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STUDIES ON EFFECT OF PHYSICO-CHEMICAL MODULATION OF MORPHOGENESIS IN FILAMENTOUS CYANOBACTERIA

Mamta Pandey*

Key words : Physico-chemical modulation, morphogenesis, filamentous cyanobacteria.

In the present investigation, effects of cytokinin (BA) on ***Anabaena doliolum*** have been studied. It has been observed that BA induced maximum cell division in this cyanobacterium.

INTRODUCTION

The basis of morphogenesis in a living organism seems to be a relation between environment and genotype. The phenotype of a system is greatly modified as the environment/ physico chemical factors to morphogenesis is altered. The study of different physico chemical factors regulating morphogenesis is therefore highly significant. The physical and chemical factors may act at any point in the cell cycle (Steward and Krikorian, 1971). The factors act by cell enlargement or enhancement or enhance metabolism by affecting transfer across membrane or by activating enzyme or it could intervene at the higher levels of control where morphogenesis is determined. In the present work attempt has been made to know the response of genotype in the presence of some physico chemical modulators of morphogenesis in filamentous cyanobacterial systems.

It is a well-known fact that in both plants and animals a right balance between different hormones is essential for harmonious growth and development. Common plant hormones include auxins, gibberellins and cytokinins. Excess production of these hormones lead to abnormal growth as is evident from studies on common gall and hairy root disease. The pathogenic bacteria i.e., the causal organism for these diseases transfer its genes controlling auxin and cytokinin production into the host cell. The infected cell after transformation produce abnormal amount of these hormones and keeps dividing and redividing leading to tumorous or hairy root growth. Hormonal manipulation to increase the productivity of plants and animals is an important component of biotechnology. There are innumerable biotechnogical applications of hormones and they are increasing rapidly.

Hormonal regulation of development and differentiation has been studied mostly with higher plants system and is well understood. The growth substances functioning as hormones in higher plants are present in micro-organism as products of secondary metabolism. The question of acquisition of hormonal function during the evolution of plants has attracted as lot of attention. The sex hormones (sex pheromones) of algae and fungi are very different from the phytohormones and some of them have been characterized chemically (Kochert, 1978; Walland, 1986). The sex pheromones are highly specific and probably each arose independently from a metabolic product. Chemical signaling through pheromones had already evolved and become established in sexual reproduction and somatic cell repair in algae. According to Kochert (1978) the pheromones of unicellular eukaryotes could be the ancestor of hormones for all multicellular eukaryotes. In present investigation, effect of cytokinin (BA) on morphogenesis has been studied on *Anabaena doliolum*.

MATERIAL AND METHODS

The material for the present study was *Anabaena dolium* a cyanobacterium. Different concentrations of cytokinin (BA) ranging from 100µm to 0.001 µm were prepared.

The cyanobacterium was grown in BGII medium and harvested by centrifugation and a thick paste was made by mixing with 6% sodium alignate and crushed. The cyanobacterial suspension were extruded dropwise in $CaCl_2$ solution to form fine beads of Calcium alginate. The specimen was kept a room temperature, 4°C and 20°C.

RESULTS AND DISCUSSION

The effect of different concentration of BA (from 100µM to 0.001 µM) was studied on the cyanobacterical system (*A. doliolum*). The blue-green algal cultures were inoculated on 15.09.03 and O.D at 660 nm for growth was taken from 3rd day of culture onwards. It shows that the growth of *A.doliolum* on 3rd and 5th day of culture was better in the presence of all concentrations of BA except 100 µm BA than the control culture. The best growth was shown by *A. doliolum* in the presence of 0.001 µM BA followed by 0.01 µM BA and 0.1 µM BA. The growth pattern is interesting in showing different trends of growth in *A. doliolum* in nine days of culture.

***Biotechnology Lab. University of Botany, B.R.A Bihar University, Muzaffarpur**

The control culture of *A. doliolum* shows steady state growth in the sense that alga shows an increase in O.D. with age upto 9th day. The alga in the presence of 0.001 µM BA shows log phase of growth for the first five days, followed by a decline phase for the next two days then again enters the log phase for the next two days to four days. In the presence of 0.01 µM BA the alga first shows the log phase of growth upto seventh day of culture followed by log phase. The growth pattern is almost same in the presence of 1.0 µM BA and 10.0 µM BA in that in both the cultures the initial lag phase of growth is followed by log phase of growth. It was also interesting to observe that the alga in the presence of 10µM BA showed a higher growth rate as compared to the growth rate of *A. doliolum* in the presence of 1.0 µM BA. It seems that the nutrient medium containing 100 µM BA did not support growth of *A. doliolum*.

The control filaments are typically blue-green long, heterocystous and the heterocyst frequency is about 12.5%. Both terminal and intercalary heterocysts are observed. The following observations were recorded in ten randomly selected slides prepared from cultures of *A.doliolum* in the presence of different concentration of BA (100 µM to 0.001 µM).

Effect of 100µM

1. In the first filament, number of cells were 58 and 3 heterocysts were observed and most of the cells in this filament were dividing. The terminal cell of the non-heterocystous end was dividing. Out of three, one heterocyst was terminal and two were intercalary.
2. In the second filament number of cells were 24 with 2 heterocysts.
3. The third filament had 10 cells with one heterocyst. The position of the heterocyst was sub terminal and the terminal vegetative cell was dividing besides other dividing cells.
4. The fourth filament has 10 cells and heterocyst was absent. Two cells were dividing.
5. In the fifth filament 12 cells are there and only one heterocyst was found. The position of heterocyst was intercalary and it was clear and prominent.
6. In the sixth filament 20 cells were observed. Heterocyst was not present. Each and every cell of the filament was in division stage.
7. The seventh filament had 10 cells, showed division stage with one heterocyst and with single polar nodule.
8. In the eighth filament there were 27 cells in filament. Only one heterocyst was noted. The position of heterocyst was terminal.
9. In the ninth filament the number of cells were 7 with only one terminal heterocyst.
10. In the tenth filament 15 cells were observed with two heterocysts.
11. 41 cells were counted in this selected filament with two heterocysts.
12. The most significant observation was spore formation in the presence of 100µM BA.

Effect of 10µM

Following observations were recorded in the randomly selected filaments of *A.doliolum*

1. 15 cells were present in the filament without heterocyst and all cells were dividing.
2. The second filament had 31 cells with one heterocyst and all cells were dividing.
3. The filament number 3 had 12 cells with 2 heterocysts. The position of one of the heterocysts was terminal with 2 polar nodule and other looked like proheterocyst. 5th cell being transformed into heterocyst / pro-heterocyst and the cells between terminal heterocyst and proheterocyst were dividing.
4. 17 cells of the 4th filament had one heterocyst. The position of heterocyst was terminal with 2 polar nodules.
5. The filament number five had 38 cells and three heterocysts were found, all were in intercalary position.

6. In sixth filament 43 cells with 2 heterocysts were observed and the position of heterocyst was both terminal with 2 polar nodules and intercalary.
7. 15 cells were counted in this filament and heterocyst was absent. Three cells were in division stage.
8. In this selected filaments 25 cells and one heterocyst were observed. The filament shows many dividing cells.
9. This filament had 17 cells with one prominent heterocyst. The filament shows many dividing cells.

Effect of 1µ MBA

Following observations were recorded in the randomly selected filaments of *A.doliolum*

(1) In one filament 15 cells were found without any heterocyst. The second filament had 3 cells dividing.

(2) 27 cells with one heterocyst were observed. Position of heterocyst was intercalary and cells were in dividing stage.

(3) The third filament consisted of 3 cells with one heterocyst in terminal position showing 2 polar nodules.

(4) 15 cells were present in this filament. Cells were dividing and no heterocyst formation was observed.

(5) 15 cells were observed in the fifth filament with one heterocyst. 5th cell was heterocyst while the 11th cell looked like proheterocyst.

(6) In the sixth filament 11 cells were recorded and no heterocyst was seen. Two cells were dividing

(7) 28 cells were noticed in this filament with one heterocyst. The position of the heterocyst was terminal with one polar nodule.

(8) 13 cells with one heterocyst were present in the eighth filament. Position of heterocyst was terminal with single polar nodule.

(9) In this filament 20 cells with one intercalary heterocyst were observed.

(10) In this tenth filament, 8 cells and one heterocyst were present. Terminal heterocyst with one polar nodule was observed.

(11) Isolated, scattered heterocysts were present. Some were with one polar nodule and some with two.

(12) The filament had 6 cells with one heterocyst. 2 cells were dividing next to heterocyst.

(13) 15 cells of the filament consisted of one heterocyst. Heterocyst was with 2 polar nodules.

Effect of 0.1 µM BA

The following observations were recorded.

(1) Thirty cells were present in the filaments with one heterocyst. The position of the heterocyst was terminal with one polar nodule. The other terminal cells were dividing.

(2) The second filament had 16 cells in the filament and no heterocyst formation was noticed. One cells in the center was the largest among all and looks like proheterocyst. The terminal cell was almost spherical and it looked like proheterocyst.

(3) Twenty three cells were found in the third filament and the filament did not show heterocyst formation. Seven cells were dividing.

(4) Twenty five cells were observed in this filament with 2 heterocysts. Cells were spherical. The first and the 3rd cells enlarge either to differentiate into proheterocyst or heterocyst prepare for division.

(5) In this filament 30 cells were observed and no heterocyst was found. 14th and 16th cells were dividing.

(6) 15 cells were observed in this filament with one heterocyst. The position of heterocyst was terminal with single polar nodule.

(7) This filament had 8 cells. No heterocyst formation took place.

(8) In this filament 6 cells were observed and no heterocyst formation took place.

(9) 17 cells were counted in this filament and no heterocyst formation was observed. The form of filament was semi-circular.

(10) 15 cells were observed in this filament with one heterocyst. 8th and 10th cell were in dividing stage. The intercalary heterocyst with double polar nodules was observed.

Effect of 0.01µ BA

The following observations were made:

(1) 30 cells were observed in this filament with one heterocyst. The position of heteocyst seems to be terminal with single polar nodule.

(2) 17 cells were present in a filament with two heterocysts. One heterocyst was present at each end. All cells were dividing.

(3) 7 cells were observed in filament number three and no heterocyst was found.

(4) This filament had 5 cells and no heterocyst formation was found. All cells are in dividing stage.

(5) This filament had 10 cells and no heterocyst formation was observed. One spherical cell seems to be proheterocyst.

(6) The filament was made up of 8 cells with 2 heterocysts. 2 cells are in dividing stage.

(7) The filament had 15 cells with no heterocyst formation.

(8) No heterocyst formation took place in this filament and three cells were dividing.

Effect of 0.001 µM BA

The following observations were made:

(1) This filament had 40 cells with 2 heterocysts. One heterocyst is intercalary while the other is terminal.

(2) There were 34 cells arranged in a filament with two heterocysts.

(3) 5 cells in a filament were counted with one heterocyst. The position of heterocyst is terminal.

(4) 15 cells in this filament were observed with one heterocyst with 2 polar nodules.

(5) 15 cells were arranged in a filament with two heterocysts.

(6) This filament had 41 cells and two heterocysts were present.

(7) Mostly, the filaments were blue-green, heterocystous and cells were frequently observed in division stage.

Differentiation in an organism is the result of the controlled sequential information in the genome. The difference in the character and location of growth which leads to structural or functional differentiation is determined not only by the genetic constitution of the plant but by the particular environment in which development takes place. Variety of external factors exist which effect the ontogenetic pattern. The ultimate basis of differentiation must be in physiological changes in the living material itself. In all the cases of cellular differentiation the portion of the cell in the developing system is highly concerned with the type of differentiation it undergoes.

The development in *A. doliolum* involves the formation of several distinct cell types such as vegetative cell, spore and heterocyst in précise time phase manner at specific locations. This phenomenon known as morphogenesis results through a series of highly co-ordinated and genetically determined process. It involves selective gene expression i.e. the right genes must be expressed in the right cells at the right time. In general a multicellular organism employs two classes of discernible mechanism to bring about growth and differentiation and to elicit growth responses to environmental stimuli :

(1) System of chemical messengers which direct cells to carry out various functions of growth and differentiation, and

(2) System of physical or field forces.

The former includes hormones or chemical regulators and the latter might include electric gradients, mechanical or structural constraints and metabolic or gas concentration gradients. Very little is known about the role of physical and field forces in regulation of growth and differentiation. On the other hand, much information has accumulated about hormone systems and chemical control mechanism in developing the biologists picture of how plant cells are regulated in growth and differentiation. However, very little work has been done on cyanobacterial responses to growth hormones. The plant growth regulators used in the present study are cytoknin (BA).

The effect of growth regulators on cyanobacterial morphogenesis has been limited to a few sporadic reports (Bunt, 1961; Ahmad and Winter, 1968a ; Bahal et al., 1974; Gupta and Agrawal, 1975 Agrawal and Kumar, 1977; Das and Singh, 1977 ; Datta and Angadi, 1990; Vijayrengam, 1990; Sunderavalu, 1990; Kumar 1995).

Kumar and Gupta (1978) who observed aseriate stage of *Hapalosiphon welwitschii* when the alga was treated with green light. It seems likely that cAMP is involved in the induction of aseriate stage by regulating the synthesis of certain photosynthetic pigment which in turn leads to change in the morphology of *N.muscorum*. In *H.welwitschii* probably the green light acts through repression of mitosis and or cell elongation as found in higher plants (Klein, 1970; Rudiger and Scheer, 1983) leading to formation of aseriate condition.

In the presence of Kinetin from 0.001 µg/ml, the pattern of heterocyst differentiation is changed which is significant. Also, abnormal vegetative cells are observed in *A.doliolum* in the presence of kinetin. The formation of abnormal bizarre forms have been reported in *A.doliolum* treated with NTG (Ladha and Kumar, 1977).

There are only a few sporadic reports on the effect of plant hormones in cyanobacteria. Hence the present work was designed to undertake a systematic study on the effect of plant growth hormones on growth and differentiation in a cyanobacterial system *A. doliolum*.

Plant hormones the powerful regulators of plant physiological processes are chemical signals that control morphogenesis i.e. growth and differentiation in plant.

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