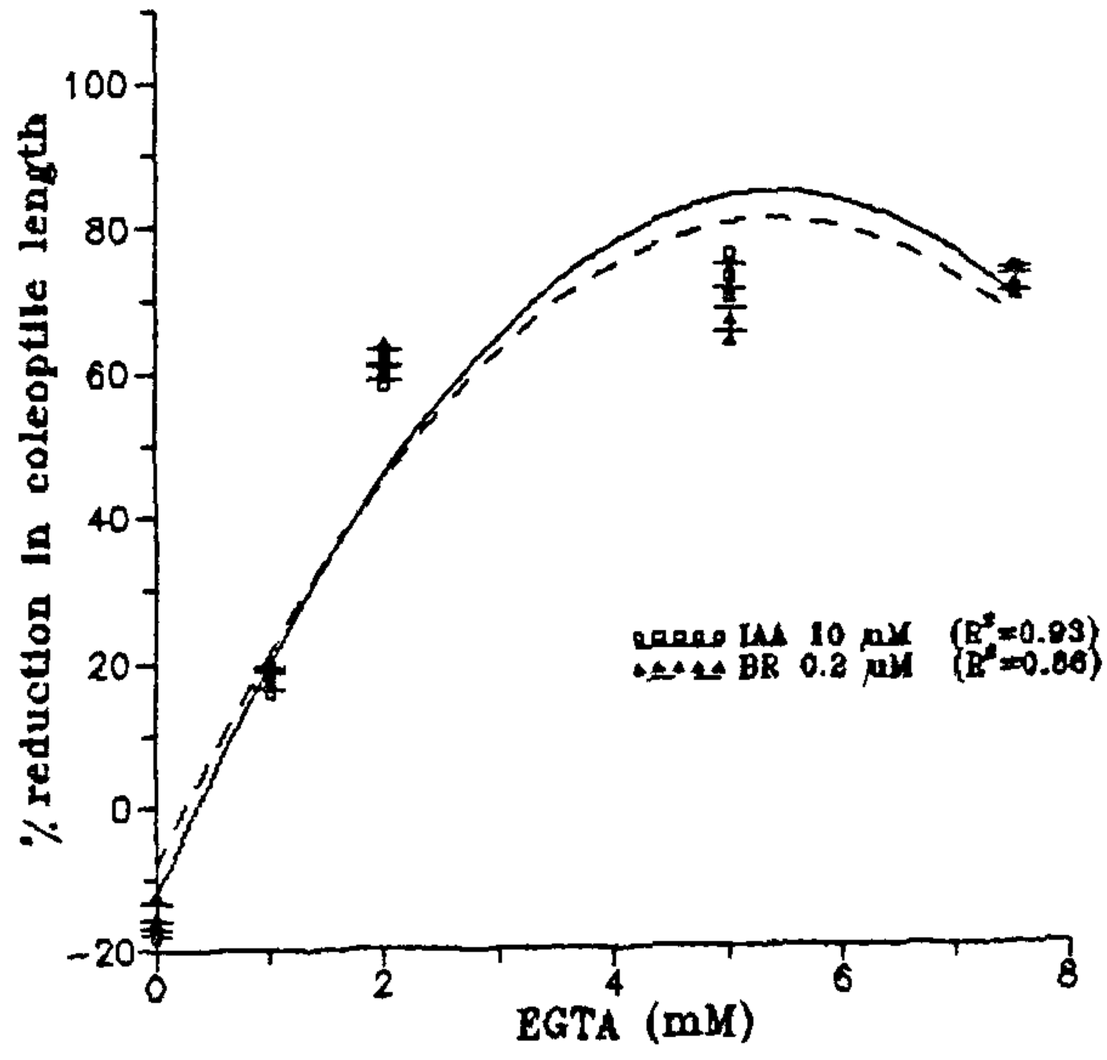

Figure 2. Effect of EGTA on IAA and BR-induced growth of wheat coleoptile segments (Coleoptile segments were incubated in medium containing IAA (10 µM) or BR (0.2 µM) with different concentrations of EGTA The final lengths of coleoptile segments were measured at the end of 24 h of incubation in dark).

Table 1. Effect of CPZ and EGTA on the extent of inhibition of growth of normal and calcium-enriched wheat coleoptile segments

Inhibitors	Calcium level in the coleoptile	Basal medium	IAA (10 µM)	BR (0 2 µM)
CPZ (50 µM)	Normal	30 60	81 30	63 93
	Enriched	84 33	92.16	81 00
EGTA (5 mM)	Normal	20 25	82 07	54 42
	Enriched	36 84	92 43	79 59

(% inhibition of growth over the respective control)

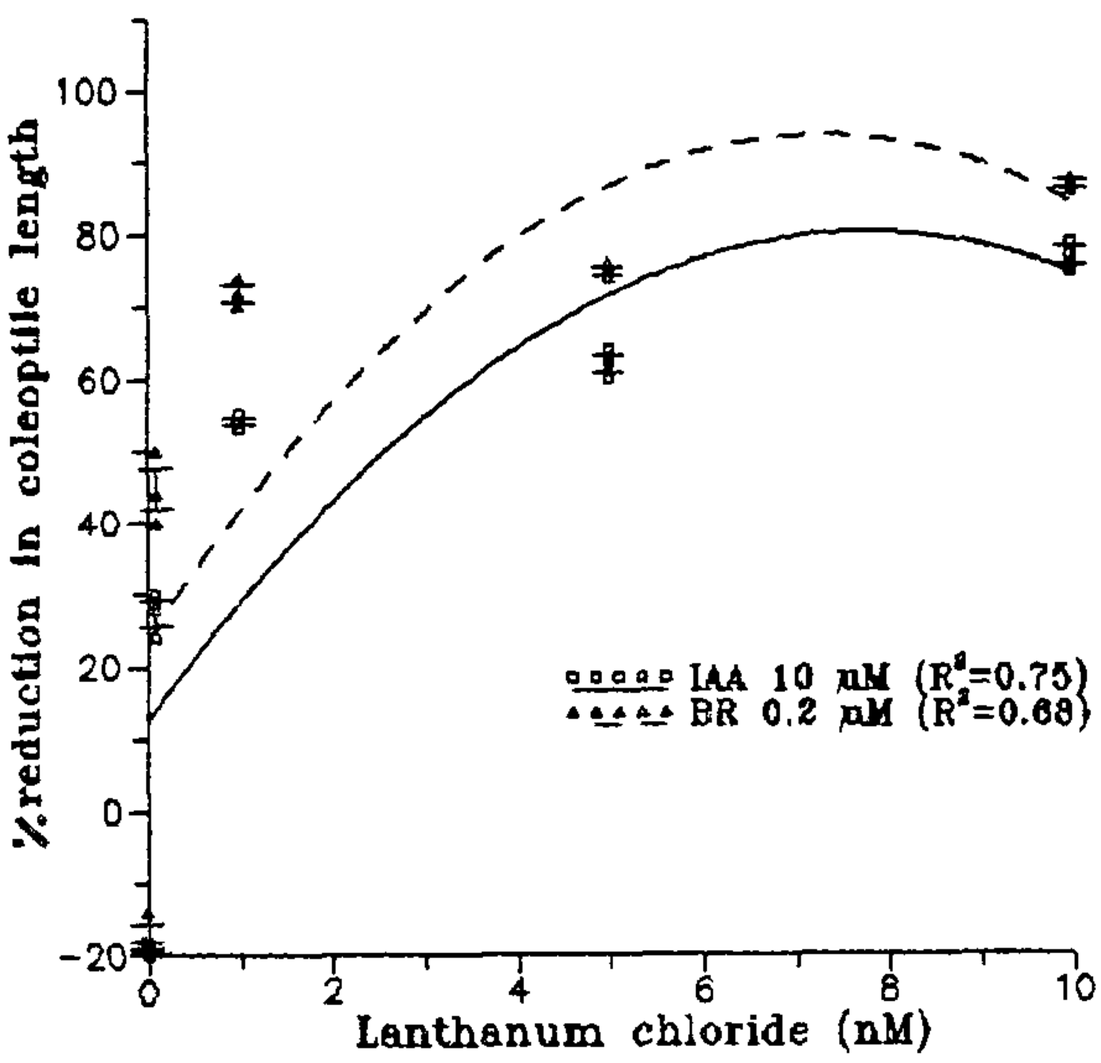

Figure 3. Effect of lanthanum chloride on IAA and BR-induced growth of wheat coleoptile segments. (Coleoptile segments were incubated in medium containing IAA (10 µM) or BR (0.2 µM) with different concentrations of $LaCl_3$. The final lengths of coleoptile segments were measured at the end of 24 h incubation in dark).

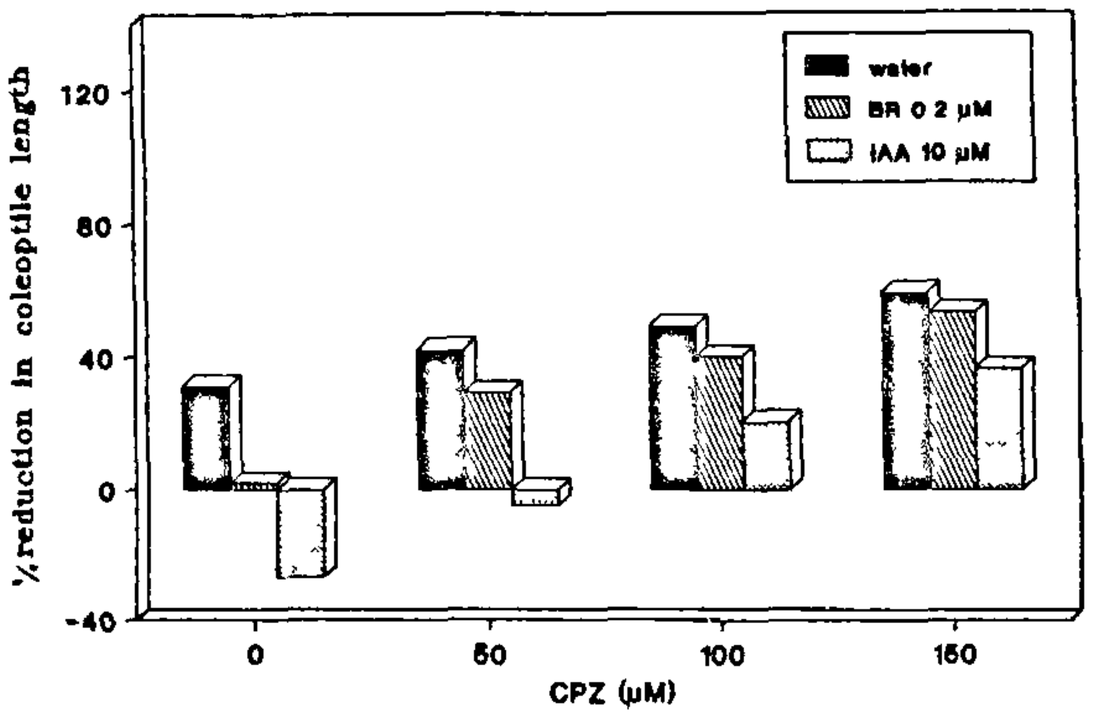

Figure 4. Effect of pretreatment of coleoptile segments with CPZ on IAA and BR-induced growth

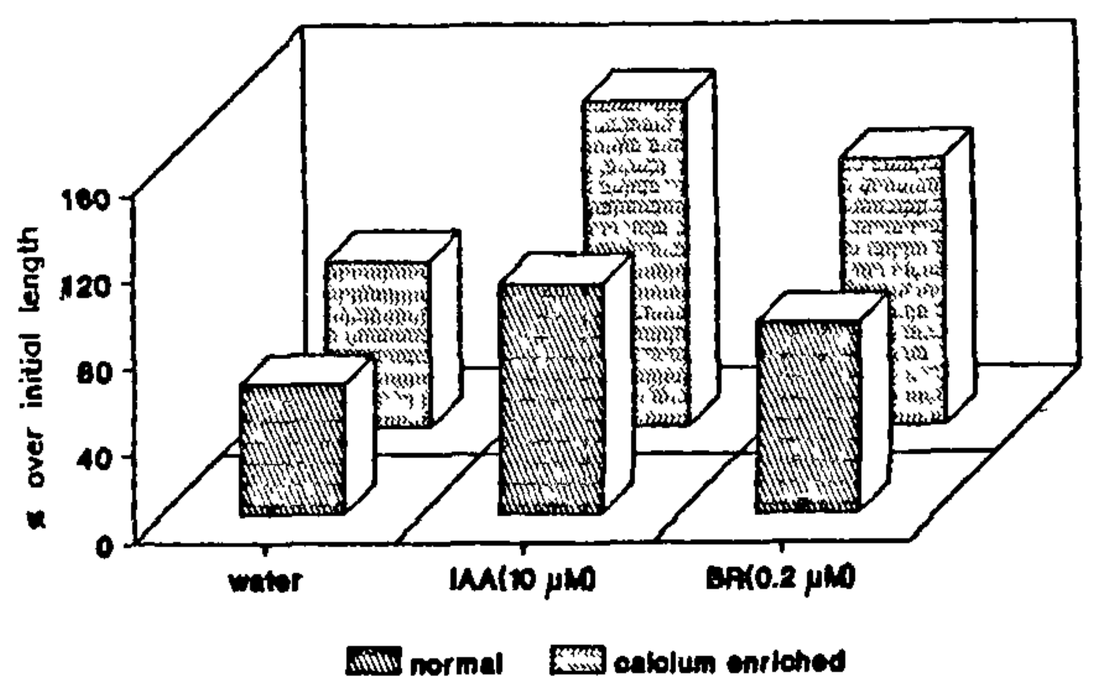

Figure 5. Effect of IAA and BR on the growth of coleoptile enriched with calcium.

medium showed 133% increase in growth. Segments pretreated with 50, 100, 150 µM of CPZ, however, showed increases of 105, 80, 64% respectively. A similar decline in response was observed when the CPZ-pretreated coleoptile segments were transferred to BR (0.2 µM) medium (Figure 4). Similar results were also obtained by pretreating coleoptiles with calcium chelating agent EGTA or calcium surface antagonist $La^{+3}Cl_3$ (data not shown).

Coleoptiles from seedlings raised in calcium-enriched medium showed significantly more elongation than coleoptile segments not enriched with calcium (Figure 5). The endogenous-free calcium in the former was nearly 27% more than the latter. Coleoptile segments enriched with calcium showed 16, 45 and 45% increase in length in control, IAA (10 µM) and BR (0.2 µM) media respectively over coleoptile segments not enriched with calcium. Under normal and enriched levels of calcium, both CPZ and EGTA were effective in reducing the elongation growth of coleoptiles-induced by IAA and BR. At 50 µM CPZ and 5 mM EGTA, complete inhibition of auxin and BR-induced elongation was noticed.

Calmodulin inhibitor (CPZ) or calcium chelator (EGTA) or surface calcium antagonist ($La^{+3}Cl_3$) reduced both auxin as well as BR-induced elongation growth of coleoptiles. Coleoptiles pretreated with calcium calmodulin inhibitor – CPZ, calcium sequestering agent and calcium channel blocker significantly reduced growth in IAA or BR medium. This indicated that reducing cytoplasm-free calcium concentration and inhibiting calmodulin activity reduces IAA as well as BR-induced growth.

Coleoptile segments with high endogenous calcium concentration showed more elongation growth in the presence of BR or IAA in the medium. All the calcium and calmodulin inhibitors used were also effective in inhibiting IAA or BR-stimulated growth of coleoptile

tissue with elevated calcium levels (Table 1). The results emphasize two points—the IAA- and BR-induced growth was more in tissues having high cytosolic-free calcium concentration and calcium inhibitors were effective in reducing the hormone-induced growth in tissues with elevated calcium concentration. This may be due to altered calmodulin content in the cytoplasm. It is opined that altered calcium levels may influence calmodulin activity and thus calmodulin-mediated cellular processes[18,19]. The range of concentration of calcium inhibitors which resulted in significant reduction in growth was comparable to the inhibition of other calcium or calmodulin-mediated responses[20].

Our results therefore indicate that the action of BR which closely mimics that of auxin in causing cell elongation could also be mediated by the release of calcium with the involvement of the calmodulin-binding protein.

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