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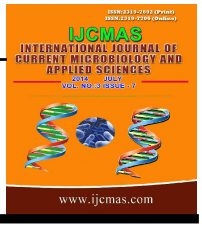

Original Research Article

Growth of Cotton fiber is enhanced by IAA and NAA under in vitro conditions

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A B S T R A C T

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 of cotton (*Gossipium hirsutum* L.) fibers under *in-vitro* conditions. The experiment was laid out in Completely Randomized Design with (CRD) 7 different concentration levels of auxins IAA and NAA. Auxins were tried at the levels of 0,5,10 and 15 µM 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 data on fiber length as well as fiber weight recorded. Different concentrations of auxins significantly influenced development of cotton ovules under *in-vitro* conditions. NAA at concentration of 15 µM was found significantly superior over rest of the levels of IAA and NAA in case of fiber length where as IAA concentration of 15 µM found significantly superior over rest other concentrations of IAA and NAA for increasing fiber weight.

Keywords

Cotton,
Ovule culture,
IAA,
NAA and
Fiber length
and weight.

Introduction

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 fiber 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 fiber 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/fibers *in vitro* (Kim and Triplett, 2001).

Cotton fibers are single celled outgrowths from individual epidermal cells on the outer integument of the ovules in the developing cotton fruit. Fibers of upland cotton (*G. hirsutum* L.) generally grow up to 30 to 40 mm in length and 15 µm in thickness at full maturity. Their development consists of four overlapping stages: fiber initiation, cell elongation, secondary wall deposition, and maturation. The thickened secondary walls of mature cotton fibers 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 fiber is composed of concentric layers. The cuticle layer on the fiber itself is separable from the fiber and consists of wax and pectin materials. The primary wall, the most peripheral layer of the fiber, is composed of cellulosic crystalline fibrils. The secondary wall of the fiber consists of three distinct layers. All three layers of the secondary wall include closely packed parallel fibrils with spiral winding of 25-35^{o} and represent the majority of cellulose within the fiber. The innermost part of cotton fiber, 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 fiber are due to the removal of this liquid. The cross section of the fiber is bean-shaped, swelling almost round when moisture absorption takes place. Cotton fiber is composed of 94% cellulose, followed by protein 1.3%, pectic substances 0.9%, 1.2% Ash, 0.6% Wax, organic acids such as malic acid and citric acid up to 0.8 %, 0.3 % total sugar and other trace elements 0.9%. (CIRCOT, Mumbai, Cotton Statistics at a glance Directorate of Cotton Development, Mumbai, (M.S.) , Ministry of Agriculture Govt. of India 2010).

In addition to basic studies on fiber 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 fiber 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).

Materials and Methods

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% 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, 1973) 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^{0}C, which promoted the overall vigorous appearance of the ovule/ Fiber units. The figure a, b, c and d explains process of cotton ovule culture.

Measurement of Fiber length

Ovules derived from the cultures induced were taken from the medium at 21 day post culture and rinsed with sterile distilled water. Submerged Fibers were excised from the chalazal end of the ovule and placed into glycerol (~80 µl) on the top of a slide. Fibers were separated from each other right after soaking in glycerol for 10 -15 min. Single Fibers 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 Fiber in mm. (Feng and Brown 2010).

Measurement of Fiber weight

Fibers were separated from each treatment separately by the above mentioned method and kept on a butter paper and weighed by using an analytical balance.

Results and Discussion

Length of Cotton Fiber (cm)

Data presented in table 02 showed Treatment T_6 with mean fiber length 2.17 cm was found significantly superior over all other treatments tried in the study i.e. T_5, T_4, T_3, T_2, T_1 and control. NAA at the conc. of 10 µM (T_5) measured significantly longer fibers with mean length 1.97 cm as compared to the rest of the treatments. Treatments T_2 (IAA at the conc. of 10 µM) and T_4 (NAA at the conc. of 5 µM) were at par with mean fiber lengths 1.83 cm and 1.73 cm respectively, whereas T_2 was found significantly superior over T_3, T_1 and T_0. The difference between T_4 and T_3 was not significant but both were found to be significantly superior to T_1 and control. Similarly IAA at the conc. of 05 µM (T_1) recorded significantly greater ovule length than control.

Weight of Cotton Fiber (mg)

Data presented in table 03 revealed that the different concentrations of IAA and NAA tried in the experiment did differ significantly from each other. Treatment T_3 (IAA at the conc. of 15µM) recorded significantly higher fiber weight (8.47 mg) in comparison to rest of the other treatments viz., T_2, T_1, T_6, T_5, T_4 and T_0. Similarly treatment T_2 (IAA with 10 µM conc.) contributed for significantly higher fiber weight (8.20 mg) than remaining treatments (T_1, T_6, T_5, T_4 and T_0). Whereas 5 µM conc. of IAA (T_1) recorded significantly superior results than T_6, T_5, T_4 and control. Treatments T_6, T_5 and T_4 did not differ significantly whereas T_6 (NAA with 15 µM conc.) and T_5 (NAA with 10 µM conc.) recorded significantly superior fiber weight than control. Similarly the difference between treatments T_4 and T_0 was not significant.

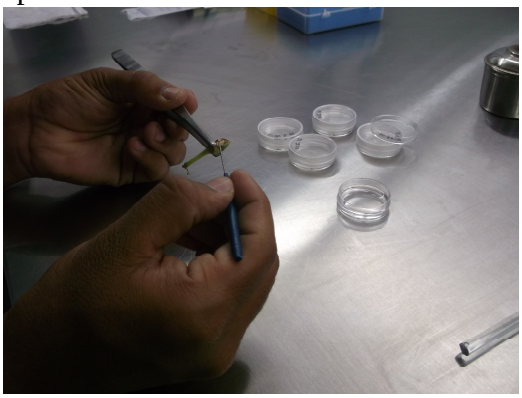
NAA and IAA significantly enhance the growth of cotton ovules and Fibers under *in vitro* conditions as they promoted fiber elongation and reasonably vigorous overall growth of ovule/fiber units as compared with control. NAA at the concentration of 15 µM influenced cotton Fiber length, Fiber weight at maximum level where as IAA showed maximum effect up to 15 µM for cotton Fiber length and for Fiber weight it was up to 10 µM for all NAA concentrations tested in the experiment. The Fiber length and fiber weight is increased with the treatment of auxins as they rapidly increases the cell wall extensibility by increasing coefficient of cell wall extensibility and turgor pressure, which are responsible factors for cell wall elongation. It also increases the activity of H^+ ATPase from plasma membrane providing H^+ ions for cell wall loosening and expansion (Taiz, L. and Zeiger, E. 2006). From above research work it is concluded that NAA is superior over IAA in increasing cotton fiber length, whereas IAA is superior over NAA for increasing the fiber weight as NAA suppress secondary cell wall deposition of cotton fibers (Singh *et al*. 2009). The results obtained from this study can be utilized for field application of auxins.

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 µM was found significantly superior over rest of the conc. of NAA.

Table.1 Treatment details

Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6
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 **b)** Aseptic extraction of cotton ovules from ovary



c) Culture of ovule in BT medium **d)** Growth of cotton ovules and fibers after 21Days

Table.2 length of cotton fiber influenced by different concentrations of IAA and NAA

Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6	
Mean length of Cotton Fiber(cm)	1.27	1.53	1.83	1.67	1.73	1.97	2.17	CD 0.1196 SE 0.0294

Table.3 weight of cotton fiber influenced by different concentrations of IAA and NAA

Treatments	T_0	T_1	T_2	T_3	T_4	T_5	T_6	
Mean Weight of Cotton Fiber(mg)	1.10	3.03	8.20	8.47	1.60	1.93	1.97	CD 0.6616 SE 0.1625

Similarly IAA at 15.0 µM conc. was proved significantly superior over rest of the conc. of IAA for development of weight of fibers. Whereas 10.0 µM 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 increases fiber development with optimum concentration of 15 µM and it can be further studied where as IAA produced highest cotton fiber weight at 15 µM and cotton fiber length at 10.0 µM. NAA is superior over IAA for increasing cotton fiber length but IAA is superior over NAA for increasing cotton fiber weight. The data obtained in this *in vitro* study can be utilized for field trials of these growth hormones.

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