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Hydrolytic enzymes in cotton ovules during early development

RICHA, AMARJIT S BASRA, KIRANJIT K AHLUWALIA and C P MALIK

Department of Botany, Punjab Agricultural University, Ludhiana 141 004, India

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Abstract. Soluble and wall-bound activities of acid phosphatase, β-glucosidase, α-galactosidase and invertase in the ovules of *Gossypium arboreum* L. and *Gossypium hirsutum* L. are implicated in the hydrolysis and translocation of substrates during pre- and post-anthesis phases. The physiological functions of these hydrolytic enzymes are discussed in relation to early ovule development and cotton fibre differentiation.

Keywords. *Gossypium* spp.; ovules; hydrolases.

1. Introduction

The biology of ovule development has been studied at various levels (Evenari 1984) but few studies have looked into the enzymic changes during early development. Dhindsa (1978) studied the hormonal regulation of some enzymes of intermediary metabolism in unfertilized cultured ovules of cotton from the day of anthesis to 5 days post-anthesis. Recently, Joshi *et al* (1985) reported the distribution of β-glycerophosphatase activity in the outer integument of cotton ovules by histochemical localization in a linted cultivar and a lintless mutant line from 1 day pre-anthesis to 3 days post-anthesis. Cotton fibres are reported to differentiate before 16 hr pre-anthesis from certain epidermal cells of the outer integument and the elongation begins on the day of anthesis (Aiyangar 1951; Ramsey and Berlin 1976a,b). The information on metabolism of cotton ovules during early development is, therefore, additionally important from the view-point of fibre differentiation. The present work was carried out to determine the activities of hydrolytic enzymes such as acid phosphatase (EC 3·1·3·2); β-glucosidase (EC 3·2·1·21); α-galactosidase (EC 3·2·1·22) and invertase (EC 3·2·1·26) in developing ovules of *Gossypium arboreum* L. cv. LD 230 and *G. hirsutum* L. cv. F 414 from 2 days pre-anthesis to 2 days post-anthesis. Soluble and wall-bound activities of the above mentioned hydrolases were correlated to the ovule development.

2. Material and methods

The cotton crop was raised according to the recommended practices for fertilizer application, plant protection, weed control and irrigations under field conditions. The ovules were sampled at 2- and 1-day pre-anthesis, anthesis and 1- and 2-days post-anthesis (−2, −1, 0, +1, +2, respectively).

Ovules (0·5 g) were homogenized in 5·0 ml of 0·1 M acetate buffer, pH 5·2, containing 1% PVP and 5 mM EDTA. The homogenates were centrifuged twice at 10,000 g for 20 min. The supernatant fluids were collected and designated as soluble fractions. The

remaining pellets were washed 3 times with 0·1 M acetate buffer, pH 5·2, and after these washings and subsequent centrifugations at 3,000 g for 15 min, the pellets were designated as the wall fractions. All stages in the preparation of the soluble and wall fractions were carried out at 0–4°C. Acid phosphatase was assayed according to Simola and Sopanen (1971) using *p*-nitrophenylphosphate as the substrate and invertase according to Singh *et al* (1978). The activities of β-glucosidase and α-galactosidase were determined according to Sharma *et al* (1981) using *p*-nitrophenyl-β-D-glucopyranoside and *p*-nitrophenyl-α-D-galactopyranoside as substrates, respectively. Results presented are average of duplicate determinations.

3. Results and discussion

The enzymes studied revealed activities both in the soluble and wall fractions (figures 1–4). Acid phosphatase (*p*-nitrophenylphosphatase) activity was located mainly in the soluble fraction than in the cell wall (figure 1). The soluble activity in *G. hirsutum* was higher in pre-anthesis ovules compared with the day of anthesis or post-anthesis stages. The activity in *G. arboreum* ovules was highest at −2 stage and after remaining relatively decreased through −1 to +1 stage increased again by 2 days post-anthesis. On the other hand, wall-bound activities in the ovules of both species kept low and did not fluctuate much except for a marked decline at +2 stage in *G. arboreum* (figure 1). The active metabolic participation of the enzyme in the change-over to the gametophytic phase and the early development of zygote has been implicated (see Evenari 1984). Histochemical localization of the enzyme in the outer integument of cotton ovules has revealed a strong relationship with the process of fibre differentiation (Joshi *et al* 1985). The biochemical function may lie in hydrolyzing various phosphate esters, intercellular transport, phosphate transfer reactions and cell wall metabolism (Sexton *et al* 1971; Onofeghara and Karoma 1974; Gahan *et al* 1978). Joshi *et al* (1985) speculate that the enzyme may function as a phosphoprotein phosphatase to regulate differentiation.

Soluble and wall-bound β-glucosidase activities in *G. hirsutum* were similar at −2 stage but at subsequent stages the wall-bound activity was higher (figure 2). In

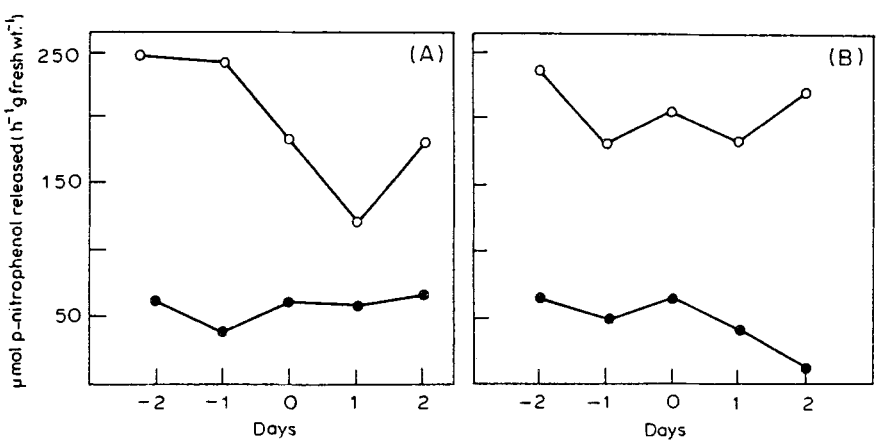

Figure 1. Soluble (○) and wall-bound (●) activities of acid phosphatase in ovules of (**A**) *G. hirsutum* L. cv. F 414 and (**B**) *G. arboreum* L. cv. LD 230.

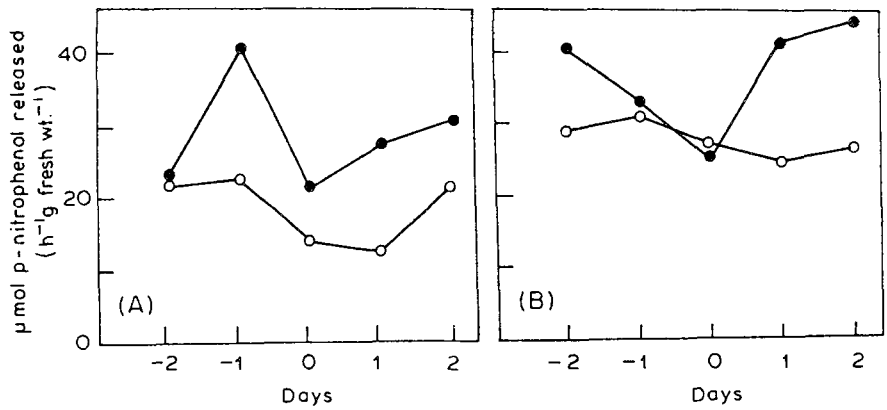

Figure 2. Activities of β-glucosidase in cotton ovules. Symbols as in figure 1.

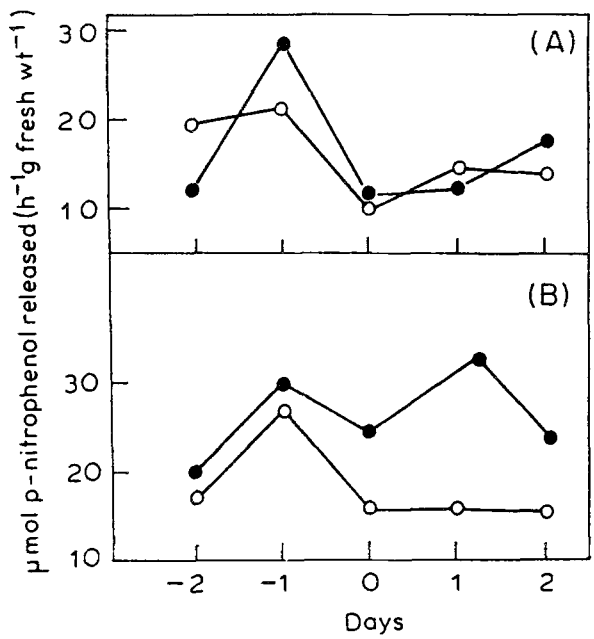

Figure 3. Activities of α-galactosidase in cotton ovules. Symbols as in figure 1.

G. arboreum, the wall-bound activity was higher at −2, +1 and +2 stages and was almost similar at −1 and 0 stages (figure 2). As reported for other systems (Murray and Bandurski 1975; Rosenfield and Matile 1979), the wall-bound β-glucosidase activity may be involved in cell wall loosening by cleaving wall linkages and thus facilitating cell growth.

In two cotton species, the activities of soluble α-galactosidase were relatively higher at −2 and −1 stages than the remaining stages (figure 3). In *G. arboreum* the wall-bound activities were higher at all the developmental stages whereas in *G. hirsutum* the wall-bound activity was higher only at −1 and +2 stages (figure 3). α-Galactosidase

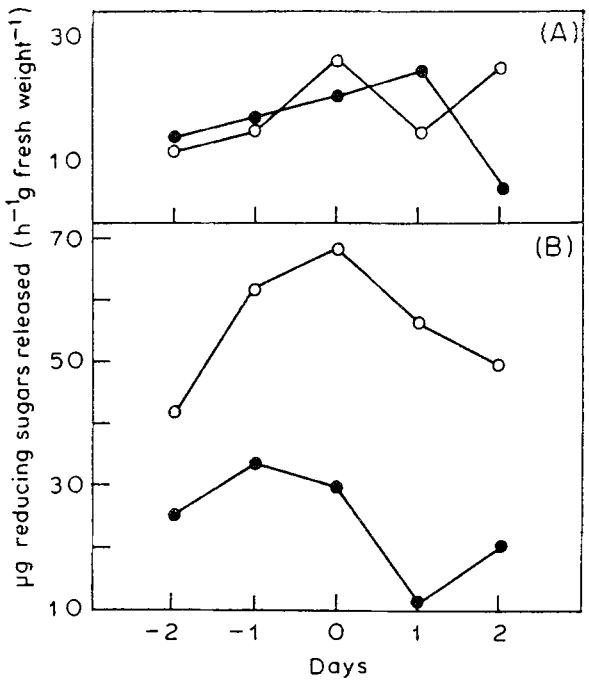

Figure 4. Activities of invertase in cotton ovules. Symbols as in figure 1.

catalyzes hydrolytic, synthetic (*de novo*) and transfer reactions (Dey 1979). The raffinose-type oligosaccharides and galactose residues of wall constituents may be the natural substrates for enzyme action. Oligosaccharides containing α-D-galactoside groups, some of which serve as an energy source, are widely distributed in the plant kingdom. The work of Labrador and Nicolás (1984) has shown that α-galactosidase is one of the enzymes involved in the autolysis of cell walls in pea epicotyls during growth and the liberation of arabinose and galactose.

It is tempting to suggest that the ability of glycosidases to liberate phenols by hydrolyzing phenolic glycosides could provide a means of controlling the growth and differentiation of ovules. Phenolic substances are considered to be potent regulators of plant development (Kefeli and Dashek 1984), which may act on their own or via altering the level of hormones. O-Diphenols released from the vacuoles of differentiating cotton fibre cells presumably inhibit IAA oxidase to allow an intracellular auxin level high enough for fibre initiation (Ramsey and Berlin 1976b).

Soluble invertase activity in *G. arboreum* was much higher than the wall-bound activity at all the ovular stages whereas in *G. hirsutum* it was higher only at 0 and +2 stages (figure 4). Maximum activity of the soluble enzyme was observed on the day of anthesis in both the species but it was substantially lesser in *G. hirsutum* ovules. Similarly, wall-bound activities were higher in *G. arboreum* ovules except at +1 stage. The enzyme catalyzes the hydrolysis of sucrose into glucose and fructose, and possibly the hexoses are then metabolized to meet the high demand for energy and carbon substrates required for biosynthetic reactions of developing ovules. Soluble activity is linked with the hydrolysis of intracellular sucrose and the wall-bound with the extracellular hydrolysis of sucrose into hexoses for cellular uptake.

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References

Aiyangar G S 1951 Origin and development of lint and fuzz in cotton: *Indian J. Agric. Sci.* **21** 293–312

Dhindsa R S 1978 Hormonal regulation of enzymes of nonautotrophic CO_2 fixation in unfertilized cotton ovules; *Z. Pflanzenphysiol.* **89** 355–362

Dey P M 1979 Transgalactosylation activity of sweet almond α-galactosidase: Synthesis of saccharides; *Phytochemistry* **18** 35–38

Evenari M 1984 Seed Physiology: From ovule to maturing seed; *Bot. Rev.* **50** 143–170

Gahan P B, Dawson A L and Fielding J 1978 Paranitrophenylphosphate as a substrate for some acid phosphatases in roots; *Ann. Bot.* **42** 1413–1420

Joshi P A, Stewart J M and Graham E T 1985 Localization of β-glycerophosphatase activity in cotton fiber during differentiation; *Protoplasma* **125** 75–85

Kefeli V I and Dashek W V 1984 Non-hormonal stimulators and inhibitors of plant growth and development; *Biol. Rev.* **59** 273–288

Labrador E and Nicolas G 1984 Autolysis of cell walls in pea epicotyls during growth. Enzymatic activities involved; *Physiol. Plant.* **64** 541–546

Murray A K and Bandurski R S 1975 Correlative studies of cell wall enzymes and growth; *Plant Physiol.* **56** 143–147

Onofeghara F A and Koroma S A 1974 Histochemical localization of enzymes in the cucurbitaceae. Acid phosphatase; *Ann. Bot.* **39** 477–483

Ramsey J C and Berlin J D 1976a Ultrastructural aspects of early stages in cotton fiber elongation; *Am. J. Bot.* **63** 868–876

Ramsey J C and Berlin J D 1976b Ultrastructure of early stages of cotton fibre differentiation; *Bot. Gaz.* **137** 11–19

Rosenfield C L and Matile Ph 1979 Glycosidases in pear pollen tube development; *Plant Cell Physiol.* **20** 605–613

Sexton R, Cronshaw J and Hall J L 1971 A study of the biochemistry and cytochemical localization of β-glycerophosphatase activity in root tips of maize and pea; *Protoplasma* **73** 417–441

Sharma S, Singh M B and Malik C P 1981 Relation of glycosidases to *Amaryllis vittata* pollen tube growth; *Plant Cell Physiol.* **22** 927–931

Simola L K and Sopanen Y 1971 Changes in the activities of certain enzymes of *Acer pseudoplatanus* L. cells at four stages of growth in suspension culture; *Physiol. Plant.* **23** 1212–1222

Singh M B, Malik C P and Thapar N 1978 Changes in the activities of some enzymes of carbohydrate metabolism in *Amaryllus vittata* pollen suspension cultures; *Plant Cell Physiol.* **19** 677–684