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IN VITRO FIBRE DEVELOPMENT IN COTTON (*GOSSYPIUM HIRSUTUM* L.) I. HORMONAL REGULATION

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SUMMARY

***In vitro* fibre development was initiated in cotton (*G. hirsutum* L.) cv LRA 5166 by culturing three day old fertilized ovules. The ovules were cultured on standard basal mediums (MS & TM) containing different hormonal combinations. Fibre initiation took place when isolated ovules were cultured in suspension or on semi-solid medium under dark incubation. Ovules responded to changes in media constituents and hormones by producing fibres or de-differentiating into callus, and in certain treatments both callus and fibres were observed simultaneously. The best result was observed in ovules cultured on TM medium with IAA (1.0 mg l^{-1}) + GA (1.0 mg l^{-1}) + BA (1.0 mg l^{-1}) for 15 days and later sub-cultured on TM medium with IAA (2.0 mg l^{-1}) + BA (0.5 mg l^{-1}) with a fibre length of 15 -17mm in 25-30 days of culture.**

***Key words*: Cotton, *in vitro* fibre development, plant growth regulators**

INTRODUCTION

Cotton fibres are technically trichomes derived from the epidermal cells of fertilized ovules. Fibres maintain a precise synchrony and homogeneity in growth during their development in cotton bolls. The fibres undergo a striking amount of elongation during their development and can end up over 1000 to 3000 times longer than their diameter (Van't Hof & Saha 1997). Ovule culture is advantageous to study the precise effects of growth regulators, radioactive labeling or controlled environmental conditions on fibre properties. Even though ovule culture as a model system for cotton fibre studies was initiated in seventies by Beasley & Ting (1973), the fibre production in culture thus far has not reached the levels recorded under *in vivo* conditions (Gokani & Thaker 2002). A fibre length of less than 50 per cent and cellulose deposition to an extent of 70-80 per cent only has been achieved under *in vitro* conditions (Feng & Brown, 2000). Even though Meinert and Delmer (1977) reported no qualitative changes between *in vivo* and *in vitro* produced fibres, recent studies suggest that fibres developed in culture may differ from fibres produced on plants in the degree of branching of carbohydrate polymers (Triplett & Timp 1995) and protein profile during fibre development (Turley & Ferguson 1996). Plant growth regulators like indole-acetic acid, gibberrelic acid, ethylene and abscissic acid play a decisive role in fibre development (Prakash *et al*. 2002; Kosmidou-Dimitropoulou 1986). Fibre initiation and development are known to be influenced by the age of the ovule (Graves & Stewart 1988), temperature (Xie *et al*. 1993), plant growth regulators (DeLonge 1986) and inorganic nutrients (Eid *et al*. 1973) in the culture medium. *In vitro* culture of cotton ovule is one of the valuable tools to investigate the fibre biochemistry. Biochemical aspects of *in vitro* fibre development in cotton have not been worked out in detail. Hence, the present investigation was conducted to study the hormonal regulation of fibre

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development *in vitro* and the associated biochemical changes in order to understand the process of *in vitro* fibre development.

MATERIALS AND METHODS

The plants of cotton (*Gossypium hirsutum* L.) cv. LRA 5166 were raised in field under optimum growth conditions by following recommended agronomic practices. The flowers were tagged on the day of anthesis. Three-day-old bolls were collected and utilized for the experiment. The ovaries were surface sterilized by treating with 0.1% mercuric chloride for 10 minutes followed by thorough washing with autoclaved distilled water. The ovary was dissected and ovules were aseptically isolated and floated on the culture medium. Each treatment consisted of at least five replications with 20 ovules per flask. The cultures were incubated in dark at 26±2°C. The basal medium was MS (Murashige & Skoog 1962) or TM (Shahin 1985). Reducing sugars, amino acids and soluble protein content in ten-day-old fertilized ovules were estimated following standard procedures (Sadasivam & Manickam 1992).

RESULTS AND DISCUSSION

Optimum hormonal combination required for effective fibre initiation has been worked out. The basal medium (MS medium) with varying PGR combinations was tested for ovule culture (Table 1). The growth response was evaluated after five days in culture. In the absence of the hormones, the ovules survived but did not show any perceptible growth. Similar responses were reported by Beasley and Ting (1973) when two-day-old cotton ovules were cultured *in vitro*. Based on the PGR combinations, the ovules either bulged or fibre initials were observed. In certain combinations, the ovules turned brown. The combination of IAA (5.0 mg l^{-1}) + GA (5.0 mg l^{-1}) + BA (1.0 mg l^{-1}) was found optimum for fibre growth.

The interaction effect of hormones on the accumulation of biochemical constituents *viz*., reducing sugars, total free amino acids and soluble protein content was quantified in ten-day-old ovules. It was observed that ovules cultured at 1.0 mg.l^{-1} of IAA accumulated 94.6 mg g^{-1} of reducing sugar, while at the same concentration of 2,4-D, the ovules accumulated 72.3 mg g^{-1} of sugars (Table 3). If auxin concentration remained constant or reduced and the GA increased from 1.0 to 5.0 mg l^{-1}, there was inhibitory effect on the ovule growth, which was reflected in lower levels of sugars.

Free amino acids also showed a similar trend with ovules accumulating higher levels of amino acid when cultured on medium with auxin and GA at 1.0 mg l^{-1}. At 1.0 mg l^{-1} of IAA and GA, the ovules accumulated 22.8 mg g^{-1} of amino acid, while at 1.0 mg l^{-1} of IAA and 5.0 mg l^{-1} of GA it was only13.4 mg g^{-1}. The free amino acid

Table 1. Response of cotton ovules (cv. LRA 5166) to PGR's under *in vitro* culture.

Treatment (IAA + GA + BA mg l^{-1})	Response	Ovular response (%)
0.0 + 0.0 + 0.0	Survive but no growth	100
0.0 + 1.0 + 1.0	Survived but browning of ovules	60±4
0.5 + 1.0 + 1.0	Ovules enlarged	75±3
1.0 + 1.0 + 1.0	Ovules bulged and small fibre initials observed	75±4
2.5 + 1.0 + 1.0	Ovules bulged and small fibre initials observed	75 ±7
5.0 + 1.0 + 1.0	Ovules enlarged and found white and healthy. Small fibre initials were observed.	80±5
5.0 + 0.5 + 1.0	Ovules found healthy	60 ±7
5.0 + 2.5 + 1.0	Ovules enlarged but turned slightly brown	55±2
5.0 + 5.0 + 1.0	Ovules healthy and enlarged with signs of fibre initiation	80±3

content was 18.6 and 11.2 mg g^{-1} when GA was varied from 1.0 and 5.0 mg l^{-1} at constant level of 1 mg l^{-1} 2,4-D (Table 3). The accumulation of protein showed an opposite trend to that of other solutes with the protein level increasing from 9.87 to 22.51 mg g^{-1} with increase in GA. But reduction of IAA from 1.0 to 0.5 mg l^{-1}, the protein synthesis was substantially reduced (Table 3).

The ovule growth has a set pattern. It is reported that the ovules undergo expansion during the first five days of culture. This is basically attributed to the turgor pressure (Smart *et al*., 1998). This turgor pressure is facilitated by high accumulation of solutes. At a given level of auxin, IAA was more responsive in accumulation of reducing sugars and amino acids than 2,4-D. Similar response was reported earlier (Ruan & Chourey 1998, Cosgrove 1997) wherein the cell turgor is achieved largely through the influx of water driven by a relatively high concentration of osmoticum within a cell. This led to the conclusion that cotton ovules grow better when IAA is used as an auxin source. Hence, in other experiments, IAA was only used as auxin source. The importance of IAA in cotton fibre development for selection of genes for fibre development has also been indicated by Gokani and Thaker (2002).

Subsequently, the ovules were cultured on two nutrient media viz., MS and TM medium. The ovules were sub-cultured after 15 days either on the same medium or using altered hormonal combinations. Subculturing eliminated the cell exudates inhibitory to ovule growth and also replenished the nutrients. IAA degrades rapidly in culture medium compared to 2,4-D and NAA (Dunlap *et al*. 1986). Based on the media and hormonal combinations, the ovular responses also changed (Table 2, Plate a-f). Irrespective of the hormonal combinations, the ovules survived and the fibre initiation was observed in all treatments (Plate b). Among the treatments, the medium with 2.5 mg l^{-1} of IAA, GA and BA led to profuse callus growth. When ovules were cultured on MS+ IAA (2.5 mg l^{-1}) + GA (2.5 mg l^{-1}) + BA (2.5 mg l^{-1}), there was callus formation and also tuft of fibre development at the micropilar end (Plate f**)**. Beasley and Ting (1973) reported that BT medium promoted fibre development with a minimum of callus formation, while the modified MS medium favoured callus formation over fibre development. When ovules were exposed to MS + 5.0 mg $l^{-1}$of IAA, GA and BA, fibre development to an extent of 7-10 mm was noticed by 30 days. Ovules cultured on TM medium with IAA (1.0 mg l^{-1}) + GA (1.0 mg l^{-1})+BA (1.0 mg l^{-1}) for 15 days and later sub-cultured on TM + IAA (2.0 mg l^{-1})+ BA (0.5 mg l^{-1}) led to fibre development with a mean fibre length of 15-17 mm (Plate a-d).

A comparative growth response curve was drawn to evaluate the growth of fibres under *in vitro* and *in vivo*

Table 2. Interaction effect of media and PGR's on fibre growth in fertilized cotton ovules (cv. LRA 5166).

Media composition	Fibre initiation	Fibre elongation	Callus	Callus + Fibre
IAA + GA + BA (mg l^{-1})				
MS + 0.0 + 0.0 + 0.0	√	-	-	-
MS + 2.5 + 2.5 + 2.5	√	-	√	√
MS + 5.0 + 5.0 + 0.0	√	√ (5-10 mm)	-	-
MS + 5.0 + 2.5 + 2.5	√	-	-	√
TM + 0.0 + 0.0 + 0.0	√	√ (2-3mm)		
TM + 1.0 + 1.0 + 1.0	√	√ (15-17mm)	-	-
TM + 2.5 + 2.5 + 2.5	√	-	√	-
IAA + GA + BA + ABA				
MS + 5.0 + 5.0 + 0.0 + 0.1	√	Fibre initiated and degenerated with time		

Note: Basal medium (MS/TM) constituted of sucrose (20 g l^{-1}), glucose (10 g l^{-1}), ascorbic acid (50 mg l^{-1}) & casein hydrolysate (500 mg l^{-1})

Table 3. Influence of PGR's on accumulation of reducing sugars, total free amino acids and total soluble protein in 10 day old ovules of LRA 5166 under *in vitro* ovule culture.

Treatment combination (mg/l)	Reducing sugars (mg/gFW)	Amino acid (mg/gFW)	Total soluble Protein (mg/gFW)
1 IAA + 0.5 BA + 1 GA	94.6	22.8	9.9
1 IAA + 0.5 BA + 5 GA	43.8	13.4	22.5
1 2,3-D + 0.5 BA + 1 GA	72.3	18.6	18.1
1 2,4-D + 0.5 BA + 5 GA	22.4	11.3	19.8
0.5 IAA + 0.5 BA + 1 GA	56.3	42.7	11.3
0.5 IAA + 0.5 BA + 5 GA	40.4	20.8	10.4
0.5 2,3-D + 0.5 BA + 1 GA	37.8	11.9	13.6
0.5 2,4-D + 0.5 BA + 5 GA	27.7	16.9	5.1
0.5 IAA + 5 GA	40.4	8.3	4.3
0.5 2,4-D + 5 GA	70.5	9.2	3.5

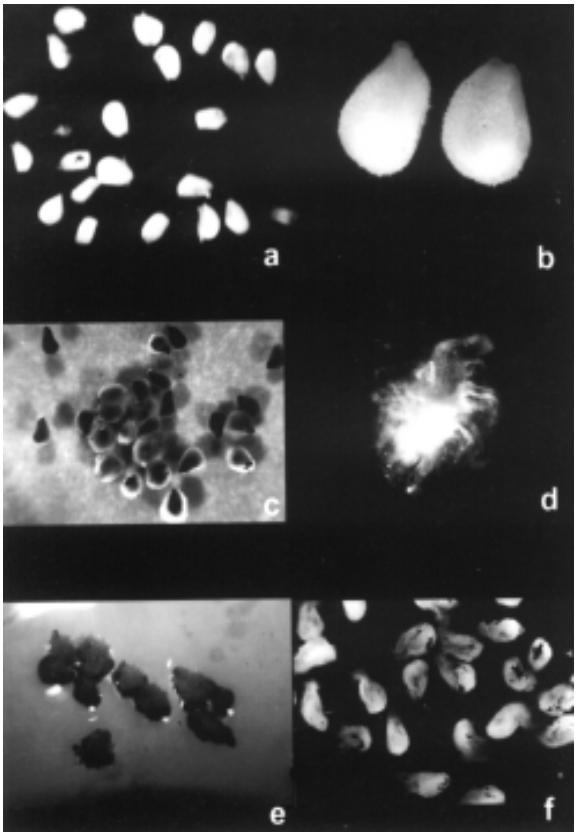

Plate (a-f). a. Freshly isolated ovules, b. Five day old ovuleswith fibre initials, c. Ten day old ovules with fibres, d. Twenty day old ovules with fibre length of 15-17 mm, e. Ten day old ovules on semi-solid medium, f. Fibre initials at micropylar end

conditions. It was observed that the rate of fibre growth was high till 20 days under *in vivo* conditions and a length of 28 mm was reached and from then onwards it slowly elongated to 30 mm by 30th day (Fig. 1). Under *in vitro* conditions, perceptible fibre growth was observed only after 5th day and then slowly elongated and reached a maximum length of 17 mm by 25-30 days. The cultured ovules required 2-3 days for acclimatization. A mean fibre length of 6 – 8 mm only has been reported in cultured ovules of certain cotton cultivars (Gokani and Thaker 2002).

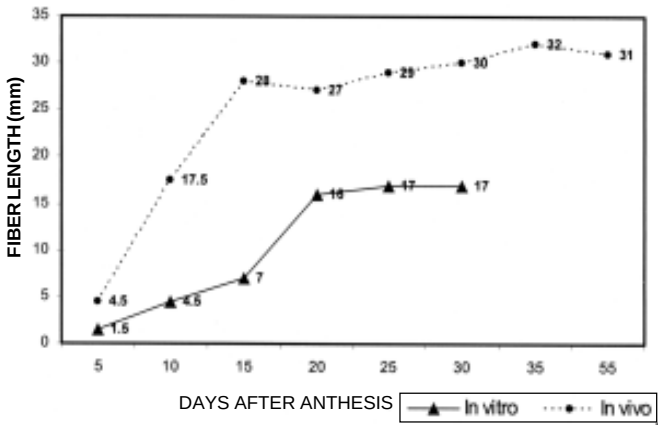

Fig. 1. Relative growth of ovules under *in vitro* and *in vivo* conditions

Attempts are underway to finalise a protocol for *in vitro* fibre development akin to that of natural fibre development under field conditions. These studies assume significance from the point of view of regulatory aspects of fibre development process vis-à-vis fibre strength and micronaire traits in cotton for tailoring to modern day processing in textile industries.

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REFERENCES

Beasley, C.A. and Ting, I.P. (1973). The effect of plant growth substances on *in vitro* fibre development from fertilized cotton ovules. *Amer. J. Bot.* **60:** 130-139.

Cosgrove, D.J. (1997). Relaxation in a high-stress environment: The molecular basis of extensible cell walls and cell enlargement. *Plant Cell.* **9:** 1031-1041.

DeLonge, E.A.L. (1986). Lint development. In: J.R.Mauney and J.M. Stewart (eds.), Cotton Physiology, pp. 325-349. Cotton foundation, Memphis, T.N.

Dunlap, J.P., Kresovich, S. and McGee (1986). The effect of salt concentrations on auxin stability in culture media. *Plant Physiol.* **81**: 934-936.

Eid, A.A.H., De Lange, E. and Waterkeyn, L. (1973). *In vitro* culture of fertilized cotton ovules. I. The growth of cotton embryos. *La Cellule* **69:** 361-371.

Feng Rong and Brown, M. R. (2000). A novel cotton ovule culture: induction, growth and characterization of submerged cotton fibers (*G. hirsutum* L.). *In vitro Cell. Dev. Biol.* **36:** 293-299.

Graves, D.A., and Stewart, J.M. (1988). Chronology of the differentiation of cotton (*G. hirsutum* L.) fibre cells. *Planta* **175:** 254-258.

Gokani, S.J. and Thaker, V.S. (2002). Physiological and biochemical changes associated with cotton fibre development. IX. Role of IAA and PAA. *Field Crop Res.* **77:** 127-136.

Kosmidou-Dimitropoulou, K. (1986). Hormonal influences in fibre development. In: J.R. Mauney and J.M. Stewart (Eds)., Cotton Physiology, pp 361-373. The cotton Foundation, Memphis, T.N.

Meinert, M.C. and Delmer, D.P. (1977). Changes in biochemical composition of the cellwall of the cotton fibre during development. *Plant Physiol*. **59:** 1088-1097.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bio-assay with tobacco tissue culture. *Physiol. Plant*. **15:** 473-497.

Prakash, A.H**.,** Gopalakrishnan,N., Khader, S.E.S.A. and Venugopal, K. (2002). Biochemical factors associated in controlling fibre elongation in different cotton genotypes. *Indian J.Plant Physiol***. 7:** 192-194.

Ruan, Y.L. and Chourey, P.S. (1998). A fibreless mutant in cotton is associated with lack of fibre cell initiation in ovule epidermis and alterations in sucrose synthase expression and carbon partitioning in developing seeds. *Plant Physiol*. **118:** 399-406.

Sadasivam, S. and Manickam, A. (1992). Biochemical Methods. A New Age International (P) Ltd. Publication, Bangalore.

Shahin, E.A. (1985). Totipotency of tomato protoplasts. *Theo. Appl. Genet*. **69:** 235-240.

Smart, L.B., Vajdani, F., Maeahima, M. and Wilkins, T.A. (1998). Genes involved in osmoregulation during turgor driven cell expansion of developing cotton fibres are differentially regulated. *Plant Physiol*. **116:** 1539-1549.

Triplett, B.A. and Timp, J.D. (1995). Characterization of cell-wall polymers from cotton ovule culture fibre cells by Gel Permiation chromatography. *In vitro Cell. Dev. Biol*. **31:** 171-175.

Turley R.B. and Freguson, D.L. (1996). Changes of ovule proteins during early fiber development in a normal and fibreless line of cotton. *J. Plant Physiol*. **149:** 695-702.

Van't Hof. J. and Saha, S. (1997). Cotton fibres can undergo cell division. *Amer. J. Bot*. **84:** 1213-1235.

Xie, W., Trolinder, N.L. and Haigler, C.H. (1993). Cool temperature effects on cotton fibre initiation and elongation clarified using *in vitro* culture. *Crop Sci.* **33:** 1258-1264.