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STUDY OF THE EXOGENOUS HORMONAL REGULATION OF LEAF SENESCENCE IN TWO MILLETS, *Setaria italica*, L. AND *Pennisefum typhoides*, BURM.

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ABSTRACT

The present project is aimed at studying the exogenous regulation of leaf senescence in two millets i.e. *Setaria italica*, L. and *Pennisefum typhoides* Burm. The present study is confined to the study of excised leaf senescence by using growth regulators as the exogenous agents. Attached leaf study was avoided for the reason that preliminary experiments showed insensitiveness of the leaves of these two millets to growth regulators in the attached condition. In this study three growth regulators were selected, each from three major groups. Benzimidazole (BZT) represented cytokinins and indole acetic acid represented the auxins. In the present investigation, the cytokinin, BZI was found to be effective in arresting the senescence process in the leaves of both P. typhoides and S. italica in the dark and in the light. Pennisetum was relatively more sensitive to BZI compared to Setaria .The event of senescence is considered concrete when the changes in the bio-molecules like amino acids, protein, DNA and RNA takes place with respect to the growth regulators. The Amino acid content an increasing trend under illumination as compared to dark condition of excised leaves. However, other biomolecules like protein, DNA and RNA decreased with respect to time of illumination in both the plants treated with hormones. The BZA was very effective in reducing the biomolecules in comparison to other hormones.

KEYWORDS : Millet, biomolecules, Hormone, Senescence, Leaf

Pennisetum typhoid's (Burm) is one of the important millets grown in the Northern tract and on the central plateau of India. Also known as Pearl millet, belonging to the family Poaceae (Gramineae), the plant is an annual herb reaching to a maximum height of 3 ft. Rooting takes place at the lower node but the upper nodes are wooly and densely pubescent under the inflorescence. The inflorescence is a spikelet and which are 3-4" long and 4" to '5" dia in flower. *S. etalica* (L) also belongs to the family Poaceae and is known as Italian millet. In Orissa it is commonly known as Kangu,: cultivated on high lands in most districts. The grain is yellow and is eaten. The plant is an erect annual with lanceolate-linear leaves. Inflorescence is a panicle which is 3-5" long and -4" in diameter. Spike lets are oval.08- 0.1" long and are found in clusters on the abbreviated branch lets of the panicles.

Studies on senescence mostly covered plants like rice, wheat, maize and barley. In developing and developed regions of the world, millets (included under cereals) form an important group of plants in contributing to increased food production. As leaf senescence is considered as a physiological determinant of yield, it is the need of the day to understand the mechanism of leaf senescence and also the activities of enzyme involved in different metabolic processes to implicate the programmes aimed at increasing the crop productivity.

In addition to the decline in chlorophyll content, a number of other changes in the bio-macromolecular contents are exhibited during senescence (Beevers, 1976,Thomas and Stoddart, 1980; Thimann, 1980; Nooden, 1980). These changes in bio macromolecules include decline in the levels of DNA and RNA in excised leaves. Schulze and Bosshard (1885) were the first to recognize the importance of proteolysis in the senescence. It has also been considered as an important aspect of senescence.

MATERIALS AND METHODS

Experimental Material

Graded seeds of *Pennisetum typhoides* Burm. And *Stearia italica* L. WERE obtained from Agricultural Research station, Ratnapur in Ganjam district. The seeds were sown in small seed bed plots (1 X 1 m^2). The plants were grown under natural conditions till the 4/5th day and the second leaves of these plants were used as the experimental material.

Selection of Effective Test Chemical Concentration

Leaves were collected, washed and randomized and floated in a range of concentration of each chemical both in the dark and under illumination. The chlorophyll content of these leaves was measured 48 h after incubation. From the texture of the leaves the toxicity of the chemical

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was assessed (loss of texture and development of dark patches was treated as a toxic response). Basing on the chlorophyll content of the leaves, the effective concentrations were chosen. . The optimum concentration of the cytokinin, BZA was0.1mM and for IAA and GA3 was 0.5mM.

Incubation

The leaves were detached from the 6/7 day, old plants, randomized and leaf samples weighing ca 100 mg fresh weight were floated in petridishes (15 cm diameter) containing 50 ml solution of the test chemicals. As the experiments with the experiments with the excised leaves were carried out both in the dark and under illuminated conditions, two sets of petridishes were incubated separately in the dark or under illumination ($20Wm^{-2}$) at RT under aseptic conditions. Leaves floated in distilled water served as controls for both dark and light.

Selection of Effective Light Intensity

Visual changes were observed in the leaves within 24 h of floating in the dark. The yellowing of the leaf was from the tip downwards. As the lower intensities were less effective and the higher ones possibly caused photo-bleaching effects, 20 W m^{-2} was chosen as the optimum intensity for further experiments

Excised leaves were collected, washed, randomized and leaf samples weighting ca 200 mg fresh weight were floated in petridishes containing distilled water. The petridishes were incubated in the dark or under different intensities of continuous light (10, 20, 30, 40, 50, 70, 100, 200 W m^{-2}). For the purpose of providing different light intensities, white light from TL 4OW/54 fluorescent lamps of Philips (India) along with 25W incandescent lamp were used and the chlorophyll content was measured 48 h after incubation.

Estimation of Biochemical Parameters

The Biochemical parameters like Protein (Lowary et al., 1951), Amino Acids (Moore and Stein, 1948), DNA and RNA (Schmeider, 1957) of the hormone treated excised leaves of *Stearia italica* and *Pennisetum typhoides* were estimated following the standard procedures.

Statistical Treatment

For all the data, deviations from means have been indicated in the form of standard errors. In a number of graphs, the points plotted are either overlapping or remain very close, hence, the standard error values have not been shown in the graph to avoid clumsiness and confusion. Wherever necessary, students t -test' were performed.

RESULTS

The results of the biochemical study are given in the Figure No. 1-16. In the dark incubated leaves, there was a sharp rise in the amino acid content upto 72 h followed by a decline 72 h period. At 72 h the amino acid content in the controls was of 439.5% of the initial and became 334% of the initial by 96 h. There was a continuous increase in the amino acid content upto 96 h in the light. At 96 h the illuminated control leaves showed a rise of 347.8 % over the initial, This was close to dark values at 72 h. (Figure 1-4)

BZI prevented the post 72 h decline in the amino acid content in the dark. Thus there was a gradual rise in amino acid content of the BZI treated leaves and by 96 h the content was 337 % over the initial. The effects of IAA and GA3 were almost similar to BZI during the pre 72 h period and both the hormones could not prevent the post 72 decline.

All the growth hormones exhibited the rising trend in throughout the incubation period upto 96 h but there was no definite trend in their individual effects on the changes in the amino acid content under illumination. (Figure1-4)).

Protein content declined parallel to the controls upto 24 h,but in the post 48 h period, BZI caused a considerable retention of protein contents. There was only 30.9% loss of protein in BZI treated leaves compared to the controls where the decline was 60.1% at 96 h. At this time the loss of protein in GA3 and IAA treated leaves was 47.4% and 60.9% respectively. This proved that GA3 was effective in protein retention (Figure 5-8)

Effect of growth regulators under illumination Presence of light caused a significant effect on the efficiency of different growth regulators in checking protein degradation. The action of all three hormones was enhanced, however, except GAg whose effect was

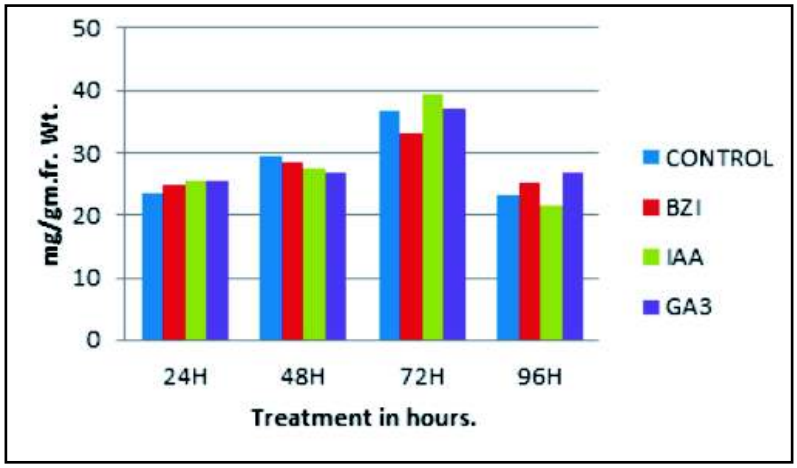

Figure 1 : Changes in Amino Acid Content in *Setaria etalica* (in light) After Different Hormone Treatments.

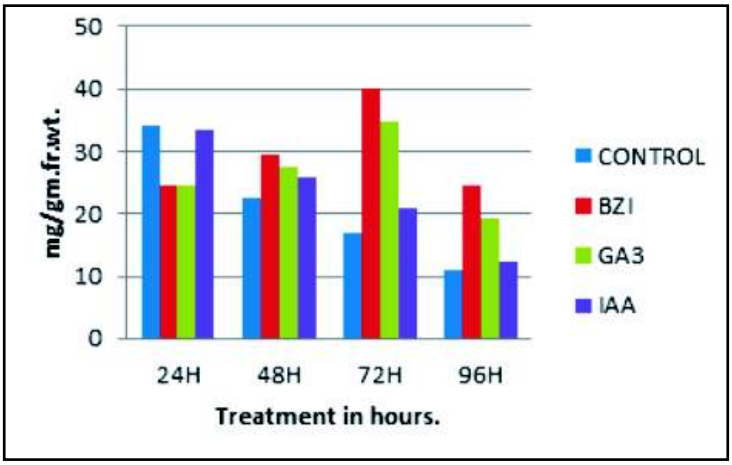

Figure 2 : Changes in Amino Acid Content in *Setaria etalica* (in dark) After Different Hormone Treatments

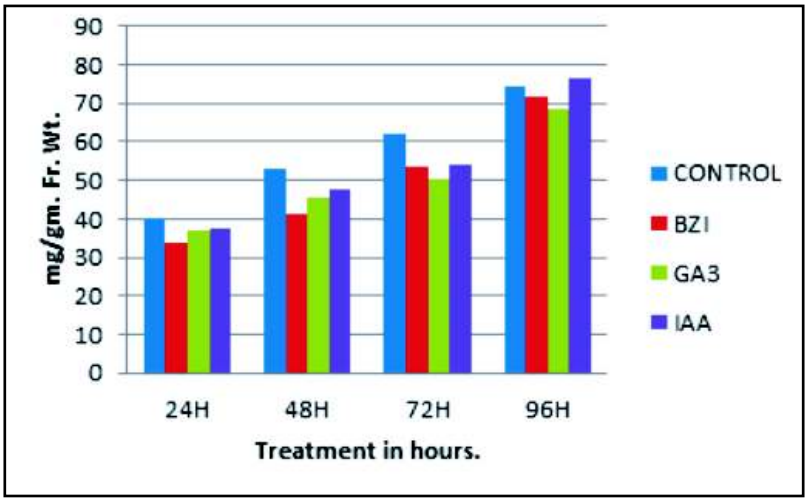

Figure 3 : Changes in Amino Acid Content in *Pennisetum typhoides* (in light) After Different Hormone Treatments

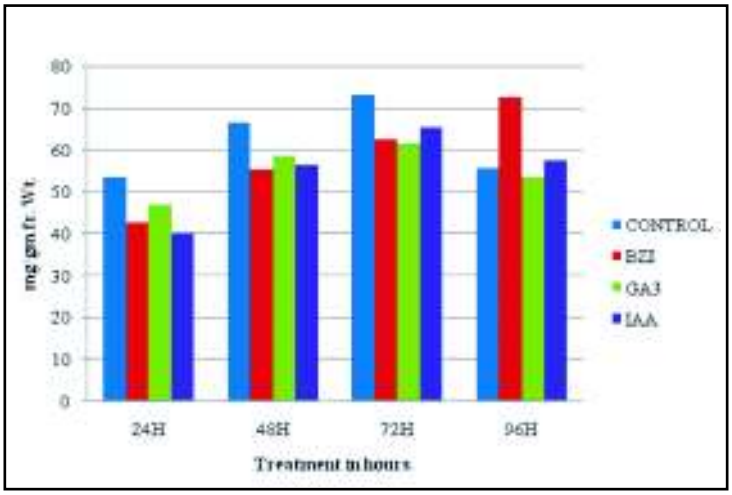

Figure 4 : Changes in Aminoacid Content in *Pennisetum typhoides* (in Dark) After Different Hormone Treatments.

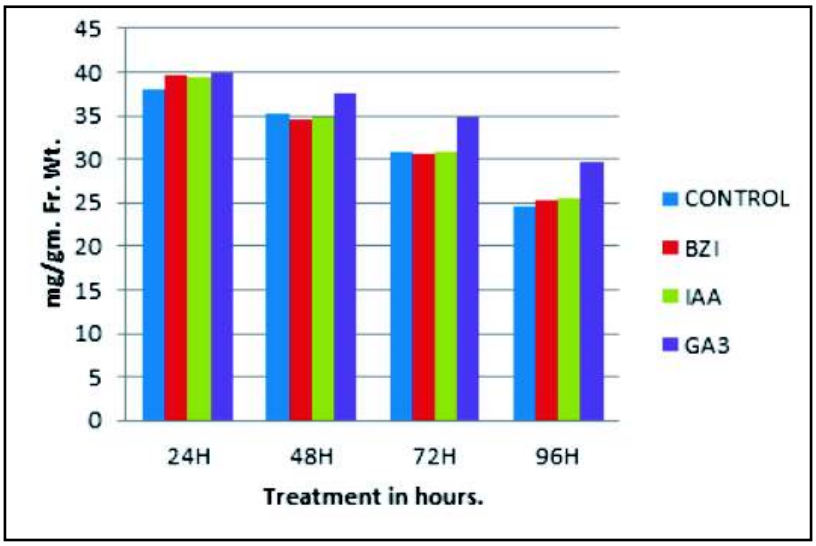

Figure 5 : Changes in Protein Content in *Setaria etalica* (in light) After Different Hormone Treatments

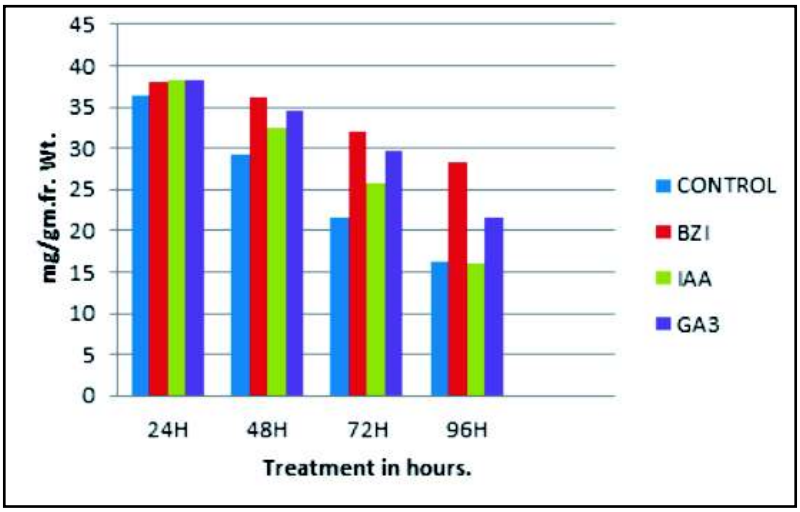

Figure 6 : Changes in Protein Content in *Setaria etalica* (in Dark) After Different Hormone Treatments

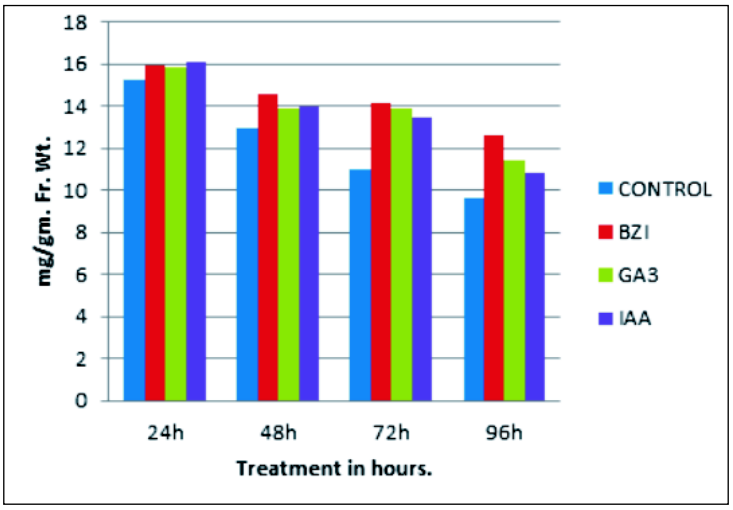

Figure 7 : Changes in Protein Content in *Pennisetum typhoides* (in Light) After Different Hormone Treatments

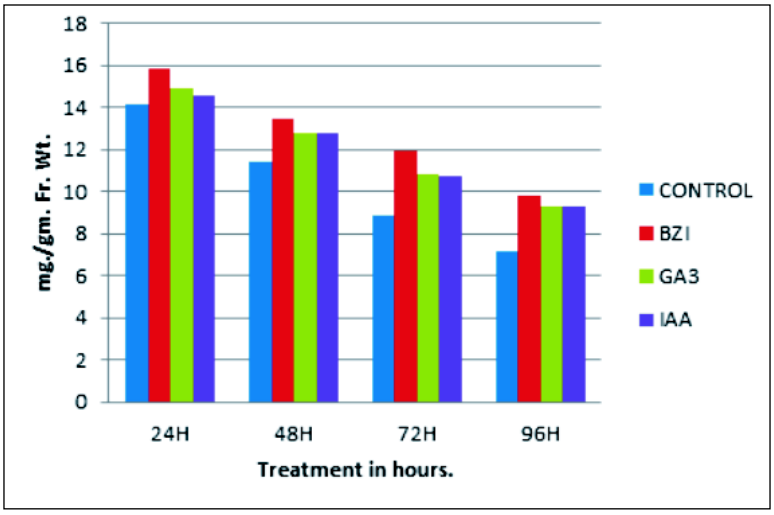

Figure 8 : Changes in protein content in *Pennisetum typhoides* (in Dark) After Different Hormone Treatments

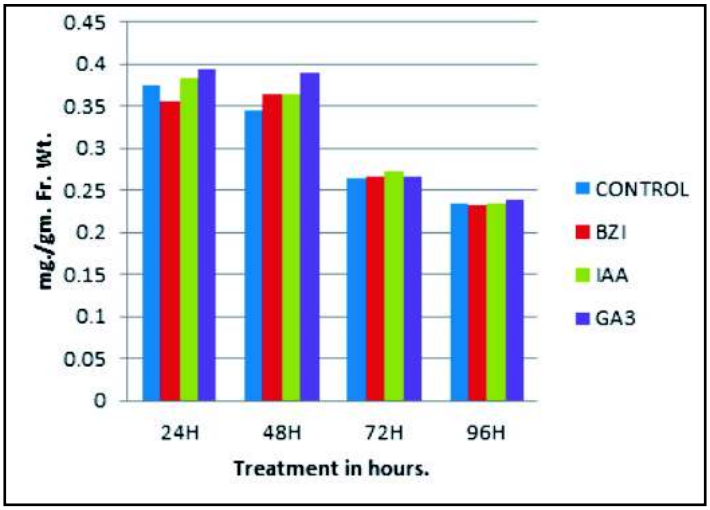

Figure 9 : Changes in DNA Content in *Setaria etalica* (in Light) After Different Hormone Treatments

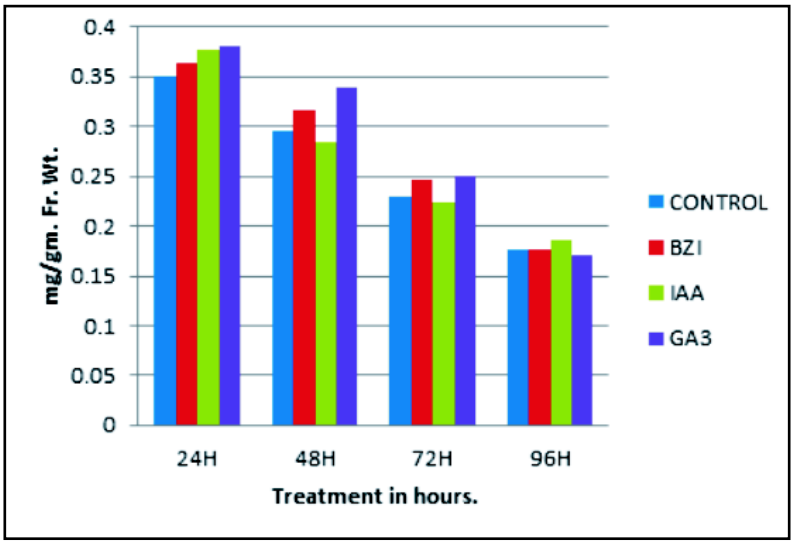

Figure 10 : Changesin DNA Content in *Setaria etalica* (in Dark) After Different Hormone Treatments

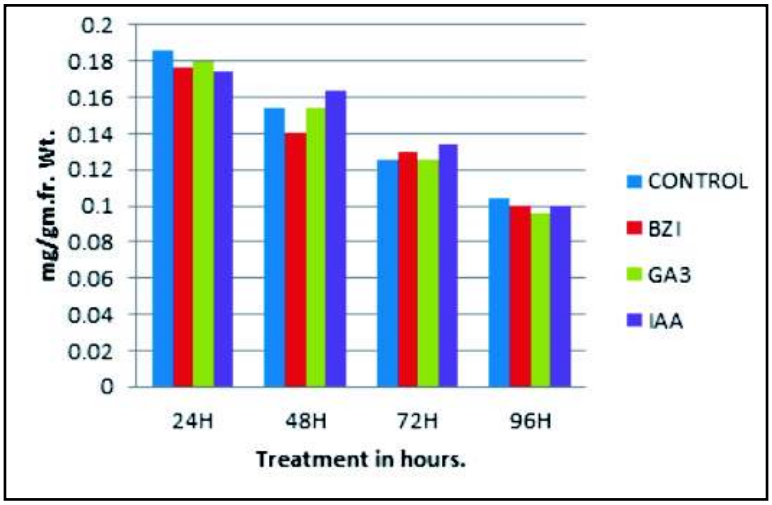

Figure 11 : Changes in DNA Content in *Pennisetum typhoides* (in Light) After Different Hormone Treatments

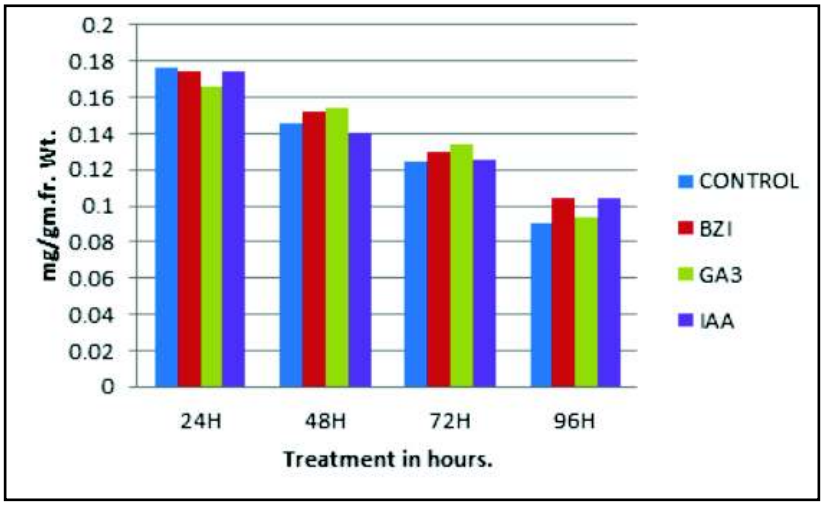

Figure 12 : Changes in DNA Content in *Pennisetum typhoides* (in Dark) After Different Hormone Treatments

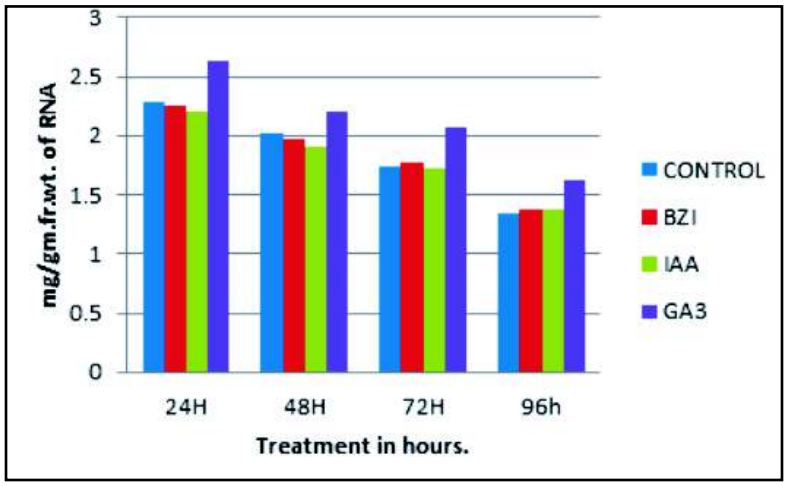

Figure 13 : Changes in RNA Content in *Setaria etalica* (in Light) in Hours

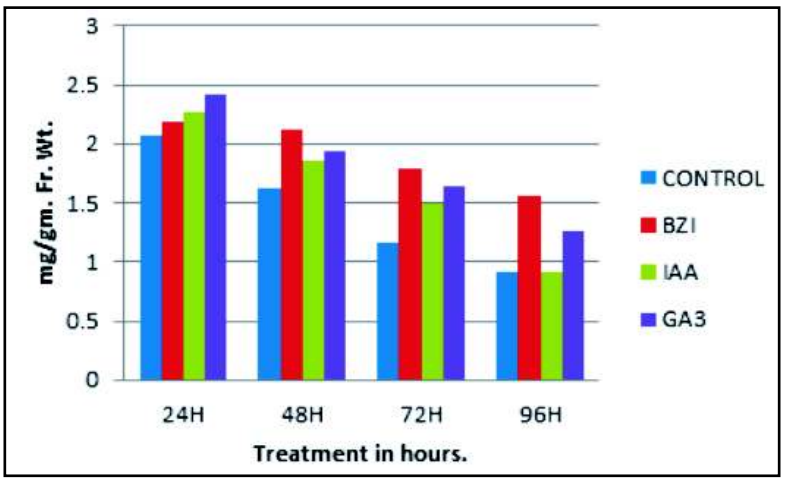

Figure 14 : Changes in RNA Content in *Setaria etalica* (in Dark) After Different Hormone Treatments

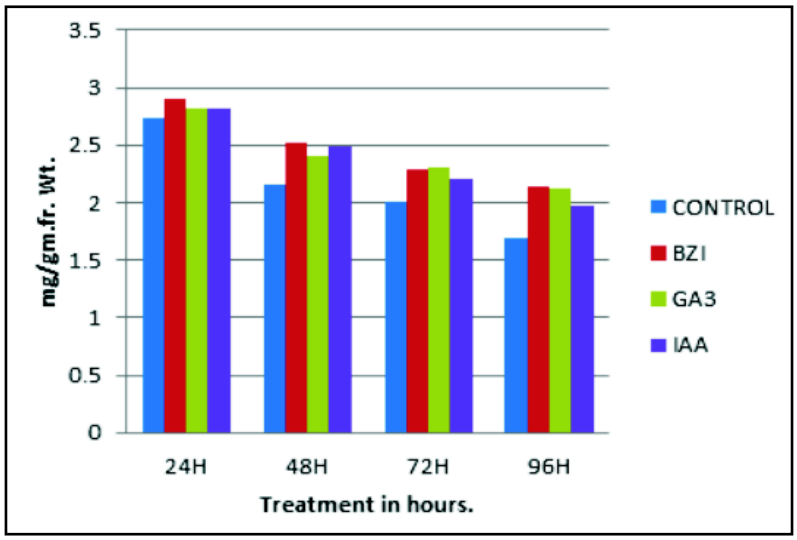

Figure 15 : Changes in RNA Content in *Pennisetum typhoides* (in Light) After Different Hormone Treatments

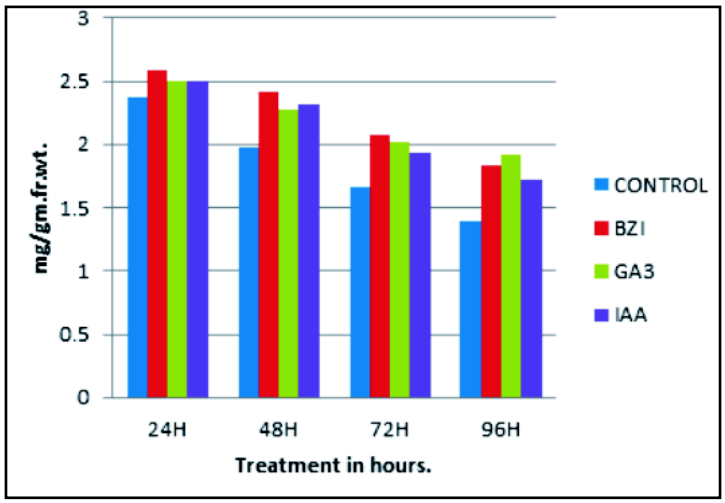

Figure 16 : Changes in RNA Content (in Dark) in *Pennisetum typhoides* After Different Hormone Treatments

enhanced in the presence of light, possibly due to synergetic effect, other growth regulators (IAA and BZI) did not show any change in their action. (Figure 5-8)

The dark incubated excised leaves exhibited a continuous decline in the DNA content with time. By the end of the experiment the DNA content in the dark incubated leaves was 45.1 % in contrast to 59.9% in the illuminated leaves indicating some influence of light on DNA retention. (Figure 9-12)

The RNA content decreased in the dark with only 36.5% of the initial at 96 hours. But light prevented the rate of decline of RNA content. In the illuminated leaves the RNA content was 53 % of the initial at the end of the incubation period.(Figure 13-16)

Both BZI and GA significantly prevented the decline in the RNA content in the dark. BZI was more effective and the RNA content in the hormone treated leaves was 61.9 % of the initial at 96 hours.IAA had little effect on the RNA content.GA3 also prevented the loss of RNA but its efficiency was less compared to BZI (Fig. 13-16).

Among the three growth regulators, GAg was most efficient in causing RNA retention under illumination. BZI and IAA treated leaves showed almost similar contents of RNA at96 h and there was not much of a difference between the RNA content of controls and BZI on IAA treated leaves. In the GAg treated leaves the RNA content was 11.4% higher than the corresponding light controls at 96 h (Figure 13-16).

DISCUSSION

Light retarded excised leaf senescence at a low optimum intensity (20 W m^{-2}). The parameters (chlorophyll, protein, RNA, DNA and amino acids) chosen as indicators of senescence exhibited a decline in their contents and the growth hormones which cheeked the loss of these parameters also prevented the change. All the three growth regulators though differed in their activity in preventing the changes to some extent and their effects differed in dark and light treatments.

The effectiveness of IAA at lower concentration and BZI at higher concentration was also observed in a number of other cases (Mishra and Mishra, 1973;; Kao, 1978). Contrary to the finding that GAg is effective at very low concentration in Maianthemvm. (Horton, 1977), the present study shows that GAg is effective only at high concentration.

The event of senescence is considered concrete when the changes in the chlorophyll are accompanied by the changes in other bio-molecules (Nooden, 1988). It is believed that the change in a single parameter like chlorophyll may be deceptive biomolecules studied in the present investigation is protein, RNA, DNA and amino acids. In, *P. typhaides* there was a decline in the content of protein, DNA and RNA. Growth regulator prevented the loss of protein and RNA." Their effect on DNA was not very significant. The changes in amino acids were not consistent. Excepting the response of amino acids there was a close

correlation between the changes in chlorophyll and other biomolecules.

There are reports that the rates of decline in different components like chlorophyll, protein, DNA, RNA are not same (Mothes et al.., 1959; Smillie and Krotkov, 1961, Hardwick and Woolhouse, 1967, Callow et al. , 1972; Callow, 1974; Nooden,1988). In the present investigation as well the rates of decline in different bimolecular components were different. With few exceptions there was a decline in, protein, DNA and RNA in both the plants.

The cytokinin used by them was kinetin, a synthetic form. Since that time, various attempts have been made at different levels to test the efficacy of exogenous agents in regulating the process. Our present state of knowledge indicates that there are a variety of exogenous agents that are capable of regulating the yellowing of a leaf. However these agents differ in their efficiency regulating the process. Both physical (light) and chemical agents can act as senescence regulators. In this connection the groups of compounds that have drawn maximum attention are the hormones, followed by light and other agents (Beevers, 1976).

In the present investigation, the cytokinin, BZI was found to be effective in arresting the senescence process in the leaves of both *P. typhoides* and *S. italica* in the dark and in the light. Pennisetum was relatively more sensitive to BZI compared to Setaria.

The possibility of growth regulator action is by checking their process of degradation involving various biomolecules including chlorophyll. One of the way by which hormones may act is by preventing the action of hydrolyzing enzymes responsible for the breakdown of chlorophyll, protein, DNA, .RNA, etc

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