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TURNOVER OF CELL WALL COMPONENTS DURING SINK DEVELOPMENT IN SEEDS OF THREE COTTON GENOTYPES

ABSTRACT

Size of the cells in developing seed is one of the major factors that regulate yield potentials. Seed of three cotton genotypes, *Gossypium hirsutum*. L cv (H-4; H-8) *G.herbaceum* L.cv (G.Cot-15) exhibits variation in their final seed size (large, middle and smaller) were studied for growth and changes in cell wall components during entire period of seed development. High and low molecular weight xyloglucan contents were extracted with 1M and 4M alkali, respectively. Initially the high and low molecular weight xyloglucan contents remained low and increased when cell size stabilized. Changes in esterified and non-esterified pectic substances were also fractionated from the cell wall of developing seed and theirs role in cell elongation phase is discussed. It was observed that bigger seed has higher xyloglucan contents than that of middle and smaller sized seed. Pectic substances were higher during elongation phase in all genotypes. The role of these polysaccharides in sink size development is discussed.

Key words: Cotton seeds, Gossypium Pectic substances, Sink size, Water amount, Xyloglucans

Abbreviation: H-4 - Hybrid-4, H-8 - Hybrid-8, G.Cot-15 - Gujarat cotton-15, DMA - dry matter accumulation, WC - Water content.

INTRODUCTION

Cotton is an important crop grown world wide in tropical and sub-tropical countries mainly for fibers and seed oil. In general, cotton fibers are extensively studied as raw materials in textile industries, cotton seed is less frequently studied. In breeding programme, the major objective is to increase yield of a crop and find out its major morphological and physiological determinants with the aim of developing new selection criteria. For the maximum yield potential, supply of assimilates (source capacity) and capacity of developing seed to continue to utilized assimilates (sink capacity) play very important role (Ho 1988). In a case, where photosynthates are not limiting, sink capacity regulates the yield potentials (Ho 1988). Sink capacity of the seed is regulated by cell numbers and cell size. A positive correlation with DMA and cell number is worked out in field grown barley (Cochran and Duffus, 1983), little is known about the cell size regulation in developing seed.

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The mechanism of cell elongation is regulated by two processes, uptake of water and cell wall turn over (Taiz 1984). The latter process can be further explained by dissemble and reassemble of cell wall components during elongation. The major components of primary cell wall are complex carbohydrates and proteins, which undergo rapid turn over during elongation. Cellulose microfibrils are linked by two groups of polysaccharide net work i.e. pectic substances and xyloglucans.

The pectic structure is elaborated by divalent cations cross-linkage and possible esterification to other cell wall polymers. In number of plant cell, wall pectic polysaccharides are a complex mixture of acidic and neutral components (Northcote 1972). They can be tightly bound to cellulose microfibrils in dicots (Seymore *et al.* 1990) or to arabinogalactans in monocots (Nishitani and Nevins 1989). The cellulose xyloglucan net work is considered to be an important load bearing structure of primary cell wall (Pauly *et al.* 1999, Whitney *et al.*, 1999); and it make–up about 20-25% dry weight of primary cell wall in dicots (Mc Neil *et al.* 1984) and about 2-5% in grasses (Kato and Masuda, 1985).

During the process of cell elongation changes in pectic polysaccharides and xyloglucan contents takes place which ultimately determine the cell size (Taiz 1984, Hayashi 1989). In this experiment, three different cotton genotypes were selected on the basis of their final seed size. An attempt is made to evaluate changes in pectic and xyloglucan components during entire period of seed development to understand their role in sink size development.

MATERIALS AND METHODS

Seeds of three cotton genotypes Gossypium hirsutum L.cv (H-4; H-8); G. herbaceum L. cv (G.Cot-15) were grown in the field. Cultural practices, including irrigation, application of fertilizers and insecticides etc., were conducted to optimize yield. On the day of anthesis, each individual flower was tagged and healthy bolls were harvested for growth analysis after the required periods. To minimize effect of environmental variations data for each set of analysis were collected from flowers that bloomed on the same day during each stage of development.

Fresh and dry weights measurement

Fibers were manually removed from the seed with a scalpel without removing the seed coat. Seeds from four locules of four bolls were used for fresh and dry weight measurement. Freshly separated seeds were weighed before and after oven drying to a constant weight at 80-90ş C.

Water amount

The water amount of seed of was determined by the difference in the fresh and dry weights at a given time. The mean dry weight and water content per tissue with ±SD (standard deviation) was calculated.

Measurement of cell size

For the measuring cell size of outer epidermal layer of he seeds, bolls of different age were collected. From the transverse section of the seed, at least 50 epidermal

cells were recorded randomly under microscope equipped with an occular-micrometer (Thaker 1999).

Extraction and estimation of wall components

Estimation of wall components was done as Breet and Waldron (1991). Freshly separated fibers of each developmental stage were powdered with liquid nitrogen in a pre-chilled mortar and stored at -20°C prior to use. From each developmental stage 500 mg of crushed fibers were suspended in 1.5% aqueous SLS (sodium lauryl sulfate) containing 5 mM $Na_2S_2O_5$, mixed thoroughly and centrifuged at 10,000 g for 10 min. The supernatant containing SLS soluble polymer of intra-cellular origin was discarded and residue was washed thrice with distilled water. The residue was then suspended in 0.5% SLS containing 3 mM $Na_2S_2O_5$, and incubated for 16 h at 2°C. The supernatant extracted after centrifugation contains cold water soluble pectic substances. The residue was again washed two times with distilled water and re-suspended in PAW (phenol, acetic acid, water, w/v/v) to remove residual proteins, lipids, adsorbed SLS and some starch. This was followed by two washes of distilled water and two washes of 90% aqueous DMSO (dimethyl sulphoxide) to remove starch from residues. For removal of adsorbed DMSO the residue was then washed six times with distilled water. The purified residue was designated as cell wall material (CWM).

Different fractions of pectic polysaccharides were extracted by using EDTA (ethylene diamine tetra acetic acid) as a chelating agent and Na_2CO_3. The CWM was stir with 0.05 M EDTA (pH 6.5) for 8 h at 20-22°C, centrifuged and washed with the same. The pooled supernatant served as source of non esterified pectic polysaccharide fraction. The residue was then stirred with 0.05 M Na_2CO_3 containing 20 mM $NaBH_4$, incubated for 16 h at 1°C and for 3 h at 20-22°C, centrifuged and washed with the same solution. Pooled supernatant was adjusted to pH 5.0 with glacial acetic acid and treated as the source sample for highly esterified pectic polysaccharide fraction. Color development procedure was adopted as Dubois *et al.* (1956). In brief, 1 cm^3 of the extract was mixed with 1 cm^3 5% phenol and 5 cm^3 98% sulfuric acid. The mixture was incubated for 10 min. at room temperature with stirring and 20 min. at 30°C in water bath. Absorbance values were measured at 490 nm.

The depectinated residue was then used for extraction of high and low molecular weight xyloglucan fractions. The residue was stirred with 1 M KOH containing 10 mM $NaBH_4$, incubated for 2 h at 1°C and for 2 h at 20-22°C, centrifuged and washed with the same solution. Pooled supernatant was adjusted to pH 5.0 with glacial acetic acid and treated as the source sample for low molecular weight xyloglucan fraction. For the extraction of high molecular weight xyloglucan fraction the residue was re-suspended in 4 M KOH containing 10 mM $NaBH_4$ for 2 h at 20-22°C, centrifuged and washed with 4 M KOH containing 3-4% boric acid. Supernatant was collected and adjusted to pH 5.0 with acetic acid. Colorimetric estimation was done by iodine staining method of Kooiman (1960) with a slight modification (Nishitani and Masuda 1981).One cm^3 of the extract was mixed thoroughly with 250 µl of I_2KI and 2 cm^3 15% Na_2SO_4. The mixture was incubated for 1 h at 4°C and optical density was measured of the resultant color solution at 640 nm.

RESULTS AND DISCUSSION

Data on dry matter accumulation water amount and epidermal cell size were fitted to appropriate polynomial equation. Best fit equation was determined by performing 't' test for different R^2 values. Changes in dry weight with age in three different genotypes are presented in Fig. 1a. In all three genotypes, a lag for nearly 10 days was observed and then entered to rapid phase of dry matter accumulation. This phase continued for 51 days and decreased there after. Maximum dry weight was recorded in H-4 (98.91 mg/seed) followed by H-8 (87.25 mg/seed) and G.cot.-15(73.45 mg/seed). Rate of dry matter accumulation was also higher in bigger seed followed by middle and short staple genotypes (Fig.1b).

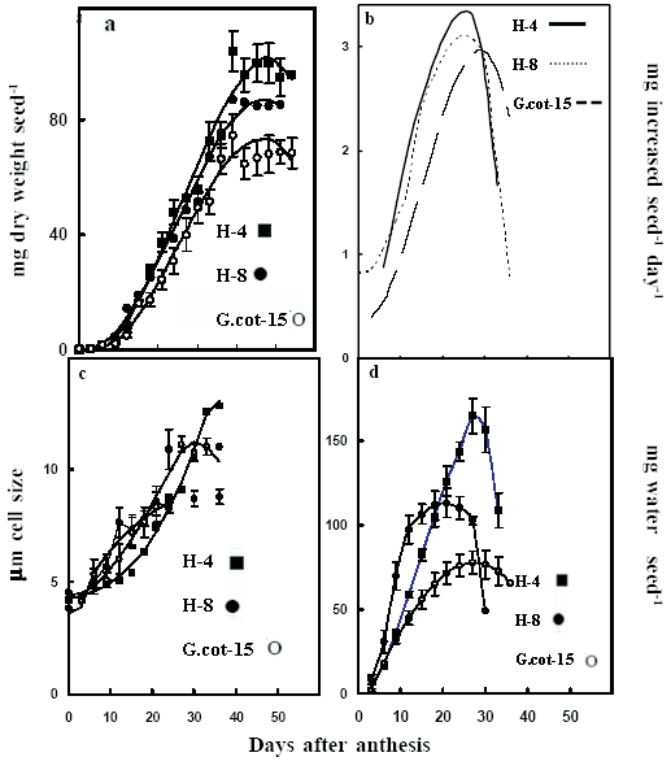

Fig. 1. Changes in dry matter accumulation (a), dry matter accumulation rate (b), cell size (c) and water amount in seeds against boll age (d)

Changes in size of the epidermal cells are presented in Fig.1c. It was observed that bigger seed had large cell size compared to middle and smaller seeds. Maximum cell size (11µm) was observed in H-4. Data on the water amount showed marked variation in three genotypes studied. H-4 had more water uptake than H-8 and G.cot.-15 (Fig. 1d). Since the increase in seed size is a primarily resultant of cell expansion, Egli (1990) concluded that the ability of cotyledon cells to take up water may play an important role in regulation of seed growth. During cell elongation turgor pressure is prereq-

uisite for drive expansion (Cosgrove 1993). A close correlation with cell elongation phase and increase in DMA is recorded (Thaker 1999).

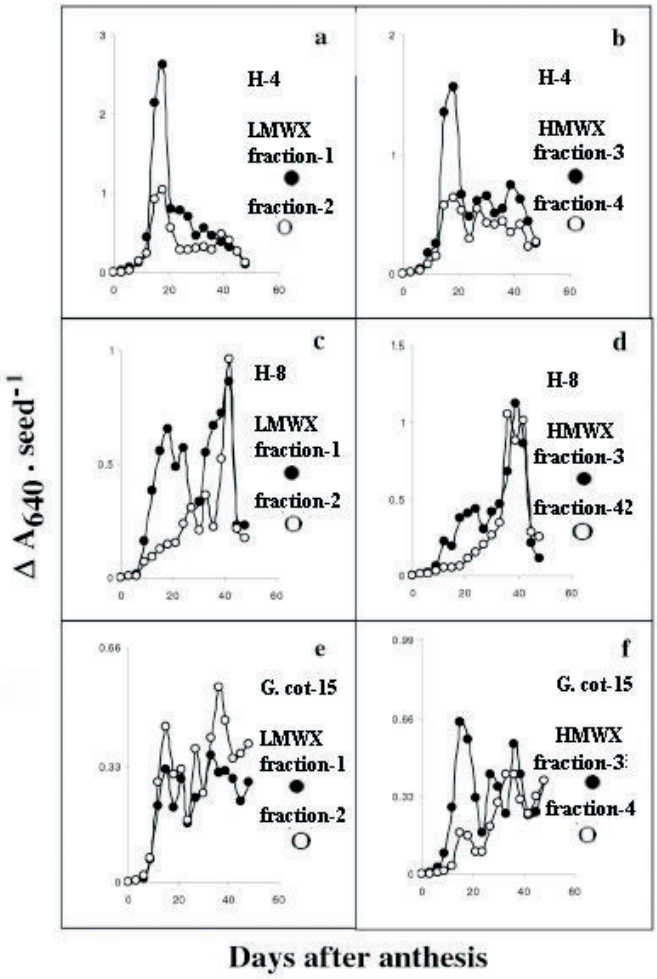

Fig. 2. Changes in low molecular weight xyloglucan (LMWX) fractions (1 and 2) and high molecular weight xyloglucan (HMWX) fractions (3 and 4) for H-4 (a ,b), H-8 (c, d) and G. cot-15 (e, f) respectively

Parallel to the process of water uptake by the cell, changes in chemical structure of cell wall polysaccharide underlie the process of cell wall loosening (Taiz 1984). The cellulose xyloglucan network is considered to be an important load-bearing structure of primary cell wall (Carpita and Gibeant, 1993). However, most of the xyloglucan in a tissue is firmly bound to the cell wall and can not be extracted by any treatment that extracts polysaccharide efficiently (Brett and Waldron, 1991). Therefore, depectinated cell wall was treated with dilute (1M) alkali for solublizing xyloglucan of low molecular weight are presented as fraction 1and 2, respectively (Fig.2a,c,e): while concentrated (4M) alkali extracted high molecular weight xyloglucan presented as fraction 3 and 4, respectively (Fig. 2b,d,f). Xyloglucan contents were remained low in all four

fractions up to 10 days in all three genotypes. In H-4, it increased gradually up to 18 days and declined thereafter (Fig.2 a b), in subsequent periods, very low contents were observed. In other two genotypes, the levels remained low during cell elongation phase and showed increasing trend at later stages. Thus both, low and high molecular weight xyloglucan contents showed low levels during cell elongation phase of seed in all three genotypes. Specific biochemical modifications of cell walls, such as adjustment in molecular weight and quantities of cell wall polysaccharides, are likely to be involved in physical process of cell wall loosening (Terry *et al.*, 1981). Similar inverse correlation with elongation growth of *Phaseolus* hypocotyl and xyloglucan contents is reported (Bagatharia and Chanda 1998; Patel and Thaker 2004). The universality of xyloglucan occurrence in the primary cell wall of higher plants and notable decrease in xyloglucan

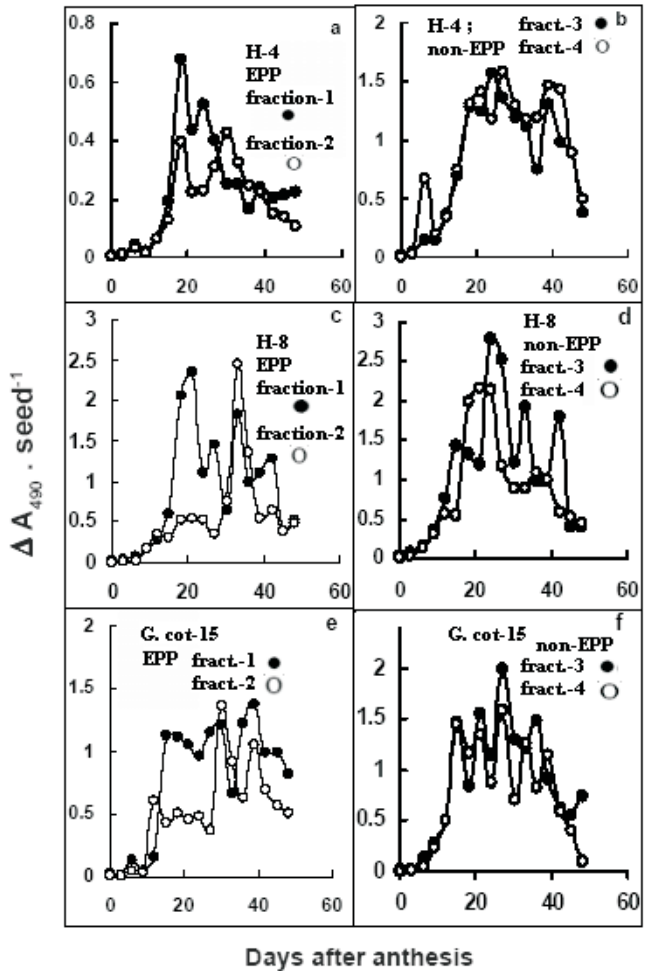

Fig. 3. Changes in esterified pectic polysaccharides (EPP) fractions (1 and 2) and non-esterified pectic polysaccharides (non-EPP) fractions (3 and 4) for H-4 (a ,b), H-8 (c, d) and G. cot-15 (e, f) respectively

contents during growth indicates its importance in cell wall metabolism (Nishitani 1998, Thompson and Fry 2001).

Nishitani and Masuda (1981) reported enhance degradation of arabinogalactan in cell wall loosening. At maximum elongation, it appears that the unesterified pectin is secreted into the medium (McCann *et al.*, 1994) and that therefore, an entire pectic net work is being replaced. Changes in the amount of pectic polysaccharides fractions/ seed are presented in Fig.3. Pectic fraction 1 to 4 showed nearly similar trend. H-8 had higher values in comparison to other two genotypes. In all genotypes, pectic fraction showed increasing trend with elongation growth. In H-4 esterified fraction increased during elongation phase and declined till maturity (Fig.3a). While non-estrified fractions, increased during elongation phase and stabilized from 24-42 days and declined thereafter (Fig.3b). In H-6, both esterified and non-esterified pectic fractions remained higher upto 21-24 days and declined thereafter (Fig.3c,d). In G-Cot-15, increasing trend was observed up to 18 days, and stabilized in subsequent periods. It declined during maturity from 42 days (Fig.3e,f). The synthesis and degradation of polysaccharides in cell wall are requires in elongation process (Nishitani and Masuda 1981; McCann *et al.*, 1994). In pea, effect of GA and IAA induced expansion showed increased in the synthesis of polysaccharides in the cell wall

In conclusion, inverse correlation with xyloglucan content and direct correlation with pectic substances and cell size in seeds of three cotton genotypes suggest that turnover of these wall polysaccharides may play an important role in sink size development.

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