

# Growth of cotton fibre and ovules enhanced by IAA and NAA under *in vitro* conditions

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**ABSTRACT :** An experiment was conducted at the tissue culture laboratory of MGM college of Agricultural Biotechnology, Aurangabad (M.S.) during 2013-14 to evaluate the effects of different concentration levels of auxins *viz.*, NAA and IAA on growth and quality of cotton (*Gossypium hirsutum* L.) ovules under *in-vitro* conditions. The experiment was laid out in Completely Randomized Design, 7 different concentration levels of auxins IAA and NAA. Auxins were tried at the levels of 0, 5, 10 and 15  $\mu$ M conc. of each in culture of 2 DPA flowers of *G. hirsutum* L using Beasley and Ting (BT) medium. Cultures were maintained for 21 days and fibre length, fibre weight, ovule length and ovule weight were recorded. Different concentrations of auxins significantly influenced development of cotton ovules under *in-vitro* conditions. NAA at concentration of 15  $\mu$ M was found significantly superior over rest of the levels of IAA and NAA in case of fibre length, ovule length and ovule weight whereas IAA concentration of 15  $\mu$ M was found significantly superior over rest of other concentrations of IAA and NAA for increasing fibre weight.

**Key Words :** Cotton, Ovule culture, IAA, NAA, Ovule length, Weight fibre length, Weight

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Cotton plant (*Gossypium* sp.) is a shrub belongs to Malvaceae family and native to sub tropical regions of the world (America, Africa and India). The history of cotton can be traced back to domestication, possibly as far back as 4500 BC. Cotton played an important role in the history of British Empire, United States and India and continues to be an important crop and commodity. It is uncommonly sensitive to vagaries of weather. Continuous rains or a long dry spell may affect the growth and development of the crop and thereby production. An annual rainfall of at least 50 cm (well distributed) is a minimum requirement for cotton. It requires black cotton soil with excellent water holding capacity, aeration and good drainage (Panda, 2008).

Cotton accounts for 40 per cent of the total global fibre production and is the most important fibre in the world. It covers around 341.15 lakh ha. area of the world under cultivation with the production of 1174.24 lakh bales (170 kg each) with the global productivity of 743 kg/ha. India is a major player in the world cotton market in terms of area and production. India is now the second largest cotton producer after China with the production of 265 lakh bales (170 kg each) on the area of 116.14 lakh ha. having productivity of 493 kg/ha. It is 2<sup>nd</sup> largest exporter

in the world following to USA, sharing 76 lakh bales (170 kg each) of the global export of 467 lakh bales (170 kg each). Maharashtra accounts for the production of 74 lakh bales (170 kg each) on the cultivation area of around 41.25 lakh ha. with the productivity of 305 kg/ha (Anonymous, 2013 and Anonymous, 2013). It is the backbone of farmer's economy of rain fed farming of tropical and subtropical parts of India. 30 lakh families who are spread over in 22,000 villages in the economically backward regions of Vidarbha and Marathwada of Maharashtra depend upon cotton cultivation.

Cotton covers around 7 per cent of the total crop coverage and is second to rice in India. Cotton textile is one of the largest industries in India. It provides livelihood of 60 million people depending on cotton cultivation, processing, trade and textiles. Textile industry contributes 4 per cent of GDP, 14 per cent of total industrial product, 20 per cent of total work force, 17 per cent of country's exports earning and employment to 30 million people (Anonymous, 2010).

Cotton fibres are single celled outgrowths from individual epidermal cells on the outer integument of the ovules in the developing cotton fruit. Fibres of upland cotton (*G. hirsutum* L.) generally grow up to 30 to 40 mm in length and 15  $\mu$ m in

thickness at full maturity. Their development consists of four overlapping stages: fibre initiation, cell elongation, secondary wall deposition, and maturation. The thickened secondary walls of mature cotton fibres have long been considered unique in that they were thought to consist of nearly pure cellulose and to be devoid of hemicelluloses and phenolics (Fan *et al.*, 2012).

Each cotton fibre is composed of concentric layers. The cuticle layer on the fibre itself is separable from the fibre and consists of wax and pectin materials. The primary wall, the most peripheral layer of the fibre, is composed of cellulosic crystalline fibrils. The secondary wall of the fibre consists of three distinct layers. All three layers of the secondary wall include closely packed parallel fibrils with spiral winding of 25-35° and represent the majority of cellulose within the fibre. The innermost part of cotton fibre, the lumen, is composed of the remains of the cell contents. Before boll opening, the lumen is filled with liquid containing the cell nucleus and protoplasm. The twists and convolutions of the dried fibre are due to the removal of this liquid. The cross section of the fibre is bean-shaped, swelling almost round when moisture absorption takes place. Cotton fibre is composed of 94 per cent cellulose, followed by protein 1.3 per cent, pectic substances 0.9 per cent, 1.2 per cent ash, 0.6 per cent wax, organic acids such as malic acid and citric acid up to 0.8 per cent, 0.3 per cent, total sugar and other trace elements 0.9 per cent (CIRCOT, Anonymous, 2010).

Nearly 30 years ago the conditions for culturing immature cotton ovules were established to serve as a working research tool for investigating the physiology and biochemistry of fibre development. Not only has this tissue culture method been employed to characterize the biochemistry of plant cell expansion and secondary cell wall synthesis, but ovule cultures have contributed to numerous other aspects of plant cell physiology and development as well. Cotton fibre is a powerful cell wall research model because it is an easily isolated single cell with distinct stages of cell wall synthesis. Other advantages include the ability to culture cotton ovules/fibres *in vitro* (Kim and Triplett, 2001).

In addition to basic studies on fibre development, cotton ovule cultures have been used to examine plant-fungal interactions, to model low temperature stress responses, to elucidate the pathways responsible for pigment formation in naturally pigmented fibre and to probe how cytoskeletal elements regulate cell wall organization. Cotton ovule cultures are an especially attractive model system for studying the effects of gravity on cell elongation, cellulose biosynthesis and embryo development and are excellent targets for examining transient expression of introduced gene constructs (Triplett, 2000).

The research work entitled as studies on effect of different concentrations of NAA and IAA on growth and quality of cotton (*Gossypium hirsutum* L.) ovules in *in vitro* conditions was carried out with following objectives :

#### Objective :

- Establishment of *in vitro* cotton ovule culture of fertilized ovules using modified Beasley and Ting (BT) culture medium.
- To study the effect of NAA and IAA on weight and length of ovules.
- To find out the effect of NAA and IAA on weight and length of fibres.

## RESEARCH PROCEDURE

### Collection of flowers and culture :

Flowers of 2 DPA (Day Post Anthesis) stage were collected during morning 9:00 to 11:00 hrs. Flowers were soaked in 70 per cent ethanol for 5-7 min for surface sterilization and rinsed 3-5 times with sterile distilled water to remove traces of sterillant. These sterilized flowers were dissected with the help of sterile forceps and needles and sepals, petals, androecia were removed in aseptic conditions. The ovaries were dissected using sterile needle and the ovules were gently isolated from the dissected ovary and cultured by floating on liquid culture medium (Beasley and Ting, 1971) supplemented with 4 different levels of NAA and IAA each *i.e.* 0, 5, 10 and 15 µM each. Cultures were maintained in the dark at 32°C, which promoted the overall vigorous appearance of the ovule/ fibre units. The Fig. A, B, C and D explains process of cotton ovule culture.

### Measurement of fibre length :

Ovules derived from the cultures induced were taken from the medium at 21 day post culture and rinsed with sterile distilled water. Submerged fibres were excised from the chalazal end of the ovule and placed into glycerol (~80 µl) on the top of a slide. Fibres were separated from each other right after soaking in glycerol for 10 -15 min. Single fibres were drawn from the glycerol with a pair of sterile needles and forceps and aligned on the slide under dissecting microscope. A plastic scale was placed underneath the slide to measure the length of the fibre in mm (Feng and Brown, 2010).

### Measurement of fibre weight :

Fibres were separated from each treatment separately by the above mentioned method and kept on a butter paper and

Treatments	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>
Concentrations of growth hormone	0 µM control no growth hormone	5 µM IAA	10 µM IAA	15 µM IAA	5 µM NAA	10 µM NAA	15 µM NAA



Fig. A : Collection of 2 DPA cotton flower

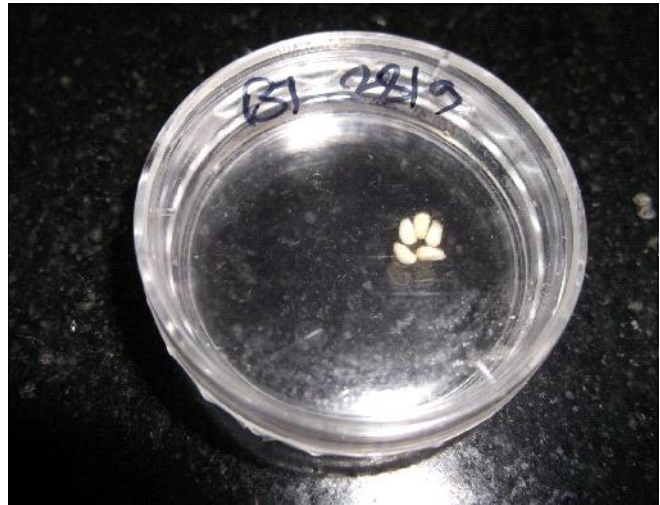


Fig. C : Culture of ovule in BT medium



Fig. B : Aseptic extraction of cotton ovules from ovary



Fig. D : Growth of cotton ovules and fibres after 21 days

weighed by using an analytical balance.

**Measurement of ovule length and weight :**

Ovules from each treatment were picked up by using fine forceps and rinsed in distilled water. These ovules were kept on tissue paper to drain out the distilled water and air dried for a while. These dried ovules were kept on a glass slide with a measuring scale underneath of it. Thus, the ovule length was measured and compared with each other. The weight of each ovule was measured using an analytical balance.

**RESEARCH ANALYSIS AND REASONING**

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

**Length of cotton fibre (cm) :**

Data presented in Table 1 showed that treatment T<sub>6</sub> with mean fibre length 2.17 cm was found significantly superior over all other treatments tried in the study *i.e.* T<sub>5</sub>, T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub>, T<sub>1</sub> and control. NAA at the conc. of 10 µM (T<sub>5</sub>) measured

Table 1 : Length of cotton fibre influenced by different concentrations of IAA and NAA								
Treatments	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	
Mean length of cotton fibre (cm)	1.27	1.53	1.83	1.67	1.73	1.97	2.17	C.D. 0.1196 S.E.± 0.0294

significantly longer fibres with mean length 1.97 cm as compared to the rest of the treatments. Treatments T<sub>2</sub> (IAA at the conc. of 10 µM) and T<sub>4</sub> (NAA at the conc. of 5 µM) were at par with mean fibre lengths 1.83 cm and 1.73 cm, respectively, whereas T<sub>2</sub> was found significantly superior over T<sub>3</sub>, T<sub>1</sub> and T<sub>0</sub>. The difference between T<sub>4</sub> and T<sub>3</sub> was not significant but both were found to be significantly superior to T<sub>1</sub> and control. Similarly IAA at the conc. of 05 µM (T<sub>1</sub>) recorded significantly greater ovule length than control.

#### Weight of cotton fibre (mg) :

Data presented in Table 2 revealed that the different concentrations of IAA and NAA tried in the experiment did differ significantly from each other. Treatment T<sub>3</sub> (IAA at the conc. of 15 µM) recorded significantly higher fibre weight (8.47 mg) in comparison to rest of the other treatments viz., T<sub>2</sub>, T<sub>1</sub>, T<sub>6</sub>, T<sub>5</sub>, T<sub>4</sub> and T<sub>0</sub>. Similarly treatment T<sub>2</sub> (IAA with 10 µM conc.) contributed for significantly higher fibre weight (8.20 mg) than remaining treatments (T<sub>1</sub>, T<sub>6</sub>, T<sub>5</sub>, T<sub>4</sub> and T<sub>0</sub>). Whereas, 5 µM conc. of IAA (T<sub>1</sub>) recorded significantly superior results than T<sub>6</sub>, T<sub>5</sub>, T<sub>4</sub> and control. Treatments T<sub>6</sub>, T<sub>5</sub> and T<sub>4</sub> did not differ significantly whereas, T<sub>6</sub> (NAA with 15 µM conc.) and T<sub>5</sub> (NAA with 10 µM conc.) recorded significantly superior fibre weight than control. Similarly the difference between treatments T<sub>4</sub> and T<sub>0</sub> was not significant.

#### Length of cotton ovule (cm) :

Data presented in Table 3 revealed that the different hormonal treatments tried in the experimental study differed significantly. Treatment T<sub>6</sub> (NAA at the conc. of 15 µM) with mean cotton ovule length 1.83 cm was found to be significantly superior over all other treatments viz., T<sub>5</sub>, T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub>, T<sub>1</sub> and control. Similarly NAA at the conc. of 10 µM (T<sub>5</sub>) recorded significantly greater ovule length (1.47 cm) than rest of the other treatments tried. Treatments T<sub>4</sub> (NAA at the conc. of 5 µM) and T<sub>3</sub> (IAA at the conc. of 15 µM) did not differ significantly and treatment T<sub>4</sub> was found significantly superior

over treatments T<sub>2</sub>, T<sub>1</sub> and control. Also IAA at the conc. of 15 µM (T<sub>3</sub>) and 10 µM (T<sub>2</sub>) were found at par and T<sub>3</sub> recorded significantly greater mean ovule length than T<sub>1</sub> and control. Likewise the difference between mean ovule lengths of T<sub>2</sub> and T<sub>1</sub> was not significant whereas, T<sub>2</sub> was found significantly superior over control. Similarly treatments T<sub>1</sub> and control were found at par.

#### Weight of cotton ovule (cm) :

Data presented in Table 4 indicated that the different concentrations of auxins tried in the experiment were found significant. NAA at the conc. of 15 µM (T<sub>6</sub>) with mean cotton ovule weight 97.13mg was found to be significantly superior over all other treatments viz., T<sub>5</sub>, T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub>, T<sub>1</sub> and control. Similarly, NAA at the conc. of 10 µM (T<sub>5</sub>) recorded significantly higher ovule weight (82.07 mg) than rest of the other treatments tried. Treatments T<sub>3</sub> (IAA at the conc. of 15 µM) and T<sub>2</sub> (IAA at the conc. of 10 µM) did not differ significantly and both were found significantly superior over T<sub>4</sub>, T<sub>1</sub> and control. Similarly, NAA at the conc. of 05 µM (T<sub>4</sub>) and IAA at the conc. of 05 µM (T<sub>1</sub>) were found at par and recorded significantly greater mean ovule weight than control.

NAA and IAA significantly enhanced the growth of cotton ovules and fibres under *in vitro* conditions as they promoted fibre elongation and reasonably vigorous overall growth of ovule/fibre units as compared with control. NAA at the concentration of 15 µM influenced cotton fibre length, fibre weight, cotton ovule length and ovule weight at maximum level whereas IAA showed maximum effect up to 15 µM for cotton fibre length, cotton ovule length and ovule weight and for fibre weight it was up to 10 µM for all NAA concentrations tested in the experiment. The cotton length, weight fibre length and fibre weight is increased with the treatment of auxins as they rapidly increases the cell wall extensibility by increasing co-efficient of cell wall extensibility and turgor pressure, which are responsible factors for cell wall elongation. It also increases the activity of H<sup>+</sup> ATPase from plasma membrane providing H<sup>+</sup>

**Table 2 : Weight of cotton fibre influenced by different concentrations of IAA and NAA**

Treatments	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	
Mean weight of cotton fibre (mg)	1.10	3.03	8.20	8.47	1.60	1.93	1.97	C.D. 0.6616 S.E. ± 0.1625

**Table 3: Length of cotton ovules influenced by different concentrations of IAA and NAA**

Treatments	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	
Mean length of cotton ovule (cm)	0.73	0.83	0.90	0.97	1.07	1.47	1.83	C.D. 0.1108 S.E. ± 0.0272

**Table 4 : Weight of cotton ovules influenced by different concentrations of IAA and NAA**

Treatments	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	
Mean weight of cotton ovule (mg)	20.03	37.17	57.10	61.77	39.20	82.07	97.13	C.D. 5.6101 S.E. ± 1.3784

ions for cell wall loosening and expansion (Taiz, and Zeiger, 2006). From above research work it is concluded that NAA is superior over IAA in increasing cotton fibre length, cotton ovule length and cotton ovule weight whereas IAA was superior over NAA for increasing the fibre weight as NAA suppress secondary cell wall deposition of cotton fibres (Singh *et al.*, 2009).

### Conclusion :

A lab experiment was conducted during summer session of academic year 2013-14 at Department of Biochemistry and Molecular Biology, MGM College of Agricultural Biotechnology, Aurangabad to study the effect of different concentrations of NAA and IAA on growth and quality of cotton (*Gossypium hirsutum* L.) ovules in *in vitro* conditions.

All concentrations of NAA and IAA tried in the experiment showed significant influence on growth and quality of cotton ovules in *in vitro* conditions. Among these NAA at the conc. of 15.0  $\mu$ M was found significantly superior over rest of the conc. of NAA. Similarly IAA at 15.0  $\mu$ M conc. proved significantly superior over rest of the conc. of IAA for development of cotton ovule weight, length and weight of fibres. Whereas, 10.0  $\mu$ M IAA was found significantly superior over rest of the IAA conc. for fibre length elongation.

From present investigation it can be concluded that the NAA increased cotton ovule and fibre development with optimum concentration of 15  $\mu$ M and it can be further studied whereas IAA produced highest cotton ovule length, weight and cotton fibre weight at 15  $\mu$ M and cotton fibre length at 1010.0  $\mu$ M. NAA was superior over IAA for increasing cotton ovule length, weight and cotton fibre length but IAA was superior over NAA for increasing cotton fibre weight. The data obtained in this *in vitro* study can be utilized for field trials of these growth hormones.

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