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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES ASSOCIATED WITH SINK DEVELOPMENT IN COTTON SEEDS. I. GLYCOSIDASES AND WATER CONTENT

ABSTRACT

Three cotton genotypes (*Gossypium hirsutum* L. H–4, H–6, *G. herbaceum* V_{797}) varying in their final seed weight were analyzed for the growth and glycosidases activity. Five glycosidases, viz., α–β–galactosidases, α–β–glucosidases and α–mannosidases were estimated in cytoplasmic and wall–bound fractions. Data on dry weight and water content were fitted to polynomial equations and third degree polynomial equation was best–fit. Rate of dry matter accumulation showed closed relationship with water content in all three genotypes. The values for correlation coefficient {r= 0.956(H–4), r= 0.892(H–6) and r= 0.983 (V_{797})}, were statistically significant. No correlation between ionically wall–bound glycosidases and sink development was observed. In cytoplasmic fraction, the activities were higher during latter phase of dry matter accumulation. Probable role of these enzymes in mobilization of storage products during seed development is discussed.

Key words: cotton, glycosidases, seed, sink development, water content,

INTRODUCTION

Seed growth, from fertilization to maturity follows a complex series of several phases which results from the integration of wide range of morphogenetic and biochemical processes. The capacity of seed to accumulate dry weight depends mainly on numbers of the cells/seed (Cochrane and Duffus 1983) or size of these cells (Chanda and Singh 1998). It is argued that genotypic differences in many crop species are regulated by the number of the cells in the cotyledons/endosperm of the seed (Cochrane and Duffus 1983, Reddy and Daynard 1983). However, the regulation of cell size of the seed is not clearly understood. The general mechanism of increase in cell size is thought to be regulated by two major processes;(i) cell wall turnover and (ii) uptake of water in growing cells (Taiz 1984). The water is absorbed because of a number of biochemical and biophysical events that make the process rapidly responsive to fluctuating environmental and metabolic process (Kutschera

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1991, Cosgrove 1993). In former, the cell wall's turn over is extensively studied either as changes in wall components like pectic polysaccha–rides network or xyloglucans and elongation growth (Bagatharia and Chanda 1998, Hadfield and Bennett 1998). It is suggested that these components are systematically disassembled and play a key role in cell expansion (Mc Cann *et al.* 1994). In another set of experiments, it is suggested that in addition to polysaccharides and structural proteins growing cell wall contain several hydrolytic enzymes like glucosidases, glucanases, phosphatases, esterases, etc., which play very important role during cell expansion (Labavitch 1981, Taiz 1984, Thaker *et al.*, 1986, 1987). Changes in these enzymes are also worked out during fruit ripening and their role in hydrolyzing of polysaccharides is discussed (Knee 1973, Knee *et al.* 1977, Wallner and Bloom 1977). However, role of these enzymes in sink development of seed is not clearly workout

Considering afore said, in this experiment, seeds of three cotton geno–types, varying in their final seed weight were studied for five glycosidases viz., α–β–glucosidases, α–β–galactosidases and α–mannosidase in both cytoplasmic and wall–bound fractions.

MATERIALS AND METHODS

Seeds of three cotton genotypes [*Gossypium hirsutum* L. cv. H–4 (big–ger seed), H–6(middle size seed) and *Gossypium herbaceum* L. cv. V_{797} (smaller size seed)] were grown under field conditions. Flowers were date–tagged on the day of anthesis and healthy bolls were harvested on desired dates post anthesis (DPA). To minimize the effect of environ–mental variations, data for each set of analysis were collected from the flowers that had bloomed during as narrow a period as possible.

Growth analysis

Freshly harvested bolls were opened with a sharp scalpel and fibers were separated from seeds. For fresh and dry weight measurements, seeds from four locules of four bolls were weighed before and after oven drying to a constant weight at 70° C. The difference in fresh and dry weights gave the seed water content in mg at each stage. The mean dry weight and water content per seed with ±SD was calculated. The data of mean water content per seed was fitted to an appropriate curve using a polynomial regression analysis program.

Preparation of enzyme extract

Randomly harvested bolls were immediately brought to the labora–tory. Seeds were separated and fibers were removed with a scalpel on an ice bath. After dissection, the ovules were counted, weighed and frozen. The frozen material was crushed in a cooled mortar with sterilized sand in pre–chilled extraction buffer: 0.1M K–phosphate buffer (pH = 6.4) containing diethyldithiocarbamate (0.15%), cysteine–HCl (0.1%), EDTA

(2.5 mM) and PEG–6000 (8%) as suggested by Thaker (1998). The ho–mogenate was centrifuged at 15000 g for 20 minutes. The resulting supernatant was passed through 15 ml column of Sephadex G–25 pre–equilibrated with 0.02M K–phosphate buffer (pH 6.4). The desalted preparations were then used for the assay of all cytoplasmic enzymes. To avoid possible loss of enzyme activities, crushing and desalting were carried out rapidly in a cold room (4˚C). As an additional precaution the desalted enzyme preparations were kept in an ice bath until assayed.

Preparation of ionically wall–bound enzyme

The wall fraction separated after PEG extraction was washed several times with 0.02M K–phosphate buffer (pH 6.4) by re–suspending the wall material and was centrifuged until no protein content was recorded in the supernatant. The residue was then extracted in 1M NaCl for 1h at room temperature with constant shaking to release ionically wall–bound enzyme and was centrifuged at 15,000 g for 20 minutes. The supernatant was decanted and the resulting pellet extracted as before. The combined supernatants were used as the source of salt–extracted enzyme. The preliminary studies showed that 1 M NaCl was appropri–ate and that higher concentrations did not improve the extraction of ionically wall–bound enzyme, any further. Glycosidases were assayed according to Thaker *et al.* (1987) and activity is expressed as n Mole p–nitrophenol released. mg protein^{-1}.h^{-1}. All biochemical analyses were done in triplicate and mean values are presented. In a preliminary as–say for the enzymatic estimation pH and conditions of linear rate were determined with respect to substrate concentration and time.

The protein content of the enzyme extract was estimated according to Bradford (1976) using the coomassie brilliant blue dye–binding method.

RESULTS AND DISCUSSION

Seed dry weight data for the three genotypes were fitted to polynomial curves of different degrees and the best–fit equations were determined statistically. In all the cases, a cubic polynomial explained the data ap–propriately (Fig. 1). In H–4, dry matter accumulation showed initial lag up to 10 DPA and then it entered a linear phase of dry matter accumu–lation, and maximum seed dry weight was achieved around 39 DPA. Subsequently, a slight decrease in seed dry weight was observed. A sim–ilar trend was noted in seeds of H–6 and $V_{797.}$ However, in the latter ge–notype, the initial lag phase was extended up to 12 DPA. Amongst the three genotypes, H–4 had maximum seed weight (102 mg/seed), at physiological maturity followed by H–6 (74 mg/seed) while V_{797} had minimum seed weight (43 mg/seed).

Data of water content was also fitted to a polynomial equation and presented in Fig. 2. In H–6 and H–4, water content was low up to 6 DPA

and increased up to 30 DPA and decreased thereafter. In V_{797}, on the other hand, the initial lag phase of water uptake was around 10 DPA, after which it increased sharply and attained a peak around 33 DPA. Amongst the three genotypes, maximum water content was recorded in H–4 (173 mg/seed) followed by H–6 (138 mg/seed) and V_{797} (110 mg/seed), respectively (Fig.2). The data on rate of dry matter accumulation are also presented in Fig.2. It showed close parallelism with the water content. H–4 had more rate of dry matter accumulation (3.9 mg/day) than H–6 (3.2 mg/day) and V_{797} (2 mg/day).

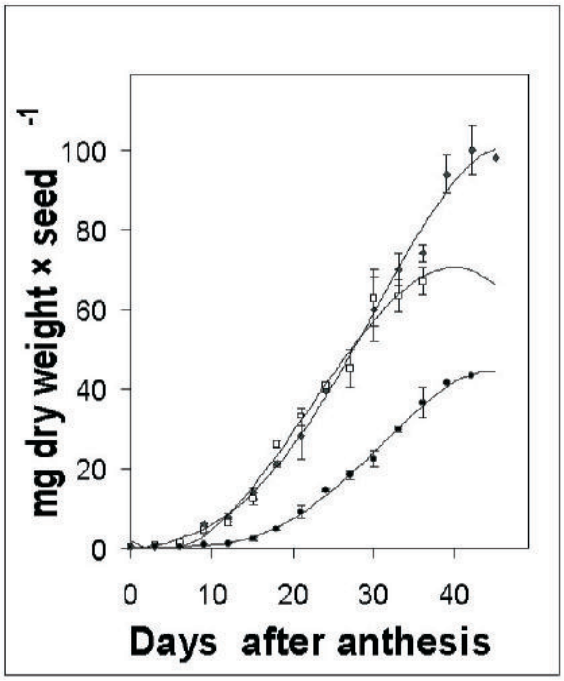

Fig. 1 Changes in dry matter accumulation against boll age (◆-◆) H-4; (□-□) H-6 and (●-●) V_{797}. Vertical bars represent ± standard deviation

Growing plant cells increase in volume is primary a resultant of cell expansion, Egli (1990) concluded that the ability of cotyledonary cells to take up water and increase in volume, may play an important role in regulating seed growth. The close parallelism between water content and rate of dry matter accumulation of developing seed reported in the present study, therefore suggests that rapid uptake of water may be required for increased cell volume and to support rapid rate of dry matter accumulation. Similarly correlation with cell size, water content and dry matter accumulation in cotton fiber (Rabadia *et al.*, 1999) and *Hibiscus esculentum* seed development (Thaker 1998) has been reported

Glycosidases are enzymes which hydrolyze oligosaccharides to monomers and show specificity in different tissue. For example,

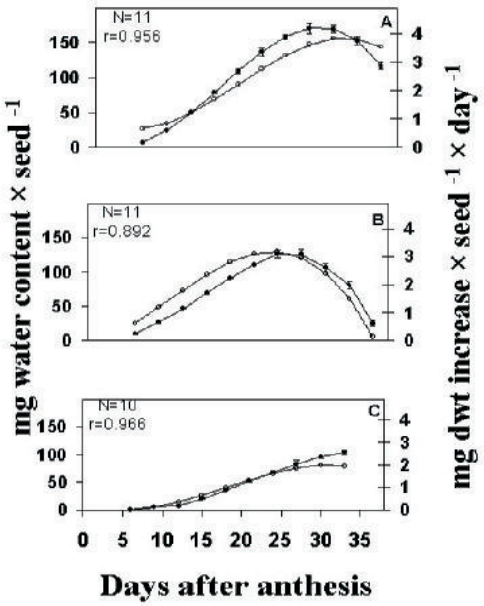

Fig. 2 Changes in water content (●-●) per seed and rate of dry matter accumulation (o-o) against boll age. Vertical bars represent ± standard deviation, or otherwise, within the symbols. r (Coefficient of correlation) and N (number of observation) in A (H-4), B (H-6) and C (V_{797})

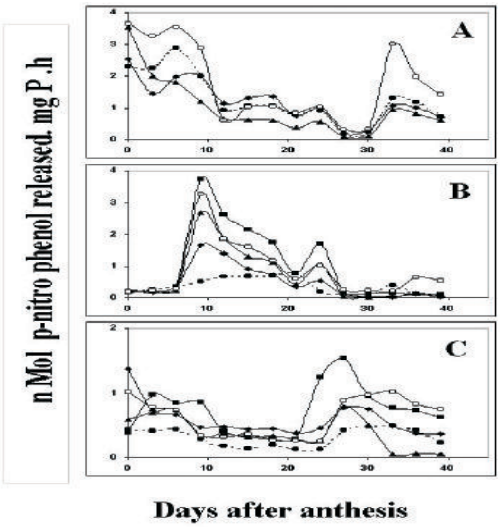

Fig. 3 Changes in wall bound glycosidases activities; (●-●) α–galactosidase, (o-o) α–glucosidase, (■-■) β–galactosidase, (▲-▲) β–glucosidase and (◆-◆) α–mannosidase against bollage. . Vertical bars represent ± standard deviation, or otherwise, within the symbols. A (H-4), B (H-6) and C (V_{797})

β–galactosidase was highly active during autolysis of cell wall in *Cicer*

arietinum (Dopico *et al.*, 1989a) while no correlation with α–galactosidase activity was recorded (Dopico *et al.* 1989b). Fruit tissue contain an impressive array of carbohydrate–degrading enzymes (Fisher and Bennett 1991; Pressey 1977). In elongating cells of Pisum sativum α–galactosidase and α–arabinosidase was abundant (Tanimoto 1985).

Ionically wall–bound glycosidases are presented in Fig. 3. In H–4 the activities were higher initially and low levels were maintained during 10 to 24 DPA and increased again at later part of the DMA phase, however, in H–6, the activities were higher initially and declined thereafter. In V_{797} also activities of glycossidases remained very low, initially up to 24 days and increased during later stages. Thus ionically wall–bound glycosidases showed no correlation with the sink development.. A consequences of the hydrolytic process of wall loosening and *in muro* alteration of polysaccharides is the recycling of sugars. In some instances recycling of sugars results in the complete turnover of some polysaccharides or specific alteration of others (Gibeaut and Carpita 1991).

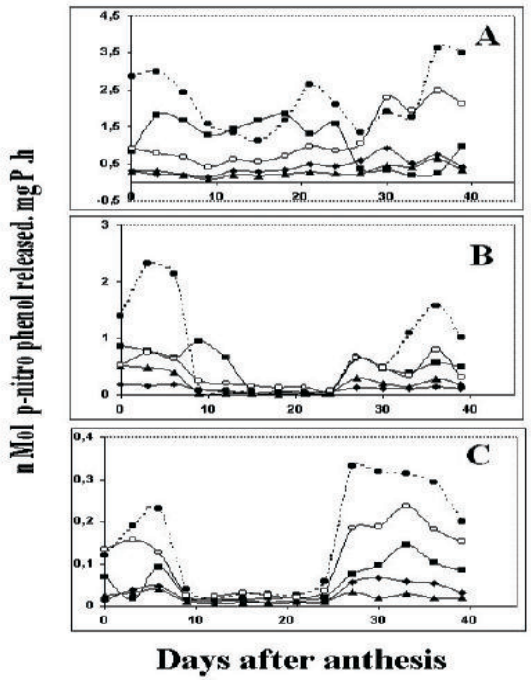

Fig. 4 Changes in cytoplasmic glycosidases activities; (●-●) α–galactosidase, (o-o) α–glucosidase, (■-■) β–galactosidase, (▲-▲) β–glucosidase and (◆-◆) α–mannosidase against bollage. Vertical bars represent ± standard deviation, or otherwise, within the symbols. A (H–4), B (H–6) and C (V_{797})

Changes in five glycosidase, i.e. α–β–galactosidase, α–β–glucosidase and α–mannosidase activities in cytoplasmic fraction, are presented in Fig. 4. Activity of β–galactosidase was maximum whereas

α–glucosidase – minimum, in all three genotypes. In general, cytoplas–mic activity was higher in bigger seed (H–4), followed by middle (H–6) and smaller size (V_{797}) seeds. In H–4, α–galactosidase activity remain higher during 3–27 DPA and declined thereafter, while β–galactosidase showed fluctuating trend. In H–6 and V_{797}, activities of all glycosidases was higher initially (during lag phase), declined during 10–24 DPA and showed increasing trend after rate of DMA has achieved the peak. Sev–eral glycosidases are active in storage organs and ripening fruits during the degradation of reserve materials or cell–wall components (Gross and Wallner 1979) In the present study, cytoplasmic glycosidase activi–ties were higher during latter part of DMA, and the bigger (H–4) seed had higher activities, followed by middle (H–6) and smaller size (V_{797}) seed (Fig.4). The reserved glycosidases may be mobilized in conversion of lipids and protein during latter phase. Role of these enzymes in mobi–lization of storage product for growth and development in developing cotton fiber have been reported (Thaker *et al.* 1987).

CONCLUSIONS

Thus it is concluded from the above results that variations in the seed size of these three genotypes studied showed significant difference in their water content and rate of dry matter accumulation (Fig.2). How–ever, no significant correlation was observed with ionically wall–bound glycosidases and sink development in all three genotypes studied. The activities of the cytoplasmic glycosidases were high during later part of seed development. This suggest that these enzymes may have a role in storage product formation during later phase of the seed development.

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