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Studies on cell elongation in GA_3 and TIBA treated *Cucumis sutivus* (cucumber) seedlings

Barai Nilaxi J., Dasani Sonal H. and Thaker Vrinda S.

Department of Biosciences, Saurashtra University, Rajkot, India

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ABSTRACT

In the present study, Cucumis sutivus (cucumber) seedlings were treated with GA_3 and TIBA. The seedlings were harvested at regular time interval and analyzed for root length, its fresh and dry weights, cell wall loosening enzymes like α and β- galactosidases and β- glucosidases in cytoplasmic as well as wall-bound fractions of GA_3 and TIBA treated seedlings with reference to control (DW). Additionally, cell wall components like low and high molecular weight xyloglucans and esterified and non-esterified pectic substances were also estimated at various developmental stages. For extraction of non-esterified pectic polysaccharide fraction, EDTA (ethylene diamine tetra acetic acid) was used as chelating agent. Depectinated residues were used for extraction of low and high molecular weight xyloglucans by alkali treatment. It was observed that TIBA has strongly inhibited growth of roots, synthesis of cell wall components and enzymes activities. In contrast, GA_3 has increased the content of cell wall components and enzymes activities, however, no influence on length was recorded. Role(s) of these enzymes and cell wall polysaccharides in treated and untreated roots is discussed.

Key words: Cell wall loosening enzymes, *Cucumis sutivus*, GA_3, TIBA, pectic polysaccharide, xyloglucans.
Abbreviations: *EDTA =ethylene diamine tetra acetic acid; GA_3 = gibberellic acid; TIBA= 2,3,5-Triiodobenzoic acid*

INTRODUCTION

Cell elongation is primarily controlled by two processes, osmotic water absorption followed by an irreversible stretching of the cell wall. The expanding cell wall maintains its thickness as it expands, even though it may increase in length or in surface area by more than 100 folds before reaching its mature size (Ryser 1985). Changes in the chemical structure of cell wall polysaccharides during growth have been studied in many plant species (Taiz 1984; Sakurai 1991). Biochemical modifications of cell walls, such as an adjustment of molecular mass and quantities of cell wall polysaccharides are likely to be involved in the regulation of the capacity of cell expansion. Cell wall contains numerous enzymes capable of hydrolyzing the major components of the wall matrix, and some of these enzymes might function to loosen the wall by breaking load-bearing links between cellulose microfibrils, thereby allowing the wall to extend (Nishitani 1997, Thaker 1998).

Hormone induced changes in wall loosening and in the content of pectic as well as xyloglucan fractions is well studied. For example, auxin induces elongation growth of stem segments by causing loosening in the growth-limiting outer cell walls of the epidermis (Kutschera et al. 1987, Masuda 1990). Breakdown of xyloglucans in the outer epidermal cell walls appears to be the event that mediates auxin induced elongation in dicotyledons. At maximum elongation, non-esterified pectin is secreted into the medium (Mc Cann et al. 1994) and therefore, an entire pectic network is being replaced.

Stem elongation is extensively studied with or without hormonal influence; however, reports on root elongation are rather scanty. In the present study, cucumber (*Cucumis sativum* L. cv. Summer best) roots were studied to evaluate the influence of gibberellic acid (GA_3) and 2, 3, 5-Triiodobenzoic acid (TIBA) on various factors that regulates cell elongation. Wall-loosening enzymes like α and β- galactosidases and β- glucosidases in cytoplasmic and wall-bound fractions of GA_3 and TIBA treated roots and changes in pectic substances and xyloglucan contents were studied in relation to growth parameters.

MATERIALS AND METHODS

Preparation of plant material

Certified seeds of *Cucumis sativus* (cv. Summer best) were purchased from local seed market. Seeds of uniform size were sorted out and soaked in distilled water for 3h. The seeds were washed thoroughly and kept in dark for germination. Germinated seeds of uniform size were selected, surface sterilized and transferred to the medium. In our preliminary experiment seeds were transferred to agar gel varying in concentrations. From the results it was clear that maximum plant growth was observed with 1.5 % agar. From the different concentrations of GA_3 and TIBA used for the experiment, maximum difference in growth was observed in 275µM and 200µM, respectively. The agar medium was sterilized poured in to culture dishes. The radical of germinated seeds were inserted in to agar gel to facilitate the seedling growth. The culture dishes were kept under constant light (1000 Lux at 28 ˚C $\pm$ 2 ˚C).

Growth Analysis

Growth analysis was performed in the terms of length, fresh and dry weights of roots from different treatments. Samples were harvested after regular time interval (i.e. 12h during initial growth period and 24h in later phases); roots were separated and analyzed for length, fresh and dry weights.

Length of roots was measured to the nearest mm with the help of a scale. Nearly 20-25 replicates were taken for each data point. For the measurement of fresh and dry weights, freshly harvested roots were weighed before and after oven drying (at 80 ºC) to a constant weight. Water content of each age group was calculated as the difference between fresh and dry weights and average with standard deviation was calculated.

Preparation of plant material

Seedlings (samples) from all the treatments were harvested at regular time interval. The samples were washed thoroughly with distilled water and kept on wet filter paper. The roots were separated and crushed (in triplicate) with pre-chilled mortar and pestle using liquid nitrogen and stored at -20 ºC until use.

Preparation of enzyme extract

Frozen samples (500mg) were homogenized in pre-chilled Na-acetate buffer (50mM, pH 4.5) and centrifuged at 10,000g for 10 min. The supernatants were separated and pellets were washed twice with the same buffer. The pooled supernatants were used as source of cytoplasmic enzyme.

The residual pellets were washed with distilled water till removal of all the cytoplasmic enzymes and suspended in 10 ml 1M NaCl for 16h at room temperature. They were centrifuged at 10,000g for 10 min to separate supernatants. Pellets were washed twice with 1M NaCl and pooled supernatants used as a source of ionically wall bound enzyme.

Estimation of enzymes activities:

Activities of glycosidases were estimated according to Patel and Thaker (2004). Absorbance of yellow color developed was measured at 405 nm and was expressed as µg PNP release/organ/min. Sample crushed in triplicates and mean from six values with ± SD was calculated.

Extraction of wall component

Freshly separated roots of each developmental stage were powdered with liquid nitrogen in a pre-chilled mortar. The samples were stored at -20 °C prior to use. From each developmental stage 500 mg of material was processed as described earlier (Patel and Thaker 2004a).

Estimation of pectic polysaccharide

For estimation of pectic polysaccharide content, extract was mixed with equal volume of 5% phenol and 98 % sulfuric acid (1:2.5, v/v). The tubes were shaken thoroughly and incubated for 10 min at room temperature and for 20 min in water bath at 30 °C. Absorbance of brown color developed was measured at 490 nm (Dubois et al. 1956). Each sample was prepared in triplicate and average values with ± SD were expressed as Δ_{A490}/organ.

Estimation of xyloglucan
The reaction mixture consisted of 1 ml of extract, 250 µl I_2KI and 2 ml 15 % Na_2SO_4. Tubes were shaken thoroughly and kept for 60 min at 4°C for color development. The optical density of the resultant solution was measured at 640 nm (Nishitani and Masuda 1981). Three replicates for each sample were prepared and average values with ±SD are expressed as Δ_{A640}/organ.

Controls were prepared by adding the solution used for extraction instead of extract.

RESULTS

Growth

Changes in length of the roots in control, GA and TIBA treated seedlings are presented in Fig. 1a. In GA treatment, gradual increase in root length was observed up to about 96h and in subsequent period length stabilized while slight increase was observed in control. No significant difference in root length was observed between DW (control) and GA treated seedlings. In comparison to GA and control, TIBA showed marked inhibition in root length (Fig. 1a). Maximum root length (115 mm) was observed in control followed by GA (106 mm) and TIBA (47 mm). ANOVA performed amongst root length of control, GA and TIBA treatments showed significant difference ($P \leq 0.01$).

Changes in Dry weight of roots are recorded in Figure 1b. After a gradual increase up to 96h, dry matter accumulation nearly stabilized in control and TIBA, while GA shows continuous promotion. TIBA treatment has inhibited dry weight (63 %) and GA showed slight promotion at later part of seedling growth (Fig. 1b).

Water content was more in GA (180 mg/root) followed by control (137 mg/root) and minimum values were recorded in TIBA (31 mg/root, Fig. 1c) treatment. Values of water content of GA_3 treated roots were higher throughout the developmental stages (up to 120h) and stabilized in later stage of development. In control, increase in water content was recorded up to 96h, where as TIBA treatment showed slight increase up to 60h after which it stabilized (Fig. 1c). Difference in water content of three treatments in root was highly significant ($P \leq 0.001$). In general, length of roots showed close correlation with water content (Fig. 1a, c).

Cell wall loosening enzymes

In the present study, changes in glycosidase activity in cytoplasmic and ionically wall bound fractions of roots treated with GA_3 and TIBA was studied. Changes in cytoplasmic α-galactosidase activity are presented in Figure 2a. GA_3 treatment proved to be promotory; the activity increased gradually after 12h and attained a peak level at 96h. In control and TIBA the levels remained low and almost equal. In wall bound fraction, a clear promotion in GA_3 treatment was observed, where as TIBA showed clear inhibition (Fig. 2d).

Similarly, changes in β-galactosidase activity in cytoplasmic fractions of root are presented in Figure 2b. Initially, up to 48h activity remained higher in control and decreased in subsequent period, while in GA_3 activity was low up to 48h and increased during later period. In TIBA treatment, the activity remained low during the entire period of study (Fig. 2b).

Changes in ß-galactosidase activity in wall-bound fraction are presented in Figure 2e. In control and GA_3 treatment showed almost similar trend in the activity. The activity increased gradually after 12h and attained a peak at 72h, after which activity declined. In TIBA treatment, a clear inhibition in the activity was observed throughout the growth period.

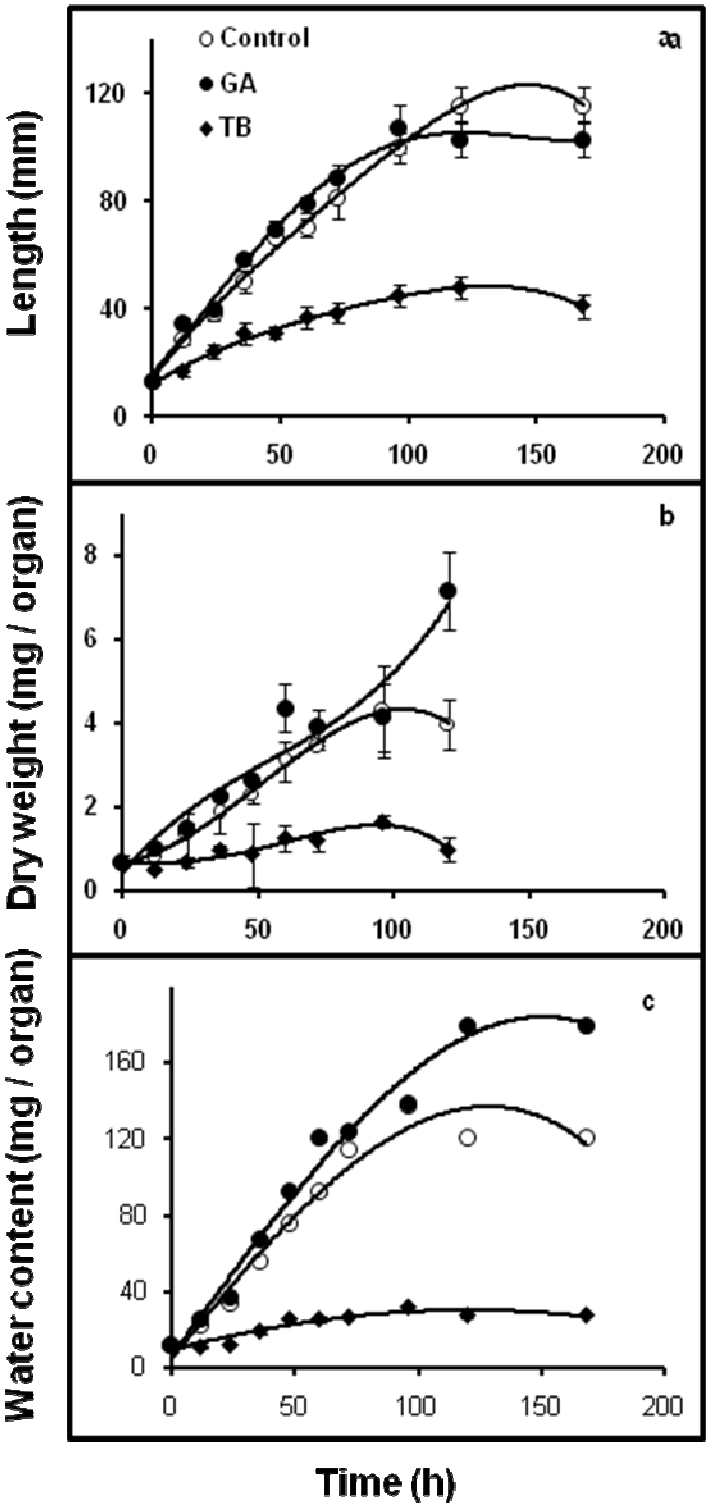
Changes in β-glucosidase activity in cytoplasmic fractions are presented in Figure 2c. In root, control showed higher activity throughout the developmental period. Initially, up to 36h, in GA treated root, the activity remained very low and increased gradually during subsequent periods. In TIBA treatment, activity was inhibited remarkably throughout the period of study. Changes in wall-bound β-glucosidase activity are presented in Figure 2f. In root, initially, up to 48 h GA_3 treatment showed more activity as compared to control and TIBA. During subsequent period in control, activity increased with a peak at 72h and declined thereafter. In GA_3 treatment, levels remained almost stable and in TIBA marked inhibition was observed (Fig. 2f).

Cell wall components

Changes in esterified and non-esterified pectic polysaccharides are presented in Figure 3 (a and b). In GA treated root, gradual increased in non-esterified pectic polysaccharide was observed with progress in root growth while in control, it remained low initially and increased sharply after 48h. In TIBA treated roots, very low levels were observed throughout the growth period (Fig. 3a). In GA treated roots, a gradual increase in esterified pectic polysaccharides was observed during the entire period of root growth (Fig. 3b). In control, initially up to 48h the

levels were low increased sharply during subsequent period, while in TIBA, very low levels of esterified pectic substances were observed through out the development period (Fig. 3b).

Fig. 1: Changes in the Length (a), dry weight (b) and water content (c) of root of cucumber seedlings against time in hours
Vertical bars denote standard deviation or are within the symbol.



Changes in the xyloglucan fractions extracted with various concentration of KOH are presented in Figure 3 (c and d). Initially the low molecular weight xyloglucan content remained low in control and TIBA as compared to GA, and increases in control and GA during later stages. However, in TIBA treated roots, it remained very low through out the period studied (Fig. 3c). High molecular weight xyloglucan content showed gradual increase in GA treated

roots, whereas in control, levels remained low initially up to 48h and increased sharply thereafter. In TIBA treated root, very low levels were recorded during the entire period of growth (Fig. 3d).

Fig. 2: Changes in the α galactosidase (a, d), β galactosidase (b, e) and β glucosidase (c, f) activities cytoplasmic (a,b,c) wall bound (d,e,f) in root of cucumber seedlings against time in hours

Vertical bars denote standard deviation.

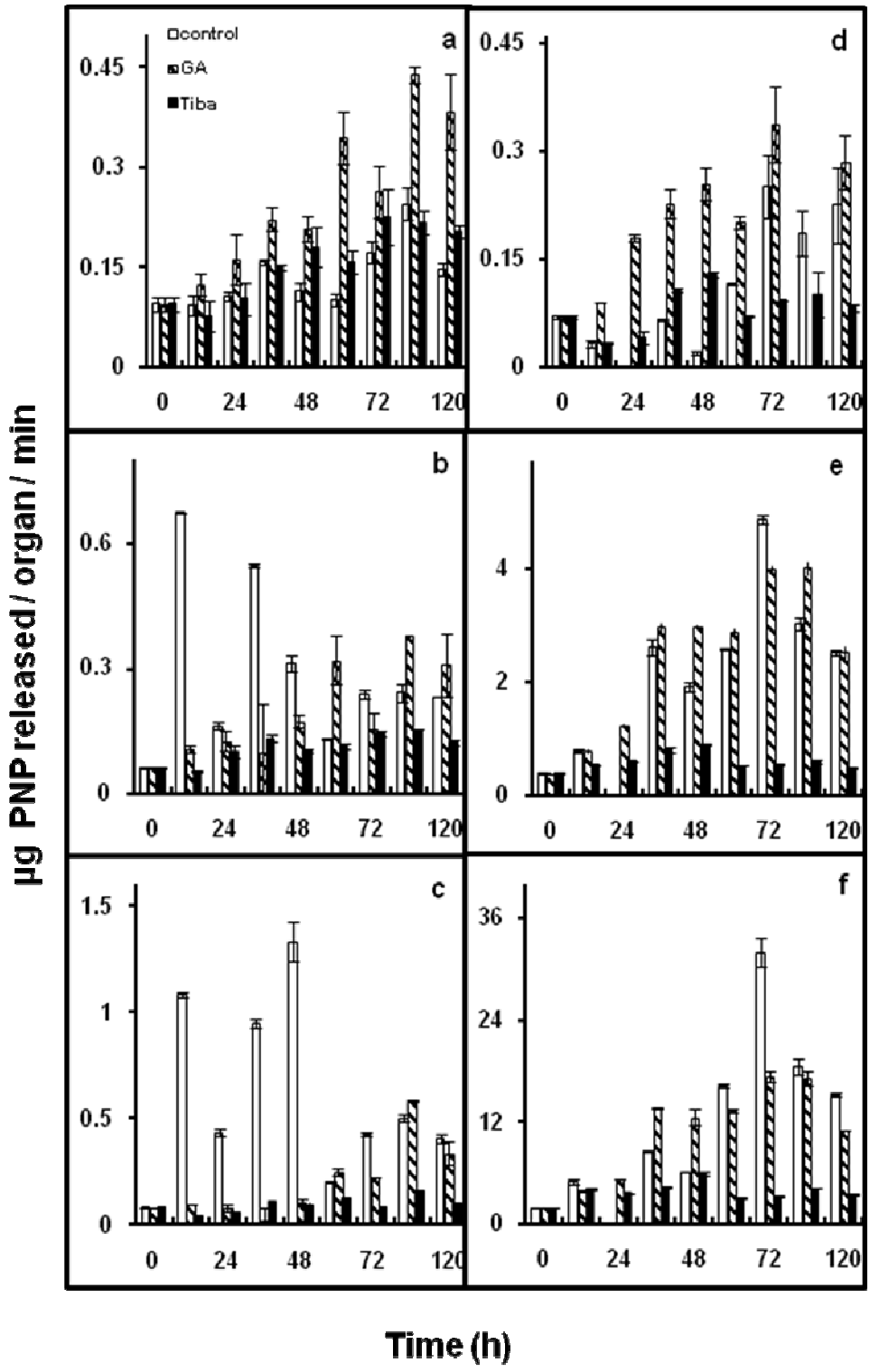
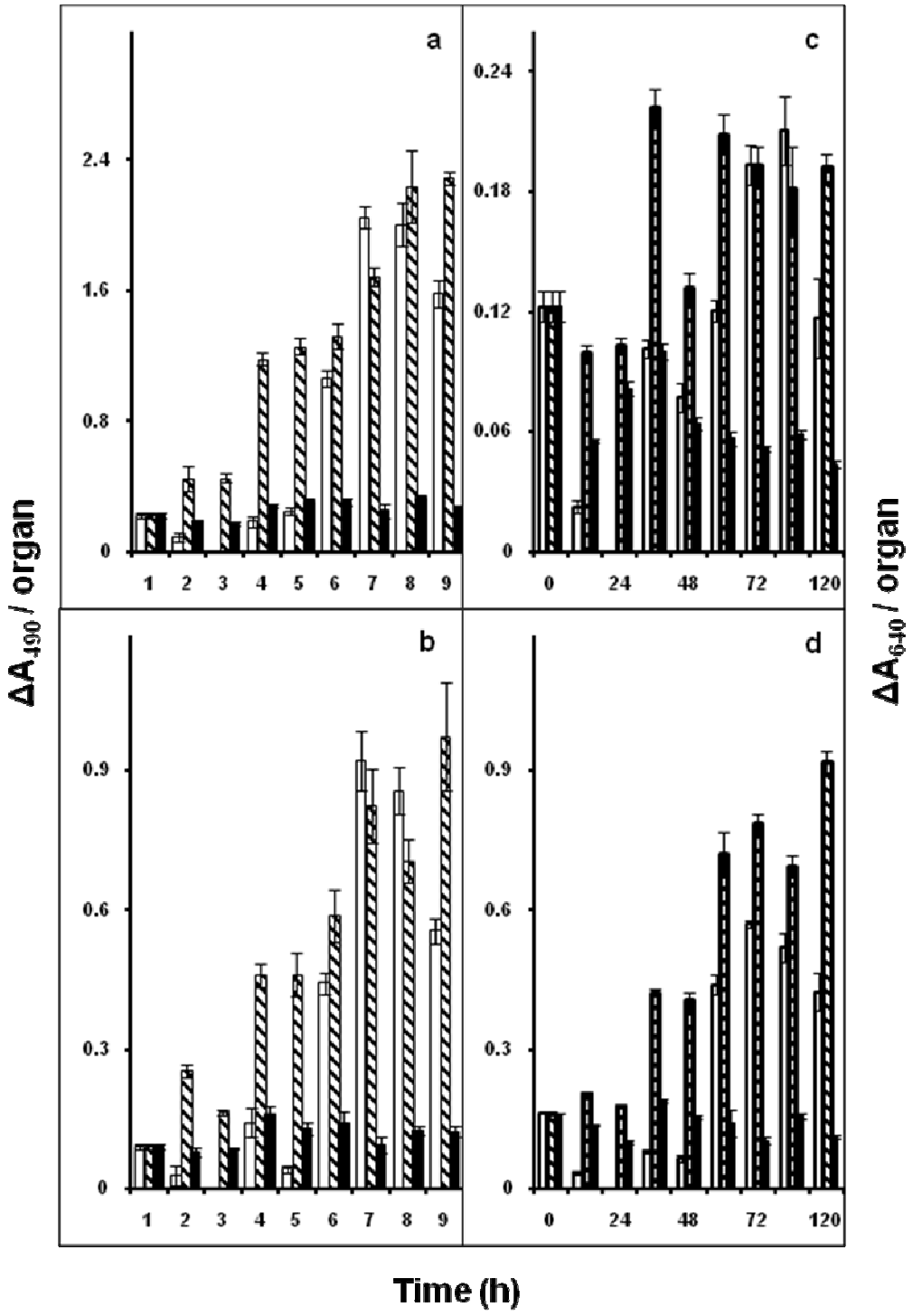


Fig. 3: Changes in the content of non esterifide (a) and esterified (b) pectic polysaccharide and low (c) and high (d) molecular weight xyloglucan contents of cucumber roots against time in hours
Vertical bars denote standard deviation.



DISCUSSION

Cell enlargement is the first physiological process to be affected when the soil water supply begins to be depleted (Nonami and Boyer 1990). In the present study, root length of control and GA_3 treated seedlings showed positive correlation with water content (Fig. 1a, c). It was observed that when plants are subjected to low Ψw, the growth of leaves and stems is rapidly inhibited (Nonami and Boyer 1990, Chazen and Neumann 1994). Continued root elongation facilitates water uptake from the soil (Spollen et al. 1993, Sharp et al. 1997).

In present study, GA treatment has increased pectic substances in root where as, a clear inhibition was observed by TIBA treatment (Fig. 3).The involvement of pectic network in controlling the metabolism of xyloglucans and on elongation growth is discussed (Carpita and Gibeut 1993). The most notable effect of GA on the cell wall composition of the fruit at harvest was the high cellulose content, which was on average 37% more than in the control fruit (Ben Arie et al. 1996). The pectin and hemicellulose component of the cell wall were also higher in the GA-treated fruit than the control (11-17%). Xyloglucan molecules that are found to be closely associated with cellulose moicrofibril as basic wall structure (Albersheim 1976) and might play a major role in cell expansion (Taiz 1984, Carpita and Gibeuat 1993).

In general, GA treated roots had higher amount of low and high molecular weight xyloglucan content as compared to control during elongation period. TIBA treated roots showed marked inhibition in low and high molecular weight xyloglucans (Fig. 3). Most xyloglucans are subjected to turnover during growth (Terry et al. 1981). These xyloglucan molecules are tightly bound to the cellulose of the cell wall (Kato et al. 1981). Further, the results suggest that TIBA has greatly reduced the enzyme activities and in contrast, GA_3 treatment has increased (Fig. 2).

Thus in general, root treated with TIBA has showed marked inhibition in length, cell wall components and enzyme activities while GA_3 has promoted both enzyme activities and cell wall polysaccharides but has no influence on the length of the root. The structure of the cell wall differs greatly amongst a variety of plant tissues and species (Masuda 1980). Hence it is possible that the rate limiting step may also differ in accordance with cell wall chemistry, and failure of an enzyme to show correlation with the rate of elongation in one plant system may not necessarily mean that it cannot act as a rate-limiting step in another. A second probability is that, wall hydrolytic enzymes might stimulate wall extension indirectly by reducing the size and viscosity of matrix polymers (Figs.2, 3) such enzymes could act synergistically to enhance the action of primary wall-loosening agents, such as expansin. Third alternative is that such hydrolytic enzymes have functions unrelated to wall loosening, e.g. in defense, in signaling (Albersheim 1974) or in polysaccharide processing of breakdown to serve cells other metabolic or energy needs (Thaker 1998). This also suggests that increased enzymes synthesis might have stimulated the release of cell wall polysaccharides; the factor(s) responsible for the elongation may be the different at least in *Cucumber* root studied.

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