

Esterase Ontogeny in Cotton Fibre

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Abstract. Growth parameters and esterase isoenzyme patterns were studied in developing cotton fibre. Fibre length and dry weight when plotted against boll age showed four distinct stages; (i) initiation, (ii) elongation, (iii) secondary thickening, and (iv) maturation. Stage specificity and changing intensities of esterase isoenzyme suggested a finely modulated control of gene expression during fibre development.

Cotton fibre which is a unicelled epidermal trichome on the seed coat is commercially important. It starts initiation on the day of anthesis and takes 40 to 50 days for its complete development into a lint fibre. On the basis of computer curvilinear analysis, JASDANWALA *et al.* (1977) reported that development of cotton fibre consists of four phases: (i) initiation, (ii) elongation, (iii) secondary thickening, and (iv) maturation. These phases are spontaneous processes which can not be delimited from one another. Further, on the basis of IAA oxidase and peroxidase levels these workers concluded that IAA in the developing fibre may regulate the termination of elongation and initiation of the secondary thickening phases. However, till date very little work has been done on the enzymatic activities taking place in a developing fibre. In the present paper changes in esterase isoenzymes during the entire period of cotton fibre development are reported.

MATERIAL AND METHODS

Seeds of cotton (*Gossypium hirsutum* cv. SRT-1) were grown in polyethylene bags filled with a 3 : 1 mixture of soil and farm yard manure. After 10 days seedlings of nearly similar cotyledon size and height were transplanted in the experimental garden. Plants were transplanted at a distance of 1 m and the rows were also spaced 1 m apart. Cultural practices including irrigation, supplying of fertilizer and spraying of insecticides *etc.*, were conducted to maximize the lint yield. Generally the field was irrigated weekly and fertilizer was added fortnightly.

On the day of anthesis each individual flower was tagged and the bolls were harvested for analysis after the required periods. To minimize the

effect of environmental factors data for each set of analyses were collected from flowers that bloomed over as narrow a time span as possible.

Growth Analysis

Fibre length and dry weight was determined as per GIPSON and RAY (1969). One locule from a boll was placed in boiling water to allow the seeds to separate from each other and each seed was placed on the convex side of a watch glass and the fibres were streamed out with a jet of water. The length of the fibre was measured from the round side of the seed adjacent to the chalazal end. All the seeds from the three locules were measured from three bolls and an average was calculated for the fibre length. The fibres were removed from the seed with a scalpel without removing the seed coat and the dry weight was determined after drying in an oven at 80 °C for 2 days. Each result represents an average of three bolls harvested randomly at a given age.

Isoenzyme Analysis

Enzyme Preparation

Bolls were harvested from healthy plants and opened with a sharp knife. Hairs were immediately removed from the seed and frozen. Two hundred milligrams of this frozen material was crushed in a cold mortar with 2 ml of borate buffer (0.2 M pH 7.6) as recommended by KING (1971) to yield maximum protein. The extracts were centrifuged at low speed and the supernatant clarified by centrifuging at 12000 *g* for 10 min. All the aforesaid operations were carried out at 4 °C. The resultant cell free extract was used as enzyme source. Till the 5th day postanthesis, it was difficult to separate fibre from the seed. Hence the young ovules were used for analyses, while at the 10th day after anthesis and in subsequent periods fibres were used.

Electrophoresis

Electrophoretic separation of esterases was performed as per the system of ORNSTEIN and DAVIS (1962), using 7.5% polyacrylamide gel. This system stacks at pH 8.9 and runs at pH 9.5. All enzyme preparations on the gels were normalised with respect to their concentrations and the space above the gels was layered by bromophenol (0.01%), a tracking dye, and buffer. A constant current of 2 mA was initially applied to each tube for ten minutes and then was raised to 4 mA. Each run was terminated after the dye reached the bottom of the gel. Gels were removed by rimming and then stained for esterases as per MARKERT and HUNTER (1959). The brownish black bands developed were immediately recorded. The results of electrophoretic separation have been presented schematically with mobilities calculated as the relative distance traversed by the band with respect to the distance travelled by bromophenol dye. The intensity of the isoenzyme bands is visually observed.

RESULTS

Growth Analysis

When the data for lint length were plotted against boll age (the number of days after anthesis) as shown in Fig. 1A, a hyperbolic pattern was obtained. The elongation started soon after fertilization without a lag phase and continued up to 20 days postanthesis after which no appreciable increase

was discernible. Maximum rate of lint length increase was obtained between 10 to 20 days.

Fibre dry weight per seed, on the other hand, yielded a more sigmoidal relationship when plotted against boll age (Fig. 1A). Lint dry weight showed

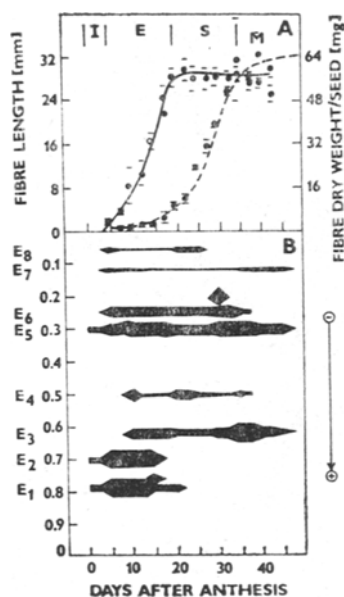


Fig. 1A. Fibre length and dry weight *versus* boll age. The horizontal bars indicate $\pm$ standard deviation. Fibre length, continuous line; fibre dry weight, broken line. I, E, S and M represent the initiation, elongation, secondary thickening and maturation phase respectively.

Fig. 1B. A schematic summary of esterase isoenzymes with changes in activity during cotton fibre development. The width of each channel represents the relative amount of activity. The activity was determined by visual observation of the staining intensity of bands on the gel.

a definite lag phase in the early periods of its development *i.e.* up to 15 to 20 days postanthesis after which it increased exponentially till 35 days. No significant increase was recorded in the subsequent period.

Esterase Isoenzyme Patterns

In all, eight esterase isoenzymes (Fig. 1B) were observed throughout the course of fibre development. The number of stage specific isoenzymes were detected *e.g.*, band E_8 appeared only after the differentiation of fibre initials and continued till secondary thickening. Similarly band E_3 was detected during elongation phase and continued till maturity, while isoenzyme E_2 and E_1 were observed only during the initiation and elongation phases.

Isoenzyme E_5 was present throughout the period of fibre development but it showed different intensities at different growth phases (less during the initiation and maturation phases and more during the elongation and secondary thickening phases). No regular trend was discernible in the case of E_8 , E_7 and E_4 isoenzymes. The elongation and secondary thickening phases, in general, had more intense and increased number of esterases than the initiation and maturation phases.

DISCUSSION

From the growth analysis, four distinct phases are discernible in the cotton fibre development; (i) initiation (0 — 3rd day), (ii) elongation (5th — 20th day), (iii) secondary thickening (20th — 35th day), and (iv) maturation

(35th — 45th day). This is in agreement with the results of JASDANWALA *et al.* (1977).

A total of eight isoenzyme of esterases were observed (Fig. 1B). Esterases are a complex family of hydrolytic enzymes which show striking multiplicity of forms within a species, and which exhibit dramatically different patterns of tissue specificity between species (MASTERS and HOLMES 1972). Reports that growth of seeds could be arrested by inhibition of their non-specific esterases are available in literature (MENDEL *et al.* 1953). Working with *Portulaca grandiflora* pollen grains SOOD (1974) suggested that non-specific esterases may participate in the formation and growth of pollen tubes. The presence of a larger number of bands during the elongation and secondary thickening phases than during the initiation and maturation phases clearly suggests that esterases play an important physiological role during the former two phases. Further, the phase specificity and intensity alteration of these isoenzymes is indicative of changes in the rates of both enzyme synthesis and degradation which require the finely modulated control of gene expression.

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REFERENCES

- GIPSON, J. R., RAY, L. L.: Fibre elongation rates in five varieties of cotton (*Gossypium hirsutum* L.) as influenced by night temperatures. — *Crop Sci.* **9** : 339–341, 1969.
- JASDANWALA, R. T., SINGH, Y. D., CHINYOY, J. J.: Auxin metabolism in developing cotton hairs. — *J. exp. Bot.* **28** : 1111–1116, 1977.
- KING, E. E.: Extraction of cotton leaf enzymes with borate. — *Phytochemistry* **10** : 2337 to 2341, 1971.
- MARKERT, C. L., HUNTER, R. L.: The distribution of esterases in maize tissue. — *J. Histochem. Cytochem.* **7** : 42–49, 1959.
- MASTERS, C. J., HOLMES, R. S.: Isozymes and ontogeny. — *Biol. Rev.* **47** : 309–361, 1972.
- MENDEL, B., MEYERS, D. K., UYLDERT, I. E., RUYS, A. C., DE BRUYN, W. M.: Aliesterase inhibitors and growth. — *Brit. J. Pharmacol.* **8** : 217–224, 1953.
- ORNSTEIN, L., DAVIS, B. J.: Disc Electrophoresis. — Distillation Products Industries, Rochester, New York 1962.
- SOOD, P. P.: Histochemical studies on the distribution of non-specific esterase in the germinating pollen grains of *Portulaca grandiflora*. — *Biol. Plant.* **16** : 102–106, 1974.