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CHANGES IN CELL WALL POLYSACCHARIDES DURING SUNFLOWER SEED DEVELOPMENT

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Summary. Developing sunflower (*Helianthus annuus* L.) seeds were analyzed for their fresh and dry weights, water content and wall components viz. low and high molecular weight xyloglucan and pectic polysaccharides. Seeds from two rows (6th and 12th) differing in their final dry weight and position on the capitulum were selected for the investigation. The dry weight of seed from 6th row was 69 mg while that of 12th row was 55 mg. Thus a clear effect of position was observed. Seed having higher dry weight had higher water content and vice versa. The xyloglucans were fractioned into low and high molecular weight fractions by extracting them in 4% and 24% KOH, respectively. Though the level of low molecular weight xyloglucan fraction was more than that of high molecular weight xyloglucan, both showed an inverse correlation with water content. Pectic polysaccharide content was maximum at the beginning of seed growth which decreased as the seed matured thus showing an inverse correlation with water content. It is suggested that pectic polysaccharides may be involved in cell wall loosening and xyloglucan may be a major component that contributes to wall rigidity.

Keywords: seed development, xyloglucan, pectic polysaccharides, *Helianthus annuus*

Introduction

Increased understanding of the growth and development of a crop species can help to identify critical periods during which yield limits are irreversibly set. Selection in a breeding programme for physiological traits will likely provide improvement in crop performance only if those traits tend to set yield limits during a critical period. Yield

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of a grain crop is a function of the production of assimilate by photosynthesis, translocation of the assimilates to reproductive sinks, and their utilization by the developing seeds for production of the storage material giving its economic value.

Weight per kernel, an important component of yield in crop plants, may be limiting in some genotypes or environment. Final kernel weight is a function of kernel growth rate and duration. In wheat, considerable differences in grain weight within a wheat ear as well as among cultivars have been reported (Bremner and Rawson, 1978; Chanda and Singh, 1997); the same is observed in sunflower. Sunflower is the most important oil crop in the world and ranks second among oil seed crops as a source of edible oil. There is considerable difference in seed weight from the peripheral row to the inner row of the capitulum. However, the physiological and biochemical basis of this difference is not known. Seed weight is a decisive yield factor and differences in this character between cultivars are frequently associated with differences in yield. Therefore in order to maximize the yield potential of sunflower, there is a need to understand various biochemical aspects of metabolism during seed development. A major factor determining the yield in sunflower as in other crops, is the potential of the seed to grow and accumulate assimilates.

Plants increase in size mostly by increasing cell water content (Boyer, 1988). Cell growth, an irreversible increase in cell volume, can occur by expansion or by elongation. During cell enlargement existing cell wall architecture must be modified to allow incorporation of new material, thus increasing the surface area of the cell inducing water uptake by the protoplast (McCann and Roberts, 1994).

The composition and properties of the plant cell walls are dynamic and variable in response to plant growth and development. The major hemicellulose is xyloglucan which typically make up 20–25% of the total dry weight of the cell wall (Hayashi, 1989). Chemical extraction of xyloglucan from the cell wall requires conditions such as strong alkaline solutions, which disrupt the crystalline structure of the cellulose microfibrils. The length of individual xyloglucan molecule (30–400 nm) generally exceeds the distance between lamellae many times over, which suggests that these polysaccharides can interconnect several microfibrils (McCann et al., 1992).

Many studies have demonstrated that an extensive turnover of cell wall polysaccharides occur during cell elongation in higher plants. However, the type of wall components whose structure is modified is different in monocots and dicots probably because of the differences in the matrix composition.

In the present work, changes in wall components, i.e. pectic polysaccharides and xyloglucans were studied during the entire period of sunflower seed development.

Material and Methods

Seeds of sunflower (*Helianthus annuus* L.) were sown in a farmer's field in black cotton soil (vertisol). The experimental plot was ploughed and layered with farmyard

manure. Cultural practices including irrigation and fertilizer application was done to maximize plant growth. Eight rows, 30 m long and 1.0 m apart were prepared. Thirty days after sowing, urea was added at the rate of 6 gm^{-3}. After a month another similar dose of urea fertilizer was added. Fertilization in sunflower starts from peripheral row of the capitulum. Every day 2–3 rows are fertilized. Seed setting occurs completely within 7–8 days. The day the peripheral row is fertilized, the flowers are tagged and this is considered as zero day. The rows from peripheral are numbered as 1, 2, 3, etc. In all there are about 24 rows.

Growth analysis

On the day of analysis, 3 to 4 capitula were cut, put in polythene bags and brought to the laboratory. Seeds from each row were separated and 50 seeds of similar size were selected for growth and biochemical analysis. For growth 25 seeds were selected, fresh weight of each individual seed was measured and subsequently they were dried in an oven at 65°C to constant weight and measured. The difference in these two weights gave the water content (mg/seed) at each time.

Cell wall isolation

Walls were isolated and fractionated according to the slightly modified method of Nishitani and Masuda (1981). The number of seeds varied from 10–100 depending on the age of the seed; approximately 500–600 mg material was taken for the estimation of wall components. The required number of seeds were selected and their fresh weight taken. Immediately the seeds were put into about 10 ml of methanol and boiled (in methanol) for 5 min. They were cooled to room temperature and then stored in methanol till the time of analysis.

Cell wall fractionation

At the time of analysis, the methanol stored seeds were homogenized in ice cold water and centrifuged at 10 000 g for 5 min. The supernatant which contained cytoplasmic enzymes was discarded. The pellet was washed many times (nearly 12–15 times) with distilled water till pellet was free from cytoplasmic enzymes. After washing of pellet 10 ml of 1 M NaCl was added for 1 h to remove wall bound enzymes. This manipulation was done twice. After centrifugation, supernatant was discarded and pellet was washed successively with ice cold water, acetone and chloroform–methanol mixture (1:1 v/v). This was also done twice after which the pellet was air dried at room temperature. The dried pellet was treated with 15 ml of dimethyl sulfoxide for 18 h. This treatment dissolved starch, and it also resulted in more facile extraction of pectic substances (Wada and Ray, 1978). After removing dimethyl sulfoxide by centrifugation, the pellet was extracted 3 times with 20 mM ammonium oxalate–oxalic acid buffer

solution (pH 4) at 70ºC for 1 h to remove pectic polysaccharides. The material was stirred every 10 to 20 min to prevent accumulation of wall material at the surface of the solution. The supernatant of all the 3 washes was collected together, mixed and the volume was made up to 20 ml. This was the source of pectic polysaccharides (Nishitani and Masuda, 1981).

Extraction of Xyloglucan

The hemicellulosic xyloglucans were fractioned into low and high molecular weight xyloglucans. The pectin free wall pellet was extracted two times with 4% KOH solution (2 h for each extraction) to obtain low molecular weight xyloglucans. The residue was then extracted with 24% KOH solution (for 24 h) to obtain high molecular weight xyloglucans. Each alkali extract was acidified (pH 5) 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 contents in pectic fraction was determined by the phenol sulfuric acid method (Dubois et al., 1956) and expressed as ΔA_{490} g fresh weight^{-1}. For analysis 0.5 ml of the extract was mixed with 0.5 ml of 5% phenol and 2.5 ml of 98% sulfuric acid with constant stirring. After 10 min, the tubes were placed on a water bath at 30ºC for 20 min. The yellow orange colour developed was read at 490 nm. The xyloglucan contents were determined by iodine staining by the modified method of Kooiman (1960) and Nishitani and Masuda (1981). Acidified extract (1.0 ml) was mixed with 0.25 ml of KI.I_2 solution (0.5% I_2 and 1% KI). The reaction mixture was then kept in dark for 1 h at 4ºC. The bluish-green colour developed was read at 640 nm.

Statistical analysis

The seed dry weight data was fitted to polynomial equation of different degrees and the best fit equation was determined statistically (Nicholls and Calder, 1973). A cubic polynomial was the best fit. The equation was of the following type:

$$Y = a + bx + cx^2 + dx^3$$

where: Y = seed dry weight, x = days after anthesis, *a*, *b*, *c* and *d* are regression coefficients.

Results and discussion

Growth of seed from flowering to maturity results from the integration of a wide range of morphogenetic and biochemical processes. In the present work, the weight of the

seeds in each row (of the capitulum) was almost same but it differed from row to row. From peripheral row to inner row, the seed number as well as seed weight decreased. In other words, the outer row had maximum number of seeds and seed weight was almost maximum. A clear effect of position of seed on capitufum was observed. Growth and biochemical analysis of seeds from every 3rd row was done but data of seeds only from the 6th and 12th row are given in this paper. Seeds of 6th row are designated as seed 1 and that of the 12th row as seed 2. In several crop species grain growth curves showed sigmoidal patterns (Sofield et al., 1977; Chanda and Singh, 1997). Cubic polynomials are used to describe the relationship between grain weight and days after anthesis. The cubic polynomial adequately described the seed dry weight of both seeds (Fig. 1a). This is in agreement with the results of Gebeyehou et al. (1982) and Chanda and Singh (1998) on wheat, Rabadia et al. (1999) on cotton ovule and Thaker (1999) on Hibiscus seed. The dry weight of seeds from both rows was low up to the 7th day. It increased linearly up to 31 days after anthesis. Maximum dry weight was attained on day 35 and subsequently a steady state was observed. All the time seed 1 had higher dry weight than seed 2. At maturity the dry weight of seed 1 was 69 mg and that of seed 2 was 55 mg. A clear effect of position was observed. The dry weight of the seeds from most inner row was very reduced (data not shown).

The water content was low initially, i.e. 3 days after anthesis but started steadily increasing and attained maximum content on 18th day in both type of seeds (Fig. 1b), subsequently the water content went on steadily decreasing reaching to very low levels by 40th day. The decrease was almost same in both type of seeds. All the time, seed 1 had more water content as compared to seed 2. Maximum water content was attained earlier than the attainment of maximum dry weight. Similar pattern of water uptake has been reported earlier in wheat (Martinez-Carrasco et al., 1988, Chanda and Singh, 1998). It has already been established that the ability of seed to continue water uptake

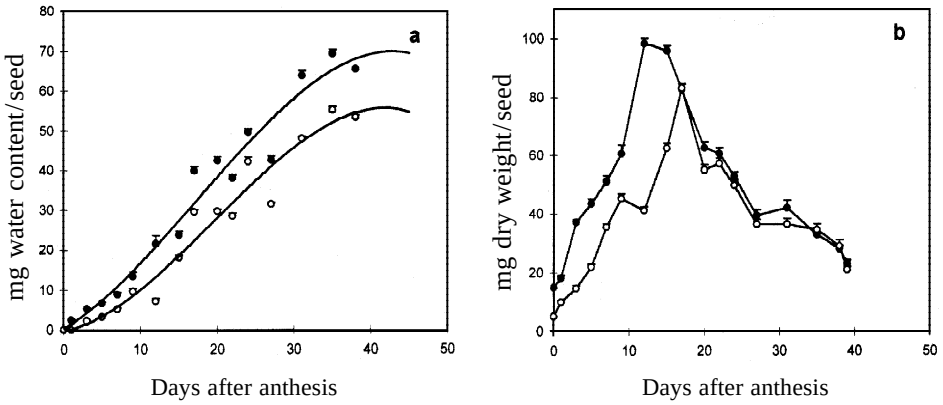

Fig. 1. Dry weight predicted from a cubic polynomial equation and actual seed mean dry weight (a) and water content (b) versus days after anthesis during entire period of seed development, seed 1 (•), seed 2 (o).

was critical to continue dry matter accumulation; and cultivars having more water content had more dry weight (Pande et al., 1992, Chanda and Singh, 1998). Further, it has been suggested that seed water status play an important role in regulating seed development (Egli, 1990).

The plant cell wall plays a critical role in water uptake. A wide range of growth regulating agents including hormones and environmental factors, apparently affect water uptake by modifying the ability of the primary wall to extend irreversibly. The modification are thought to be due to alteration in matrix polymers of the wall (Talbott and Ray, 1992). In fact, much evidence indicates that auxin modifies the visco elastic properties of some matrix polymers by stimulating their metabolic degradation when auxin increases cell wall extensibility (Inouhe et al., 1984). In monocots the cell wall loosening mechanism has been associated with the degradation of B(1-3)(1-4) glucan (Yamamoto et al., 1980) and depolymerization of an insoluble xyloglucan (Inouhe et al., 1984). On the other hand, in dicots, cell wall loosening has been associated with the degradation of a galactan and a depolymerization of a xyloglucan (Nishitani and Masuda, 1980, 1981).

Most of the xyloglucan in a tissue is firmly bound to the wall and cannot be extracted by any treatment that extract pectic polysaccharides efficiently. The xyloglucan can however be solubilized from the cell wall with alkali solutions. In the present work, xyloglucans were fractioned into low molecular and high molecular weight xyloglucans by extracting with 4% KOH and 24% KOH respectively.

Changes in low molecular weight xyloglucan in both seeds are shown in Fig. 2a. The content was low in both type of seeds on day 1, slightly increased on day 5; subsequently the content showed a steady increase till day 27, low levels between 30–35 days and again showed high levels on day 38. Maximum content was in the matured seed. Changes in high molecular weight xyloglucan are shown in Fig. 2b. The trend was similar to that of low molecular weight xyloglucan, though the content was half of the former. The content showed a gradual increase from beginning to maturity, maximum content being on day 38. Thus, both low and high molecular weight xyloglucan showed an inverse correlation with water content.

It can also be seen that when rate of dry matter accumulation is more, the xyloglucan content was less and as the seed matured, the rate of dry matter accumulation decreased and xyloglucan content increased. The metabolism of xyloglucan is undoubtedly regulated by different processes since it is subject to turnover during growth. Xyloglucan in the cell walls could be a major component that contributes to wall rigidity.

The pectic polysaccharides of the cell wall have been related to the loosening processes that occur during growth (Goldberg et al., 1989). Changes in pectic polysaccharides are shown in Fig. 3. The pectic polysaccharides were high on day 1 and showed a decreasing trend during the entire period of sunflower seed development. Mini-

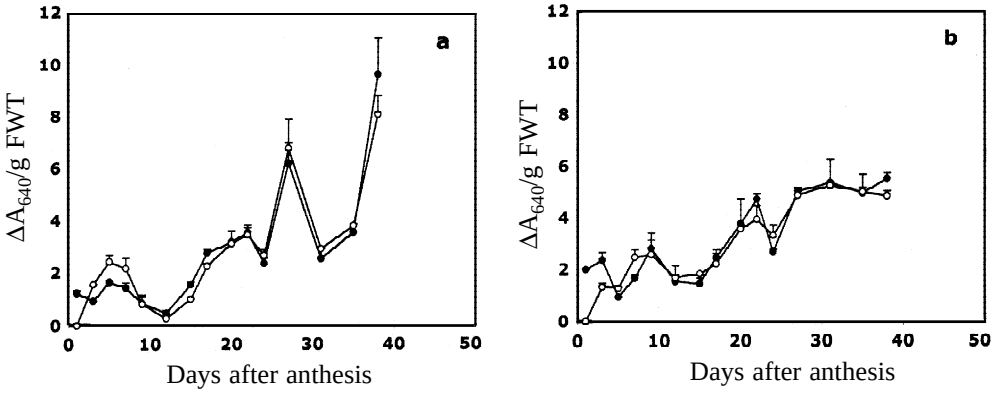

Fig. 2. Low molecular weight (a) and high molecular weight (b) xyloglucan content versus days after anthesis during entire period of seed development, seed 1 (•), seed 2 (o).

mum pectic polysaccharides content was present on day 38. Modification of pectic polysaccharides have also been implicated in cell wall loosening (Aspinall, 1980). Inverse correlation between elongation growth and pectic polysaccharides has been reported earlier in Phaseolus (Chanda et al., 1995; Bagatharia and Chanda, 1998). Here also during sunflower seed development, pectic polysaccharides showed a declining trend recording low levels in the mature seed. Thus supporting the conclusion that pectic polysaccharides may be involved in cell wall loosening. The pectic polysaccharides interact through non-covalent chemical bonding as well as through covalent bonding. Indeed non-covalent interactions may provide the most important interconnections between the pectic polysaccharides and the other cell wall polymers. It is clear that wall composition is modified during sunflower seed development and it is suggested that pectic polysaccharides may be involved in cell wall loosening and xyloglucan may be a major component that contributes to wall rigidity.

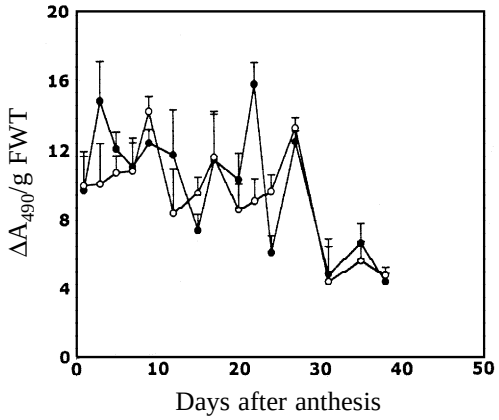

Fig 3: Pectic polysaccharides content versus days after anthesis during entire period of seed development, seed 1 (•), seed 2 (o).

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