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Sumitra V.Chanda

Department of Biosciences, Saurashtra University, Rajkot 360 005, India,
E–mail: sumitrachanda@yahoo.com

CHANGES IN CELL WALL POLYSACCHARIDES DURING WHEAT GRAIN DEVELOPMENT

ABSTRACT

Changes in low and high molecular weight xyloglucan and pectic polysaccharide content were estimated during grain development in wheat (*Triticum aestivum*) cultivar Lok–1. Three grains differing in their final dry weight and position on the spike were selected for the investigation. The low molecular weight xyloglucan and high molecular weight xyloglucan were isolated by extracting in 4% KOH and 24% KOH, respectively. Changes in the xyloglucan content (low and high) showed an inverse correlation with water content. However, low molecular weight xyloglucan was more than high molecular weight xyloglucan at all stages of grain growth. Pectic polysaccharides also showed an inverse correlation, with highest content being in the smallest grain and vice versa. In–crease in xyloglucan and pectic polysaccharide content coincided with termination of elongation growth thus suggesting its probable role in wall rigidity. It is suggested that cell wall is a dynamic compartment where reorganization occurs by turnover and alteration of wall polysaccharides.

Key words: cell wall, grain development, pectic polysaccharides, *Triticum aestivum*, xyloglucan

INTRODUCTION

Hormone induced increases in wall extensibility are unequivocal and considered by many as the rate limiting factor in expansion, but cell wall synthesis is indeed necessary for continuous plant growth (Edelmann *et al.*, 1989). The primary cell wall is subjected to continuous develop–mental processes that govern cell size, shape and function (Cassab and Varner, 1988). Work of Shedletzky *et al.* (1992) revealed that walls can be modified to a remarkable degree and still maintain structural integ–rity.

Auxin induces cell elongation in plant tissues and its mechanism has long been investigated. Rheological analyses of the cell wall have re–vealed that auxin induced enlargement of a plant cell results from in–crease in irreversible extensibility of its cell wall which permits the wall to be deformed by the cell's turgor pressure. In fact, cell elongation in

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plants is due to an irreversible yielding of cell wall to stress produced by cell turgor which appears as a result of biochemical modification of cer–tain components of the cell wall. Plant cells are thought to regulate their expansion during growth by adjusting the mechanical properties of their walls (Fry, 1989).

Plant cells rearrange the load–bearing network in their walls to re–duce wall stress and cell turgor pressure, thereby enabling the cells to take up water and extend the wall. Physical and chemical evidence points to matrix polymers as the site of these wall rearrangements. The primary cell wall is composed of at least two independent polysaccharide networks. The cellulose–xyloglucan network is considered to be an im–portant load bearing structure of the primary cell wall. Pectic polysaccharide network fills in the gaps of this network and may be it controls the porosity of the cell wall (Carpita and Gibeaut, 1993).

Xyloglucans are the hemicellulosic polysaccharides that account for 20–25% of dry weight of the primary cell wall in dicots and 2–5% in Poaceae (McNeil *et al.* 1984, Hayashi 1989). The xyloglucans all possess a 1,4–β–glucan backbone with 1,6–α–xylosyl residues attached to the 6–position of β–glucosyl residues. Species specific differences occur ac–cording to the distribution of additional branching galactosyl or fucosyl–galactosyl residues. The 1,4–β–glucan backbone can bind spe–cifically to cellulose microfibrils by hydrogen bonding and extends the *in vivo* xyloglucan–cellulose network. Fucose–binding lectins and anti–bodies raised against xyloglucan hepta– and octasaccharides suppress the auxin–induced elongation and cell wall loosening as well as break–down of xyloglucan (Hoson *et al.* 1991). This hypothesis further suggests that the breakdown of xyloglucan is associated with the wall loosening that is responsible for the auxin induced cell elongation.

Several reports suggested that xyloglucans play a major role in cell wall extension (Fry, 1989; Carpita and Gibeaut, 1993 and references therein). The mechanism was hypothesized to be associated with xyloglucan degradation by auxin induced endoglucanases (Hayashi, 1989; Fry 1989). On the other hand, pectins seem to play an important role in cell wall extensibility. Pectins are a highly heterogeneous group of polymers including poly galacturonic acids, rhamnogalacturonan I and rhamno– galacturonan II. The latter one is a highly branched, complex polysaccharide which has been found in the primary cell walls of all higher plants so far examined (Thomas *et al.*, 1989). These polyanionic galacturonan backbones are able to bind Ca ions resulting in their aggregation and the formation of gels (Jarvis, 1984). The esterification and de–esterification is correlated with elongation growth (Kim and Carpita, 1992). Several authors (Carpita and Gibeaut, 1993 and references therein) suggested the involvement of the pectin matrix in controlling the metabolism of xyloglucan and therefore elongation growth.

The complex process of cell elongation is mediated by a series of meta–bolic events co–ordinated within the wall matrix. Some of the factors re–sponsible for controlling grain weight in wheat have already been worked out (Chanda and Singh, 1997; 1998). In the present work, changes in pectic polysaccharides and xyloglucan (low and high) content are estimated during the entire period of wheat grain development.

MATERIAL AND METHODS

Seeds of wheat (*Triticum aestivum*) cultivar Lok–1 were sown in a farmer's field in black cotton soil (vertisol). The experimental plot was ploughed and layered with farmyard manure. At the time of sowing it was fertilized with 10 g × m^{-3} of diammonium phosphate as a basal dose. A plant density of 60 plants × m^{-2} was maintained. There were prepared 15 rows, 20 m long and 0.3 m apart . After 18 days, the plants were thinned and all unwanted plants were hand removed. Two doses (one after 30 days and another after 70 days) of fertilizer urea (10 g × m^{-3}) were added. Anthesis took place after 51 days of sowing. On the day of an anthesis, the main tiller spikes with 13 spikelets were tagged. The tagged spikes were harvested every 3–4 days after anthesis until maturity for growth and wall component analysis. Three grains were selected as stated and justified earlier (Chanda and Singh 1997); i.e. apical grain of 4^{th} spikelet named as grain 1 (G 1), basal grain of 8th spikelet named as grain 2 (G 2) and apical grain of 12^{th} spikelet named as grain 3 (G 3).

Growth analysis

Growth analysis was done exactly as described earlier (Chanda and Singh 1998). On the day of analysis, the tagged spikes were harvested and brought to the laboratory. Ten times more than the required num–ber of spikes were harvested and grains of the same morphological shape and size were separated from the respective florets. The fresh weight of 10 grains was individually taken and subsequently they were dried to a constant weight at 65˚C and then dry weight was measured. The difference in these two weights gave the water content (mg/seed) at each time.

The grain dry weight data was fitted to polynomial functions and the selection of the appropriate polynomial regression was made statisti–cally by the 'lack of fit' method.

Extraction of pectic polysaccharides

After separation of the grains from the florets, required number of them was taken in a Petri dish, immediately their fresh weight was taken. The grains were then killed by boiling in methanol for 5 min and then stored in methanol. At the time of analysis the methanol boiled segments were homogenized in ice cold water and centrifuged at 10,000

g for 10 min. The supernatant containing soluble enzymes was discarded. Several such washes (about 20) were done till all soluble enzymes were removed. The pellet was then treated with 1 M NaCl for 1 h (2 times) to remove bound enzymes. The pellet thus obtained was washed successively with ice cold water, acetone and methanol–chloroform mixture (1:1 v/v) and finally air dried at room temperature. The dried wall pellet was treated with 15 ml of dimethylsulfoxide (DMSO) for 12 h, to remove starch. After DMSO treatment, the cell wall pellet was extracted 3 times with 20 mM ammonium oxalate–oxalic acid buffer solution (pH 4.0) at 70˚C for 1 h each to remove pectic polysaccharides. The supernatant of all the washes was collected together, mixed and the volume made up to 30 ml or as required. This was the source of pectic polysaccharides.

Extraction of hemicellulosic xyloglucans

The hemicellulosic xyloglucans were fractioned into low and high molecular weight xyloglucans. The pectin free wall pellet was extracted 2 times with 4% KOH solution (2 h each time)to obtain low molecular weight xyloglucans. Then the residue was extracted with 24% KOH solution (24 h) to obtain high molecular weight xyloglucans. Each alkali extract was acidified (pH 5.0) with 5% and 33% acetic acid respectively. The acidification caused no precipitation of hemicellulosic polysaccharides.

Determination of total polysaccharides and xyloglucan content

The total polysaccharide content in pectic fraction was determined by the phenol sulfuric acid method (Dubois *et al.* 1956). 1.0 ml of extract was mixed with 1.0 ml of 5% phenol and 98% sulfuric acid with constant stirring. After 10 min, the tubes were placed in a water bath at 30˚C for 20 min. The yellow orange colour developed was read at 490 nm. The xyloglucan content were determined by iodine staining method of Kooiman with a slight modification (Kooiman 1960, Nishitani and Masuda 1981). 1.0 ml of acidified extract was mixed with 0.25 ml of aqueous solution containing 0.5% I_2 and 1.0% KI and 2.0 ml of 15% sodium sulfate. The reaction mixture was then kept in dark for 1 h at 4˚C. The bluish–green colour developed was read at 640 nm.

RESULTS AND DISCUSSION

There are many examples in literature which indicate that there are three processes that are important in cell elongation and continuous plant growth viz. cell wall loosening, cell wall synthesis and appropriate microfibril orientation (Adams *et al.*, 1975; Mendu and Silflow, 1993; Montague, 1995). In the present work changes in wall components is looked into.

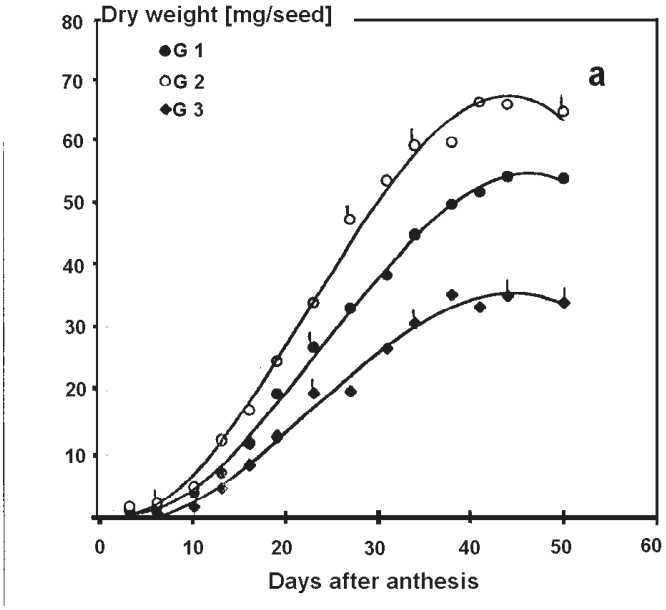

Fig.1a Grain growth curves predicted from the cubic polynomial regression equation and actual mean grain weight of three representative grains. G 1 is apical grain of 4th spikelet, G2 is the basal grain of 8th spikelet and G3 is apical grain of 12th spikelet. Vertical bars represent ± standard deviation and wherever they are absent they are within the symbol

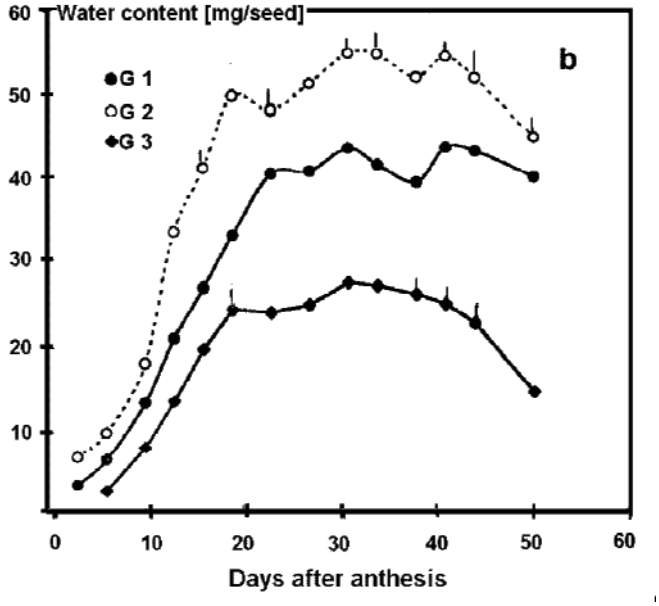

Fig. 1b The weight of water content in developing wheat grains, during the entire period of grain growth

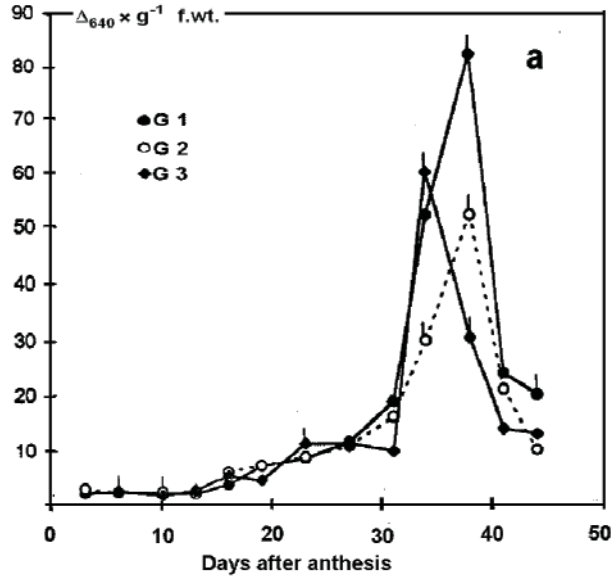

Fig. 2a Changes in high molecular weight xyloglucan content in the three developing wheat grains during the entire period of grain growth. Other details as per Fig. 1

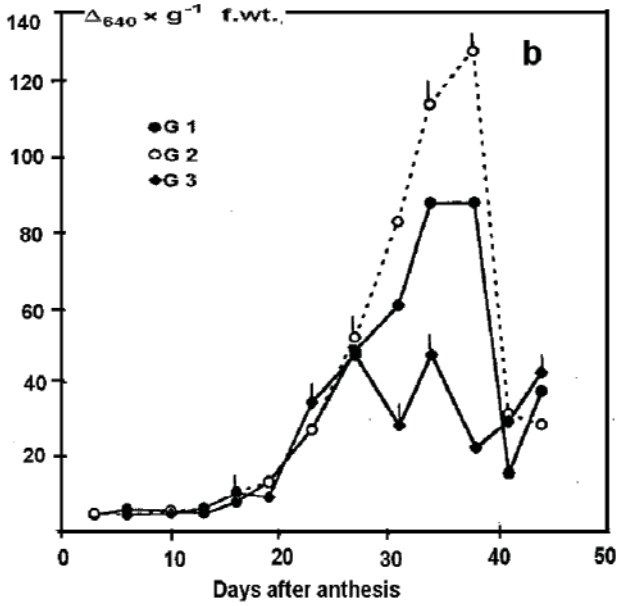

Fig. 2b Changes in low molecular weight xyloglucan content in the three developing wheat grains during the entire period of grain growth. Other details as per Fig. 1

The grain dry weight data was fitted to a polynomial equation and the best fit equation was determined statistically. A cubic polynomial adequately described the grain dry weight data of all the three grains (Fig. 1a; r = 0.99). In several crop species grain growth curves showed sigmoidal patterns (Sofield *et al.*, 1977; Chanda and Singh, 1997). In general, the dry matter accumulation showed an initial lag phase up to 10 days post anthesis. This was followed by a phase when grain dry matter increased linearly, and subsequently there was a slight decline in seed dry weight. The grains attained maximum dry weight around 44 days after anthesis. Amongst the three grains, grain 2 recorded maximum dry weight and was followed by grain 1 and grain 3 respectively.

Changes in water content per grain are shown in Fig. 1b. Lowest water content was on day 3. It gradually increased up to 31 days and subsequently the water content almost remained constant and after 40 days it declined. In this case also, grain 2 always had maximum water content and grain 3 minimum. Similar pattern of dry weight and water content in wheat has already been reported (Chanda and Singh, 1998). During the grain development, the water concentration declines steadily, causing differences in grain growth rate and final grain weight (Egli, 1990). The final weight of a wheat grain is dependent upon endosperm cell number (Cochrane and Dufffus, 1983); however much of the increase in grain size during development is associated with increase in grain volume, as cell division is usually completed early in grain development (Guldan and Brun, 1985). This increase in cell volume requires a net uptake of water and it has been suggested that seed water status may play an important role in regulating seed development (Egli, 1990). Our earlier work (Chanda and Singh, 1998) also suggests that cell enlargement is an important factor in controlling grain weight in wheat.

The cell elongation is a process, which is promoted by high concentration of xyloglucan oligosaccharides. The promotion results in a decrease in cell wall viscoelastic properties. Xyloglucan is degraded into oligosaccharides in growing plant cell wall and these oligosaccharides provide either positive or negative feed back control during cell elongation. Potential enzymes involved in xyloglucan solubilization may be expansin, xyloglucanase, xyloglucanendotransglycosolase and cellulose.

Most of the xyloglucan in a tissue is firmly bound to the cell wall and cannot be extracted by any treatment that extracts pectic polysaccharides. Strong alkaline solution is required to dissolve xyloglucan from cell wall preparations, dilute alkali extracting low molecular weight xyloglucan while concentrated alkali extracting higher molecular weight xyloglucan. In the present study, hemicellulosic xyloglucan were fractioned into low molecular and high molecular weight xyloglucans by extracting with 4% KOH and 24% KOH respectively.

Changes recorded in high molecular weight xyloglucan content are as shown in Fig. 2a. The xyloglucan content was very low in all the 3 grains. This low content was recorded till d 19 after which there was

a slight increase in the content till d 30 post anthesis. After 30 days, the content increased significantly in all 3 grains. The high molecular weight xyloglucan content was low during cell division and elongation phases which subsequently increased till d 38. The maximum content was found at 38 days (dry matter accumulation and maturation phases) i.e. high molecular weight xyloglucan content showed an inverse correlation with the water content of the grains. Thereafter the content drastically decreased.

Changes in low molecular weight xyloglucan content in all the three grains are presented in Fig. 2b. The low molecular weight xyloglucan content was low till d 19 in all the three grains which gradually increased and showed increasing trends with the development of the seed. Here also the content was maximum between 30 – 40 days and decreased thereafter; thus showing an inverse correlation with the water content of the grains. Though both high and low molecular weight xyloglucan content showed inverse correlation with water content of the grains, the low molecular weight xyloglucan content was more in all the grains than high molecular weight xyloglucan.

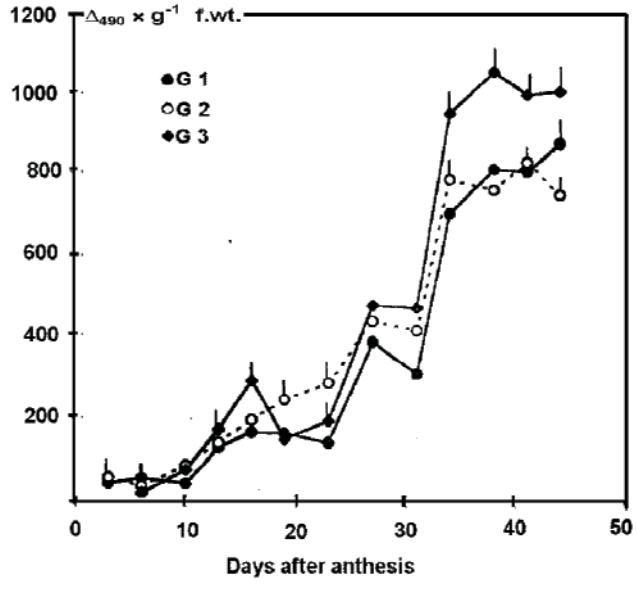

Fig. 3 Changes in pectic polysaccharide content in the three developing wheat grains during the entire period of grain growth. Other details as per Fig. 1

Xyloglucan appears to function as a cementing matrix material which contributes cross–links and rigidity to the cell wall. The results are similar to those found with auxin oxidizing system where it was shown that bound peroxidases restricted elongation growth of wheat grain partly due to the formation of diphenyl cross–links while IAA oxidase regulated growth by limiting the concentration of IAA (Chanda and Singh,

1997). Hoson and Masuda (1989) demonstrated that the antibodies and lectins specific for xyloglucan inhibited the IAA–induced elongation, cell wall loosening and decrease in molecular weight of xyloglucan. This in–dicates that changes in molecular weight of these polysaccharides are associated with the cell wall loosening process which leads to the elon–gation growth. The metabolism of xyloglucan is undoubtedly regulated by different processes since it is subject to turnover during growth (Labavitch and Ray, 1974). Xyloglucan in the cell walls could be a major component that contributes to wall rigidity.

Modification of pectic polysaccharides has also been implicated in cell wall loosening. In *Vigna* epicotyl segments, degradation of arabinogalactan has been demonstrated by Nishitani and Masuda (1981). Changes in pectic polysaccharides in the present study are shown in Fig. 3. The content in all the three grains was low on d 3. The pectic polysaccharide content thereafter gradually increased and showed increasing trends with the development of the grain. Maximum pectic polysaccharide content was during dry matter accumulation and maturation phases. At maturity, the smallest grain had maximum pec–tic polysaccharide content while the biggest grain had lowest content.

It is argued that the primary cell wall is not a simple sink for various polysaccharide components, but rather a dynamic compartment exhib–iting long–term reorganization by turnover and alteration of specific polymers during development.

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