EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 1 (January-2015) pp. 30-35

www.ijcrbp.com

Original Research Article

Study of the Exogenous Hormonal Regulation of Leaf Senescence in Two Millets, *Setaria italica* L. and *Pennisetum typhoides* Burm. 1. Plant Pigments

Mamata Mahapatra, K. Bijay Kumar and Bijay Kumar Mohanty*

P.G. Department of Botany and Biotechnology, Khallikote Autonomous College, Berhampur-760 001, Odisha, India

**Corresponding author.*

Abstract

The present work is aimed at studying the exogenous regulation of leaf senescence in two millets i.e. *Setaria italica*, L. and *Pennisetum typhoides* Burm. The present work 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 chlorophyll are accompanied by the changes in other bio-molecules.

Keywords

Hormone

Leaf

Millet

Pigments

Senescence

Introduction

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, 1968 and 1976; Thomas and Stoddart, 1980; Thimann, 1980; Nooden, 1988). These changes in bio macromolecules include decline in the levels of DNA and RNA in excised leaves. Schulze and Bosshard (1985) were the first to recognize the importance of proteolysis in the senescence. It has also been considered as an important aspect of senescence.

Pennisetum typhoides (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. italica* 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.

Materials and methods

Experimental material

Graded seeds of *Pennisetum typhoides* Burm. and *Setaria italica* L. were obtained from Agricultural Research station, Ratnapur in Ganjam district. The seeds were sown in small seed bed plots (1×1 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 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.

Three growth regulators, one from each major group (cytokinins, gibberellins and auxins), were tested for their efficiency in preventing chlorophyll loss. All the concentration of BZI under trial exhibited significant chlorophyll retention capacity. Concentrations beyond 0.1 mM caused toxic effects (the leaves developed dark patches with a loss of texture). The effect of the cytokinin was more significant under illumination. While light itself resulted additional chlorophyll retention compared to the dark, BZI caused synergetic effect leading to higher chlorophyll retention. As a result of this, chlorophyll retention in BZI treated leaves was higher than the corresponding light controls. The optimum concentration of the cytokinin was0.1 mM and IAA did not show any chlorophyll retention at very low concentration but with the increase in concentration, there was a corresponding rise in chlorophyll in leaves in the dark (up to certain concentration). The optimum concentrations chosen for both GA_g and IAA were 0.5 mM.

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 dia.) 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. By 48 h the chlorophyll content was 30% of the initial in *S. italica* and 53% of the initial in *P. typhoides.* There was significant chlorophyll retention when the leaves were exposed to different intensities of light. Such an effect increased with the increase in light intensity up to 20 Wm^{-2} beyond which there was a decline in the pigment content. As the lower intensities were less effective and the higher ones possibly caused photo-bleaching effects, 20 Wm^{-2} was chosen as the optimum intensity for further experiments

Excised leaves were collected, washed, randomized and leaf samples weighing 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 and 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.

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 study are given in the Figs. 1 to 4. There was a rapid decline in the chlorophyll content of the dark incubated leaves. By 72 h the pigment content was decreased by 2.3% in case of the *S. italica*. Illuminated leaves of *S. italica* also showed a decline which was slow and gradual and by 72 h the chlorophyll content was 39.2% of the control values. This showed that light prevented the chlorophyll loss to a significant extent. In case of *P. typhoides* under dark condition, the chlorophyll content was decreased by 1.9% only, but under illumination, the pigment content rose upto48.3% of control values showing that the light prevented pigment loss in the experimental plant.

Fig. 1: Changes in chlorophyll content in *Setaria italica* after different hormone treatments (in dark).

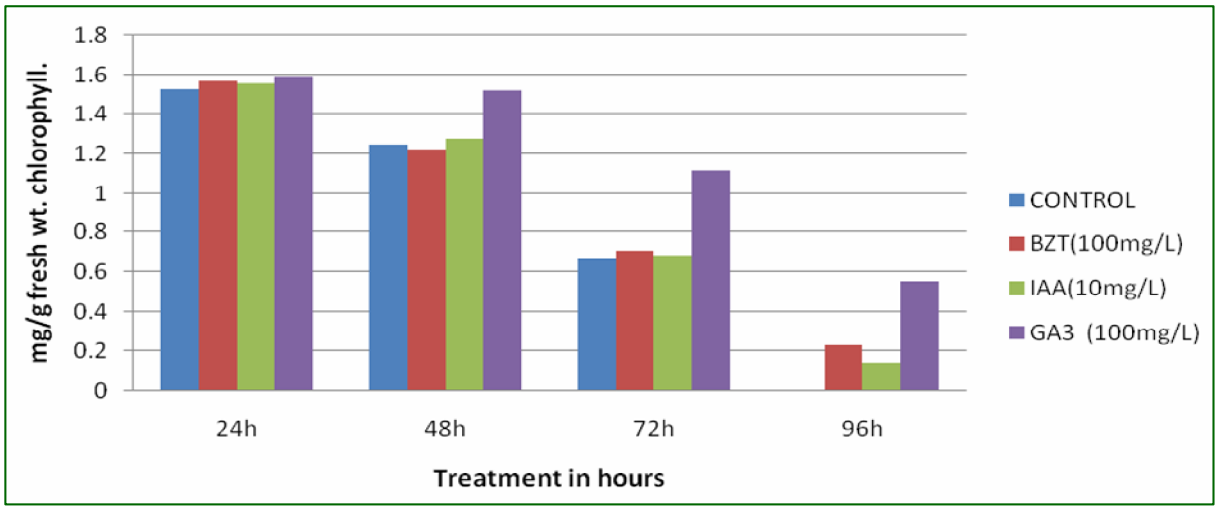


Fig. 2: Changes in chlorophyll content in *Setaria italica* after different hormone treatments (in light).

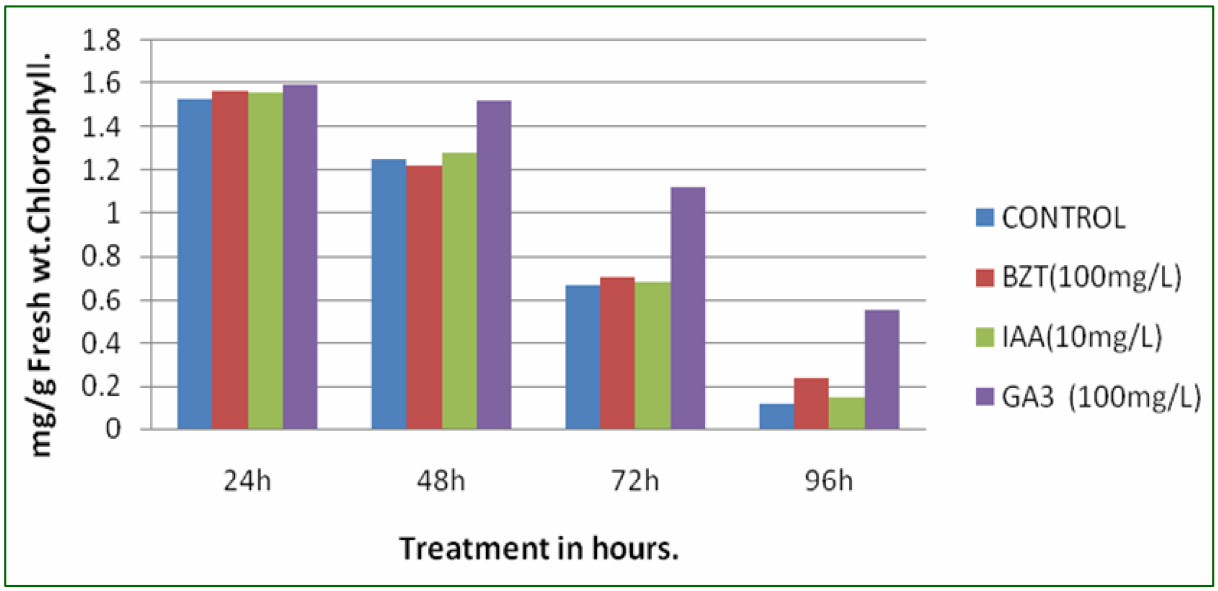


Fig. 3: Changes in chlorophyll content in *Pennisetum typhoides* after different hormone treatments (in dark).

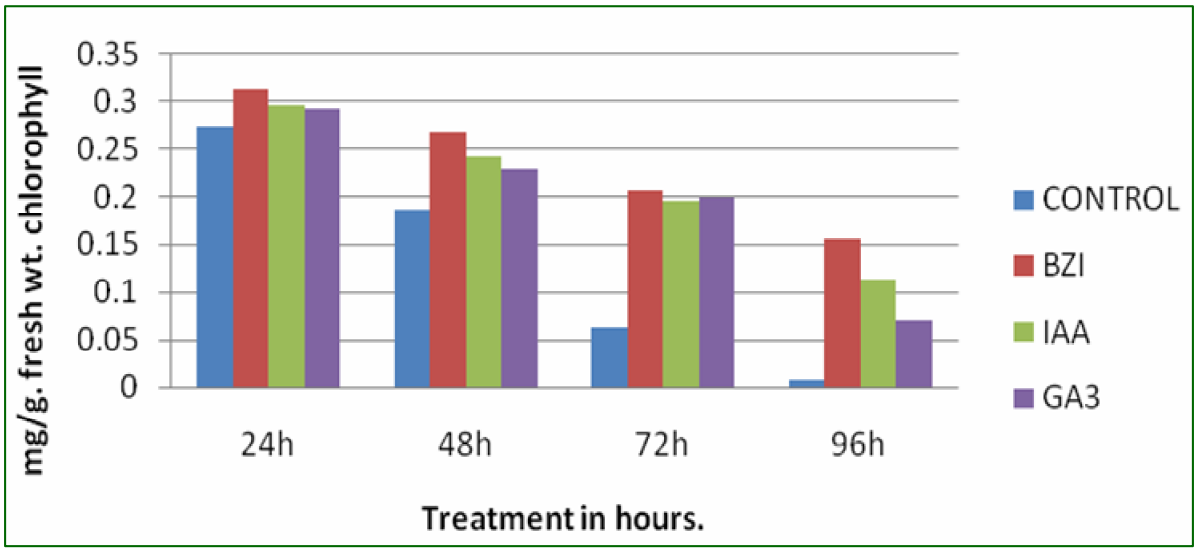
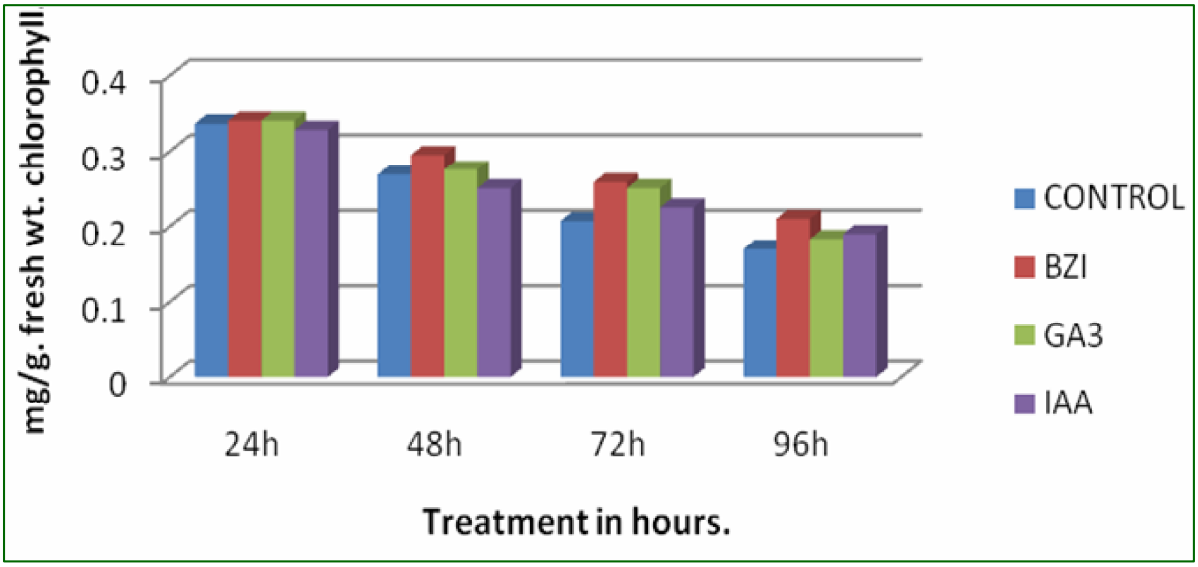


Fig. 4: Changes in chlorophyll content in *Pennisetum typhoides* after different hormone treatments (in dark).



Effect of growth regulators in the dark

Among the growth regulators, BZI could retain significant amount of chlorophyll. At the end of the experiment the chlorophyll content in the BZI treated leaves of *S. italica* was 26% of the initial values. GA_g could retain only 6% of the initial content at this time; whereas IAA was the least effective in case of *S. italica* retaining only 2% of the initial chlorophyll content. In case of *P. typhoides* leaves, The BZI was most efficient hormone in retaining the chlorophyll and the order of efficiency of hormone was, BZI>GA_3>IAA.

Effect of growth regulators under illumination

Though growth regulators could not check the declining trend of chlorophyll loss, the magnitude of loss was less compared to the corresponding dark incubations. Among the growth regulators tried, GA_g was the most efficient and was able to retain 32.4% of chlorophyll content in case of *S. italica*. The efficiency of BZI in retention of chlorophyll under illumination was less compared to dark incubation. In the dark incubation of *S. italica*, BZI could retain 26.2% at 96 h compared to 13.6% retention of the chlorophyll content in illuminated

leaves. In IAA treatments the loss was about 91.5% at 96 h of the control which was 98% in the dark incubation.

In case of *P. typhoides* leaves, the BZI and GA_3 were equally efficient in preventing chlorophyll loss as compared to IAA. All the treated leaves of both the plants showed higher chlorophyll content under illumination.

Discussion

Senescence is such a study area in which lot of work has been done since the 50s. Leaf being a chlorophyllous organ, the major change during its senescence is the loss of chlorophyll. Thus visual yellowing of the leaf is treated as an index underlying physiological and bio-chemical changes associated with senescence. This change is often accompanied with the changes in various bio-molecules and enzymes. The first successful attempt the direction of reversing the yellowing process is by the application of exogenous agents (Osborne, 1967).

Richmond and Lang (1957) were the first to observe the anti senescence action of a cytokinin in *Xanthium* leaves. 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 (Beevens, 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. This observation corroborates the reports that cytokinins show universality in their antisenescence properties (Kende, 1971; Hall, 1973; Thomas and Stoddart, 1980; Goldthwaite, 1987; Nooden, 1988; Van Staden et al., 1988). The mechanism of hormone action is extensively studied and a number of reviews have been published that are related to the hormonal regulation of senescence (Woolhouse, 1967; Beevers, 1976; Letham, 1978; Leopold, 1980; Nooden, 1988; Thimann, 1980, 1985; Thomas and Stoddart, 1980; Sabater, 1985; Nooden and Letham, 1988; Goldthwaite, 1987; Brady, 1988).

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. The possibility of growth regulator action is by checking their process of degradation involving various biomolecules including chlorophyll.

Acknowledgements

Authors are thankful to Principal, Khallikote Autonomous College, Berhampur and HOD, Botany Department for encouragement for research activity and laboratory facilities.

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