



Study of the exogenous hormonal regulation of leaf senescence in two millets, *Setaria italica*, L. and *Pennisetum 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 *Pennisetum 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 (BZI) represented cytokinins and indole acetic acid represented the auxins and GA₃ represented Gibberelic Acid. In the present investigation, a significant negative correlation between RNA loss and RNase activity was found in both *S.italica* and *P.typhoides*. This suggests RNase like chlorophyllase responsible for bio-molecular breakdown. DNase activity was stable, it's activity increasing either in the first 72 h or up to the end of the incubation, but DNase activity decreased with time. Thus there was no correlation between the two. Acid phosphatase activity did not show any correlation with the established parameters of senescence, thus in both the plants the activity of the enzyme cannot be treated as an indicator of senescence. Among the two pyrophosphatases, alkaline enzyme showed a parallelism with the changes in chlorophyll and can be used as a reliable indicator of the process. On the other hand acid inorganic phosphatase activity increased during the incubation, but had no significant relation with other senescence related changes, suggesting more investigations to ascertain the role of acid inorganic pyrophosphatase.

Keywords: Enzymes; Millets; Senescence; Hormone.

1. Introduction

Reports on the activity of the enzyme during senescence are controversial. While some found no correlation between the enzyme activity and chlorophyll changes (Holden, 1974; Sudhakar et al, 1991), others stressed the importance of the enzyme in senescence. Ellsworth et al., 1976; Moll et al., 1978; Sabater and Rodriguez et al., 1987; Garcia, and Galindo, 1991; Majumdar et al., 1991; Drakiewicz and Zbighiaw, 1992). Dephnylation of chlorophyll as an early event of senescence has been investigated in the relevant years (Amir et al., 1986, 1987; Thomas 1987, et al., 1989).

Different types of proteases such as neutral alkaline and acid proteases are well marked during leaf senescence (Balz, 1966; Martin and Thimann, 1972) and the study on these has not yet taken a complete form.

RNase activity increases during leaf senescence (Bagi and Farkas, 1967; Lewington et al., 1967; Udvardy et al., 1967; Truelson, 1967; Mc Hale and Dove, 1968; Sodek and wright, 1969; Deleo and Sacher, 1970; Upadhyaya et al., 1985, Brady, 1988; Balnk et al., 1991. But the rise of RNase was not observed in both attached and detached leaves of certain plants (Srivastava and Ware, 1965). The enzyme acid phosphatase is used as a marker of lysosome in animal tissue (Beever, 1976). This enzyme is detected in chloroplasts and suggestions are there that the enzyme may be involved in the degradation of the later (Balz, 1966). There is unanimity of opinion concerning the role of enzyme (Balz, 1966; Sodek and, Wright, 1969; Deleo and Sacher, 1970; Hodge and sacher, 1975;

Parida and Mishra, 1980; Kenis and Trippi, 1982; Huang and kao, 1991) and certain reports suggest involvement of two acid phosphatases (Sodek and Wright, 1969; Parida and Mishra, 1980). Some suggest this enzyme to be wound induced where as others suggest a de novo synthesis. The possibility that the enzyme may be released from a bound form cannot be ruled out. These aspects prove that the knowledge about this enzyme is still incomplete. Pyrophosphate is released in different biosynthetic pathways. Pyrophosphatases hydrolyze the pyrophosphates and by measuring the relative levels of inorganic pyrophosphates, attempts have been made to measure the biosynthetic capacity of leaf during development and Senescence. This is accomplished to the role of acid and alkaline inorganic pyrophosphatase during senescence (Rausser, 1971; Kar and Mishra, et al, 1981; Sen et al., 1984; Lin and Kao, 1990 a,b).

2. Materials and methods

2.1. 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 X 1 m²). 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.

2.2. 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 (Mahapatra et al, 2015).

Three growth regulators, one from each major group (cytokinins, gibberellins and auxins), were tested for their efficiency in preventing chlorophyll loss. The optimum concentration of the cytokinin was 0.1 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 GA3 and IAA were 0.5 mM.

2.3. 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 (20 W m⁻²) at RT under aseptic conditions. Leaves floated in distilled water served as controls for both dark and light.

2.4. Selection of effective light Intensity

Visual changes were observed in the leaves within 24 h of floating in the dark. 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 weighing ca 200 mg fresh weight were floated in petridishes containing distilled water. The petri dishes 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 40W/54 fluorescent lamps of Philips (India) along with 25W incandescent lamp were used and the chlorophyll content was measured 48 h after incubation.

2.5. Estimation of enzyme activity

The enzyme activities Ascorbic acid oxidase, Chlorophyllase, Acid protease, Alkaline protease, RNase, DNase, Acid inorganic pyrophosphatase, Acid phosphatase, alkaline inorganic pyrophosphatase in the excised leaves of *Pennisetum typhoides* Burm. and *Setaria italica* were estimated following the methods described by Mahapatra (1992).

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.

3. Results

3.1. Changes in acid phosphatase activity (fig. no.01-04)

The activity of the enzyme acid phosphatase increased considerably up to 48 h (220.5%). There was a sharp decline in the enzyme

activity during the post 48 h. By 72 h the enzyme activity was lower than the initial and further decrease was observed during the last 24 h. An almost similar trend was observed under illumination. Among the three growth regulators, GA3 was the only one that could retain the same enzyme activity and the trends in enzyme change were to some extent different from others. The effects of IAA closely followed GA3 but the values in BZI were lower than the controls throughout the experiments.

Growth regulators could not change the trends in enzyme activities and there was no consistency in the relative action of the enzyme.

3.2. Changes in chlorophyllase activity (fig. no.05-08)

There was a slow and gradual rise in the enzyme activity till 72 h. At 72 h the rise was 59% over the initial value and was followed by a decline bringing the levels to 132% by the end of the experiment. A trend similar to dark incubation was exhibited in the illuminated leaves but the post 72 h decline was not seen. The rise was 32% over the controls at 72 h which rose to 42% by the end of the experiment.

All the growth regulators increased the Chlorophyllase enzyme activity with time, but in GA3 and IAA treated leaves there was a post 72 h decline like the controls, a trend which was not evident in BZI treated leaves. BZI treated leaves showed a continuous rise in the enzyme activity till the end of incubation where it was 139% of the initial activity.

All the growth regulators increased the Chlorophyllase enzyme activity slowly and gradually with time. All the hormones were almost equally effective and unlike dark incubation there was no post 72 h decline either in the controls or in response to any other treatment.

3.3. Changes in RNase activity (fig. no.09-12)

The activity of RNase was different under the dark incubation and illumination. In the dark incubated controls, the enzyme activity increased during the first 48 h, where it became 76% higher than the initial activity. But in the post 48 h period the enzyme activity experienced a continuous increase in the enzyme activity with time and by the end of the experiment the increase was 179% over the initial activity.

All the growth regulator treatments checked the rise in RNase activity during the first 72 h of incubation, though there was an inconsistency in the effect of growth regulators on the whole. IAA seemed most effective among the three growth regulators.

All the growth regulators increased the RNase enzyme activity to a considerable extent. BZI was most effective and caused 250% rise in enzyme activity over the initial activity. In this respect IAA and GA3 were close i.e. they caused 184% and 194% rise in enzyme activity.

3.4. Changes in DNase activity (fig. no. 13-16)

DNase activity increased with time under dark incubation and illumination. However, the dark incubated controls experienced a mild post 72 h fall (15%). At 96 h the enzyme activity was 121% of the initial activity in contrast to 152% in the corresponding illuminated controls.

Except IAA, all other growth regulators caused an increase in the DNase enzyme activity in the first 72 h, then a decline was evident in the last 24 h of the experiment. In BZI and GA3 treated leaves the enzyme activity at 96 h were 114% and 117% of the initial activity respectively. The IAA treated leaves exhibited a gradual increase in enzyme activity resulting an increase of 19% over the initial activity at 96 h.

The growth regulators caused an increase in the DNase enzyme activity. BZI treated leaves exhibited rise in enzyme activity up to 72 h, but it experienced a mild fall in the last 24 h of the experiment. In GA3 treatments unlike BZI and IAA treatments a post 48

h decline in enzyme activity was observed which again increased in the last 24 h of incubation.

Changes in Acid inorganic pyro-phosphatase activity (Fig. No.17-20).

In the dark, the activity of Acid inorganic pyro-phosphatase increased during the first 24 h. This was followed by a decrease in next 24 h but finally there was again an increase in enzyme activity till the end of incubation. At 96 h there was a 95.2% increase in enzyme activity over the initial. Trends similar to dark incubation were observed under illumination where the final activity (at 96 h) was 114.3% over the initial activity.

In all the growth regulator treatments the pattern of change in enzyme activity was similar to that of controls. The difference was only in the magnitude in increase. BZI treated leaves showed an increase of 138% in the enzyme activity over the initial activity by the end of dark incubation. In GA3 and IAA treated leaves this level was 133.3% and 109.5% respectively.

As in the dark the growth regulators increased the activity of enzyme over the controls. However the magnitude of their effect was higher. There was no change in the pattern of enzyme activity.

3.5. Changes in alkaline inorganic pyro-phosphatase activity (fig. no.21-24)

In the dark incubation there was a slow and gradual decline in the activity of alkaline inorganic pyro-phosphatase with time. At 96 h, the activity was only 32.4% of the initial activity. Under illumination the same declining trend was evident. However the rate of decline was slow compared to the dark treatments. At 96 h the enzyme activity was 43% of the initial.

All the growth regulators could check the decrease in enzyme activity to some extent throughout the experiment. BZI treated dark incubated leaves at 96 h showed 57.45% enzyme activity against 32.4% activity observed in the corresponding controls. GA3 and IAA also had some effect in checking the rate of decline resulting in retention of 43% and 50.3% of initial enzyme activity respectively by the end of the experiment.

All the growth regulators caused retention of enzyme activity under illumination. Their effects were quantitatively higher than the dark incubations. In contrast to the 57.45% retention in the dark incubation, BZI under illumination caused 62.6% retention of enzyme activity at 96 h. GA3 and IAA treated leaves at 96 h. GA3 and IAA treated leaves at 96 h exhibited 57.4% and 59.7% retention of enzyme activity which was higher compared to the corresponding values in the dark incubation. This suggests that the light had an enhanced effect in retaining the enzyme activity.

3.6. Changes in ascorbic acid oxidase activity (fig. no.25-28)

Ascorbic acid oxidase activity increased gradually up to 72 h both in dark incubated and illuminated controls. At 72 h it was 216.2% over the initial enzyme activity in dark treatments. During the post 72 h period the activity declined showing a difference of 31%. The illuminated leaves showed an increase in enzyme activity of 193% over the initial activity at 48 h, which remained stable during the next 24 h resulting in 285.2% of initial enzyme activity at the end of the experiment.

At the first 24 h the Ascorbic acid oxidase activity in all the growth regulator treatments became more than double of initial activity which still increased up to the end of the experiment. At 96 h the enzyme activity in BZI treatments became four times as that of initial activity compared to 231.5% and 254.6% rise over initial activity in IAA and GA3 treated leaves respectively.

An increasing trend of Ascorbic acid oxidase activity was clearly evident in all the three growth regulator treatments under illumination. But in contrast to dark incubation in BZI treated leaves a post 72 h fall was noticed. At 72 h the enzyme activity was 377.7% of the initial activity in BZI treated leaves which was lowered to 362.5% in the last 24 hours of the experiment. Though BZI was most efficient in the first 24 h of the experiment, at the end of the

experiment GA3 became most efficient causing 385.6% of the initial enzyme activity which is almost 100% more than the corresponding controls.

3.7. Changes in acid protease activity (fig. no.29-32)

Acid Protease activity increased by 73% during the first 24 h in the dark. This rise was followed by a decline till the end of the experiment. At 96 h the activity was 8% lower than the initial. The trends under illumination were almost similar.

The growth regulators had no significant effect on the changes on the Acid Protease enzyme activity. The action of BZI was slightly different from other hormones quantitatively.

Time course change in the Acid Protease enzyme activity under illumination was almost identical to dark incubated leaves. No hormone could alter the changes in enzyme activity to any significant extent under illumination.

3.8. Changes in alkaline protease activity (fig. no.33-36)

Alkaline protease activity increased with time both under dark incubation and illumination. However under dark the increase in enzyme activity up to 72 h was followed by a post 72 h decline. At 96 h the enzyme activity was 168% and 226% of the initial in dark and light incubation respectively.

In the dark incubation alkaline protease activity increased with time in all the growth regulator treatments. Thus the hormones had no significant effect on the pattern of time course change. However the growth regulators prevented the post 72 h decline. Thus there was a continuous rise in the activity of alkaline protease in hormone treatments. But the rise lowered the extent of increase during the first 72 h of incubation. They also prevented the post 72 h decline in enzyme activity. The ultimate rise was minimum in BZI treated leaves followed by IAA and GA3.

All the growth regulators under illumination checked the rise in alkaline protease activity except at 72 h where the activities were almost closer to the controls; the activity of the enzyme in hormone treated leaves was always lower throughout the experimental schedule.

4. Discussions

Chlorophyll depletion is the most striking change during senescence (Beevers, 1976) and the enzyme chlorophyllase may be responsible for such a loss. Till 1980 no biological role was assigned to the enzyme (Simpson et al., 1976). Rudiger et al.(1980) concluded that chlorophyllase is involved only in the breakdown of chlorophyll.

Ascorbic acid oxidase activity increased with time but a mild post 72 h fall was noticed in both *S. italica* and *P. typhoides*, with an exception to light treatment of excise *P. typhoides* continuous rise was observed. Thus it is difficult to comment on the nature of the enzymes and its involvement in senescence. More investigations are needed to confirm conclusion drawn by using the said chemicals with other plant species.

The acid and alkaline pyrophosphatases are involved in various metabolic pathways (Naganna and Sripathi, 1954; Naganna et al., 1955a; Simmons and Butler, 1969). But limited work done on the change in these two enzymes in senescing leaf tissue provide conflicting results. Kar and Mishra (1975) went to the extent the activity of considering alkaline pyrophosphatase as one of the indicator of leaf senescence. Hence, this pair of enzymes need special attention till some meaningful conclusion is drawn regarding their role in the process of senescence.

Phosphate is the key compound in bioenergetics and hence the enzymes involved in phosphate metabolism play a vital role in the plant metabolism. Study of phosphatase is usually confined to only the acid phosphatase as there is no report regarding the presence of alkaline pyrophosphatases in higher plants.

There are two types of RNases both capable of hydrolyzing RNA. This makes the problem still critical (Beever, 1976). The activity of DNase increases during senescence but work done on this enzyme is very limited (Srivastava and ware, 1965; Simon, 1967; Kumar and Khan, 1984; Meyer and Wagner, 1986).

Increase in protease activity is also exhibited during senescence (Anderson and Rowan, 1965; Beevers, 1968; Kar and Mishra, 1977; Peoples and Dalling 1988; Vander Valk and vanloon, 1988). But in most of the cases no correlation was observed between proteolysis and protease activity (Anderson and Rowan, 1966). A significant negative correlation between RNA loss and RNase activity was found in both *S.italica* and *P.typhoides*. This suggests RNase like chlorophyllase responsible for bio-molecular breakdown (Woolhouse, 1968; De Jong, 1972).

DNase activity was stable, it's activity increasing either in the first 72 h or upto the end of the incubation, but DNA content decreased with time. Thus there was no correlation between the two. Acid phosphatase activity did not show any correlation with the established parameters of senescence, thus in both the plants the activity of the enzyme cannot be treated as an indicator of senescence. Among the two pyrophosphatases, alkaline enzyme showed a parallelism with the changes in chlorophyll and can be used as a reliable indicator of the process. On the other hand acid inorganic phosphatase activity increased during the incubation, but had no significant relation with other senescence related changes, suggesting more investigations to ascertain the role of acid inorganic pyrophosphatases.

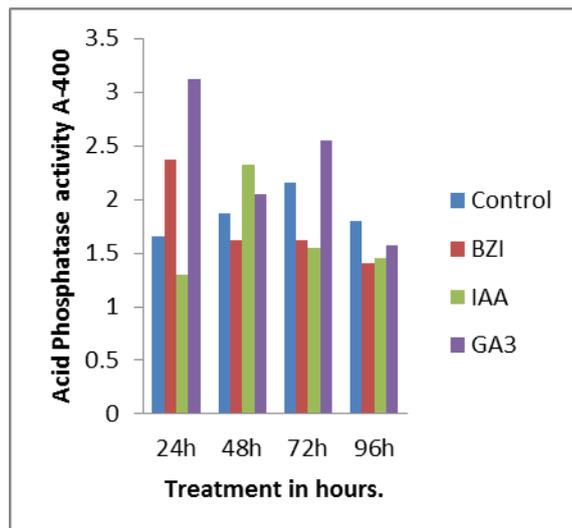


Fig. 1: Effect of Growth Regulators on Changes in Acid Phosphatase Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

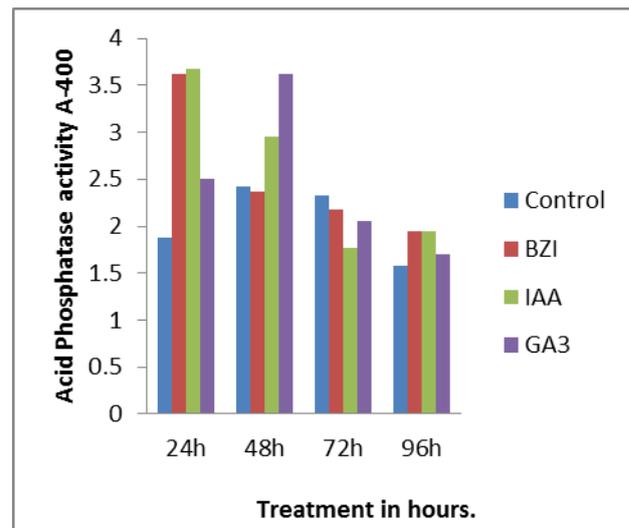


Fig. 2: Effect of Growth Regulators on Changes in Acid Phosphatase Activity in Excised *Setaria italica* L. Leaves During Senescence in Light.

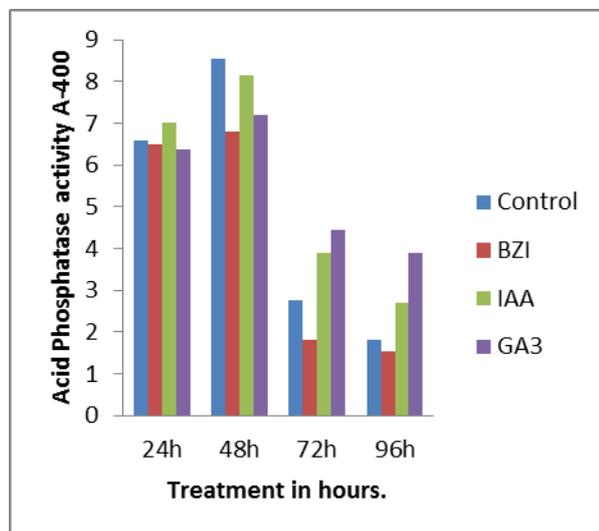


Fig. 3: Effect of Growth Regulators on Changes in Acid Phosphatase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Dark.

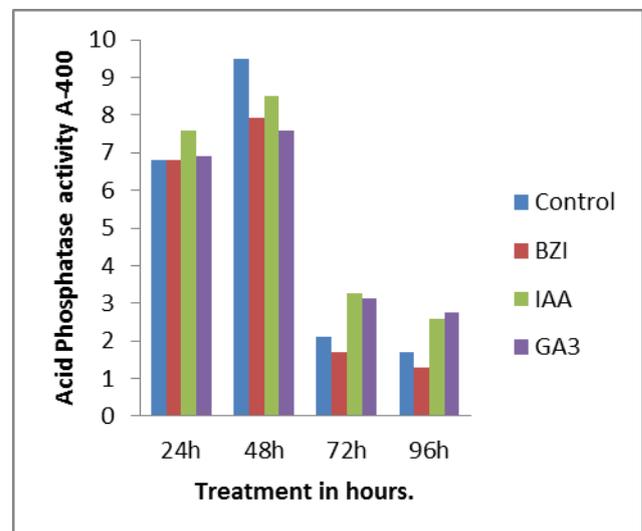


Fig. 4: Effect of Growth Regulators on Changes in Acid Phosphatase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Light.

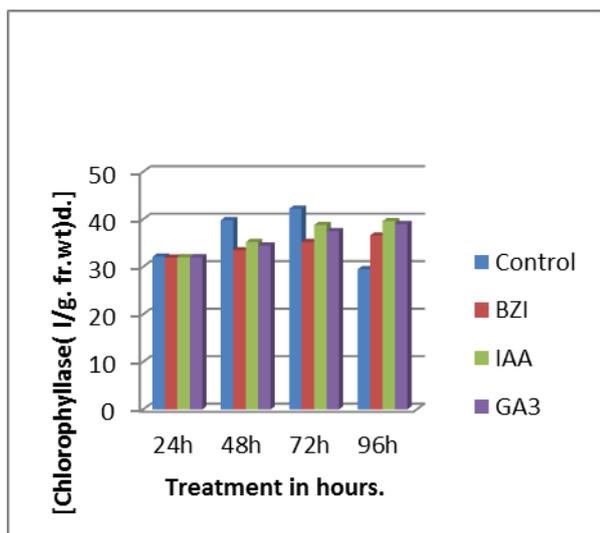


Fig. 5: Effect of Growth Regulators on the Changes in the Chlorophyllase Activity of Excised Leaves of *Setaria italica* L. During Senescence in Dark.

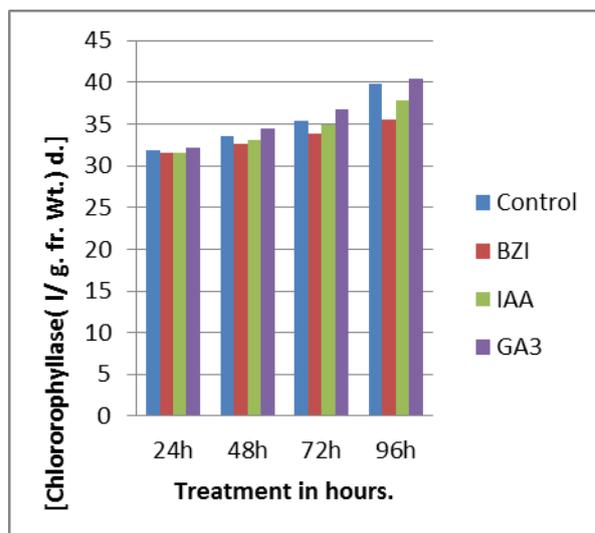


Fig. 6: Effect of Growth Regulators on the Changes in the Chlorophyllase Activity of Excised Leaves of *Setaria italica* L. During Senescence in Light.

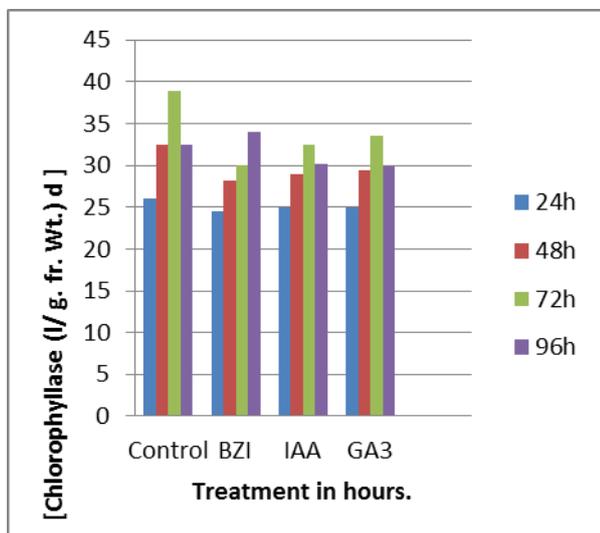


Fig. 7: Effect of Growth Regulators on the Changes in the Chlorophyllase Activity of Excised Leaves of *Pennisetum typhoides* Burm. During Senescence in Dark.

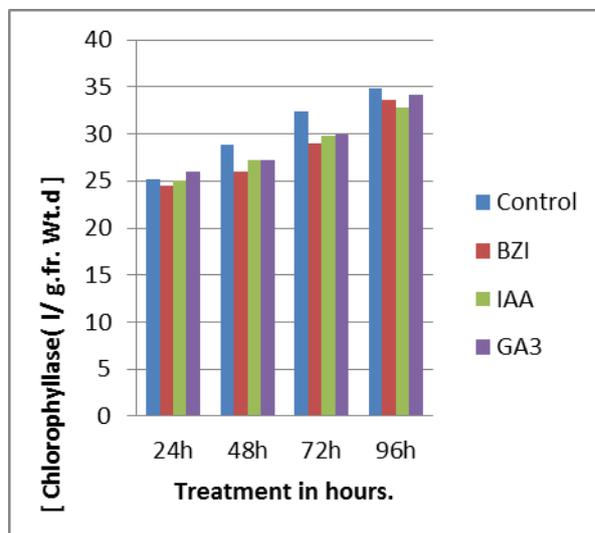


Fig. 8: Effect of Growth Regulators on the Changes in Chlorophyllase Activity of Excised Leaves of *Pennisetum typhoides* Burm. During Senescence in Light.

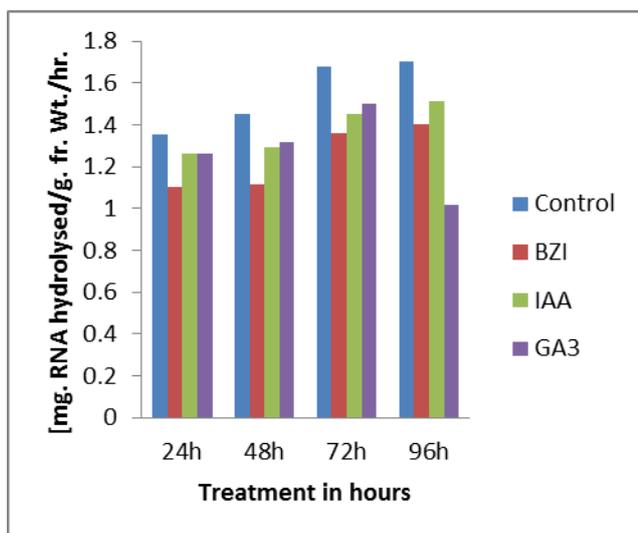


Fig. 9: Effect of Growth Regulators on Changes in Rnase Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

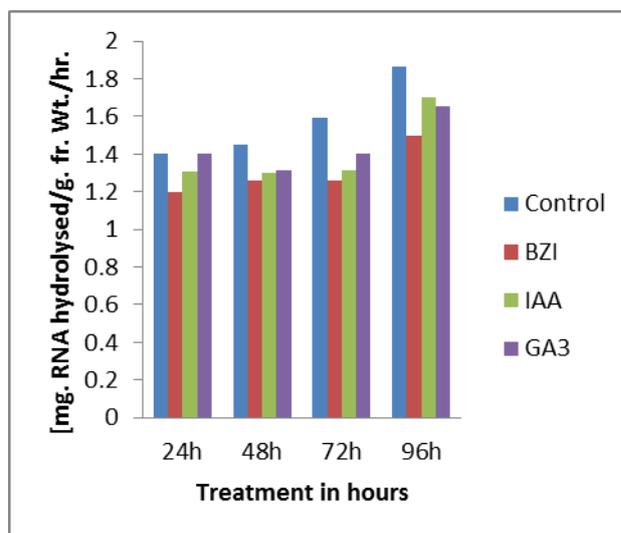


Fig. 10: Effect of Growth Regulators on Changes in Rnase Activity in Excised *Setaria italica* L. Leaves During Senescence in Light.

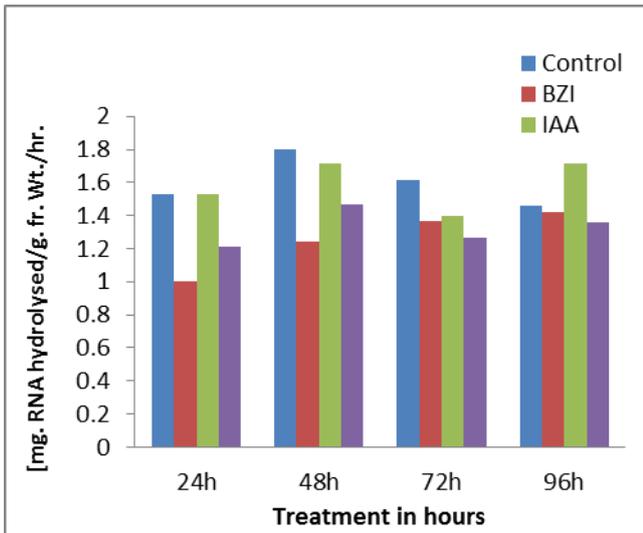


Fig. 11: Effect of Growth Regulators on Changes in Rnase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Dark.

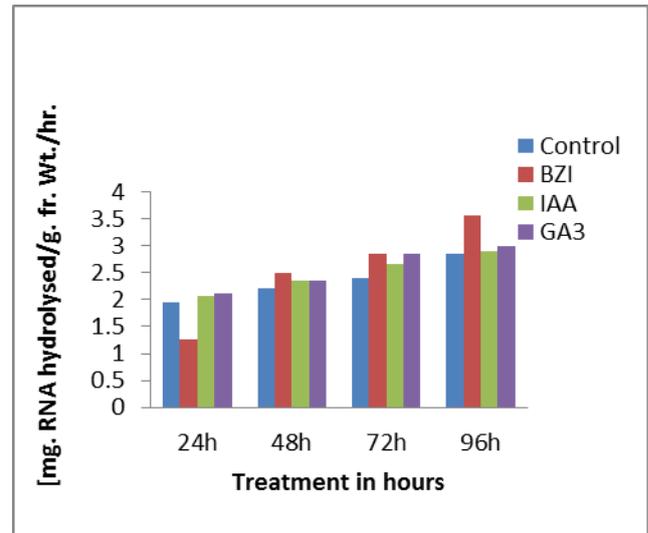


Fig. 12: Effect of Growth Regulators on Changes in Rnase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Light.

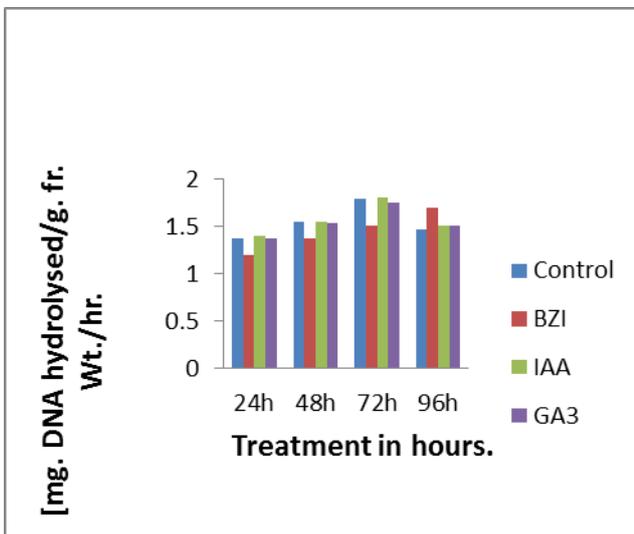


Fig. 13: Effect of Growth Regulators on Changes in Dnase Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

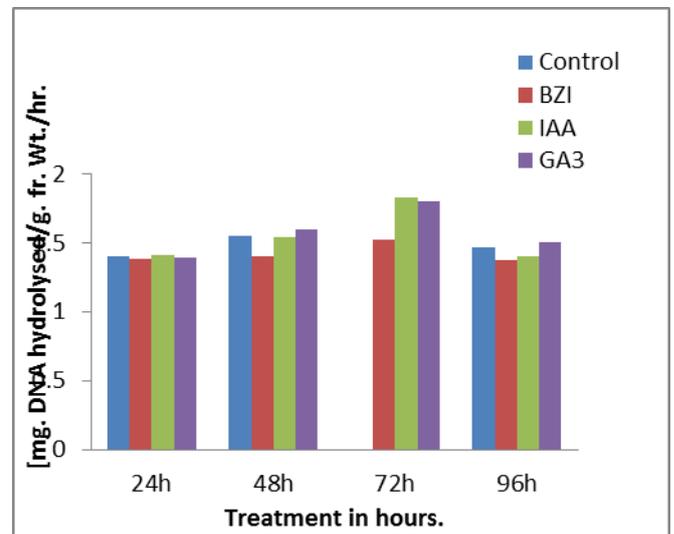


Fig. 14: Effect of Growth Regulators on Changes Dnase Activity in Excised *Setaria italica* L. Leaves During Senescence in Light.

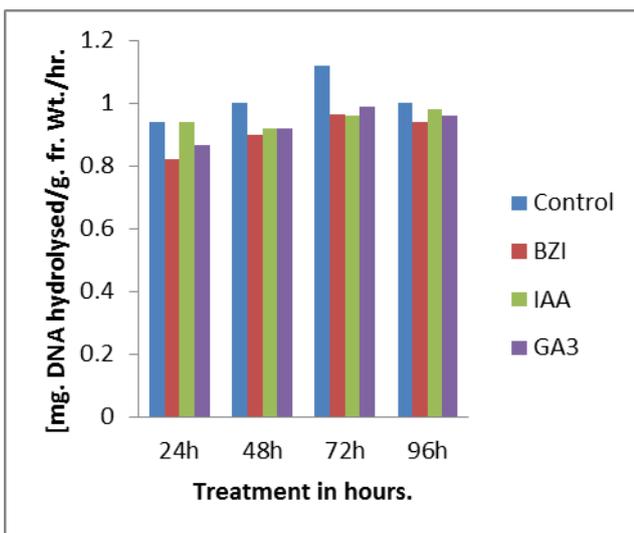


Fig. 15: Effect of Growth Regulators on Changes in Dnase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Dark.

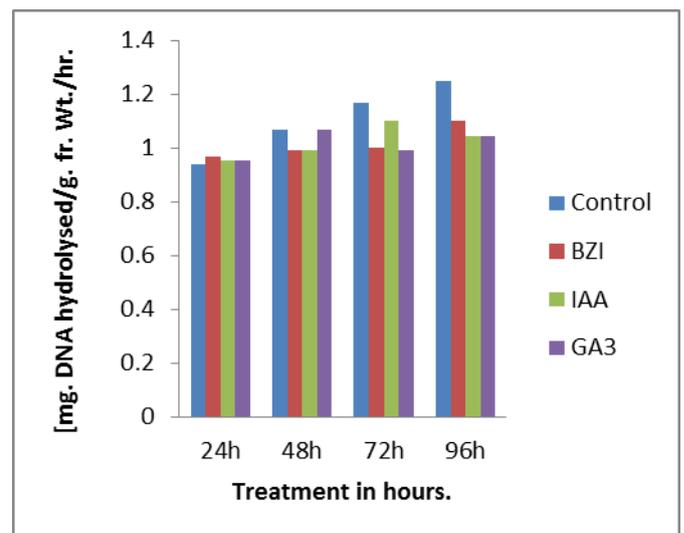


Fig. 16: Effect of Growth Regulators on Changes in Dnase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Light.

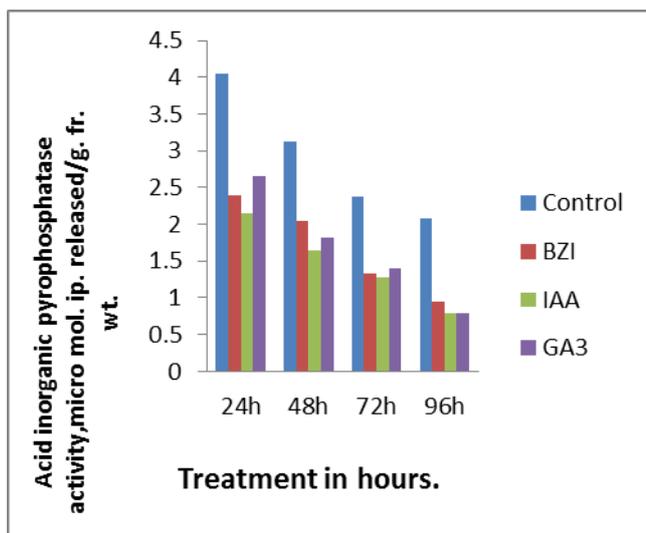


Fig. 17: Effect of Growth Regulators on Changes in Acid Inorganic Pyro Phosphatase Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

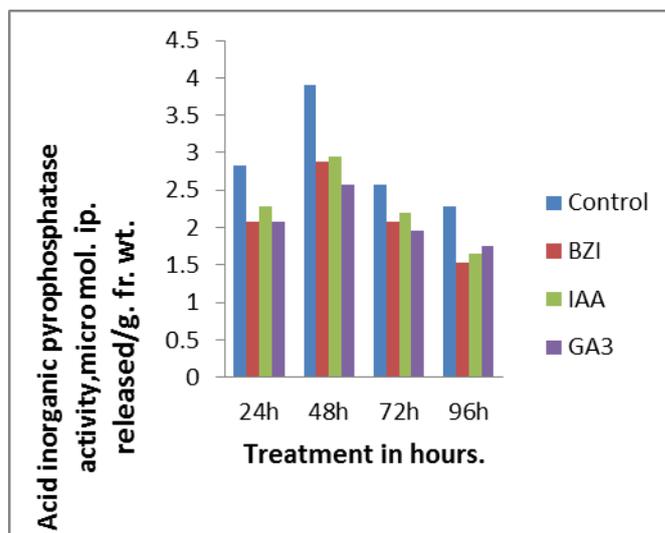


Fig. 18: Effect of Growth Regulators on Changes in Acid Inorganic Pyro Phosphatase Activity in Excised *Setaria italica* L. Leaves During Senescence in Light.

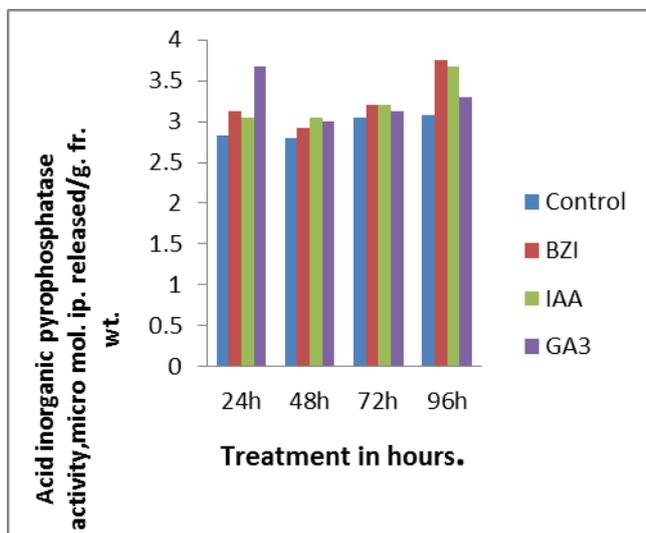


Fig. 19: Effect of Growth Regulators on Changes in Acid Inorganic Pyro Phosphatase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Dark.

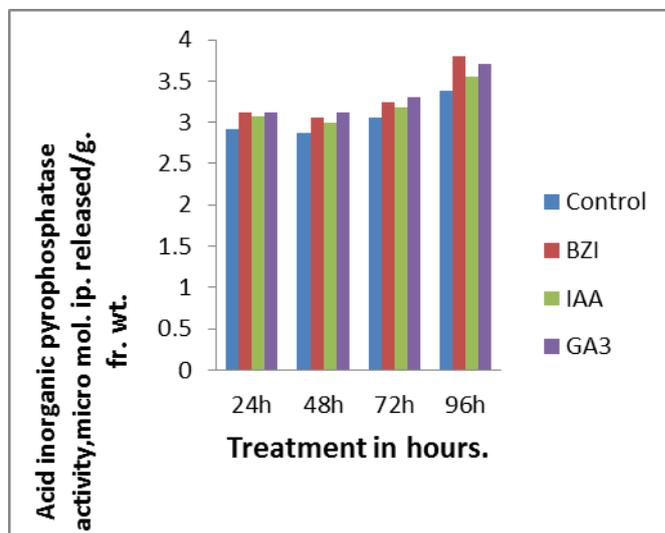


Fig. 20: Effect of Growth Regulators on Changes in Acid Inorganic Pyro Phosphatase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Light.

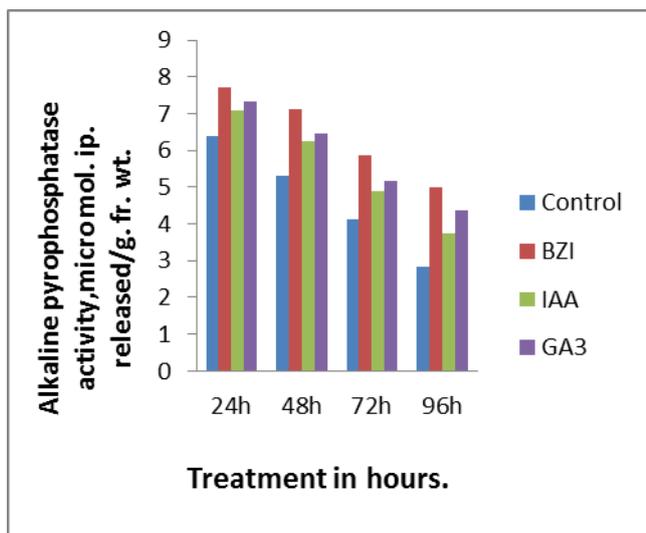


Fig. 21: Effect of Growth Regulators on Changes in Alkaline Pyro Phosphatase Activity in Excised *Pennisetum Typhoides* Burm. Leaves During Senescence in Dark.

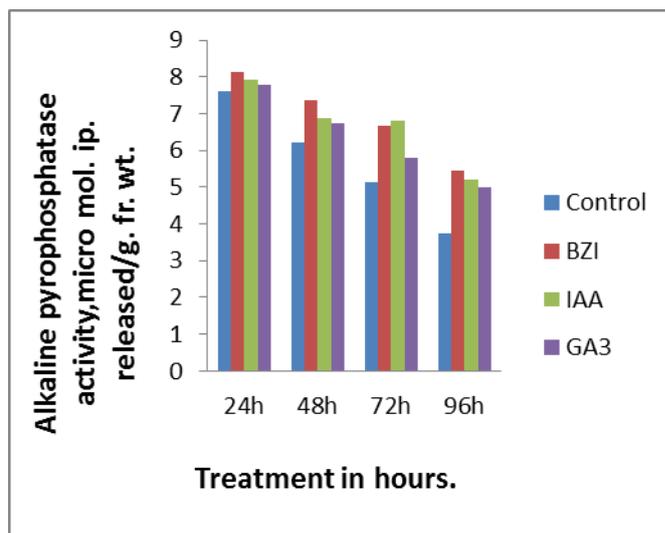


Fig. 22: Effect of Growth Regulators on Changes in Alkaline Pyro Phosphatase Activity in Excised *Pennisetum Typhoides* Burm. Leaves During Senescence in Light.

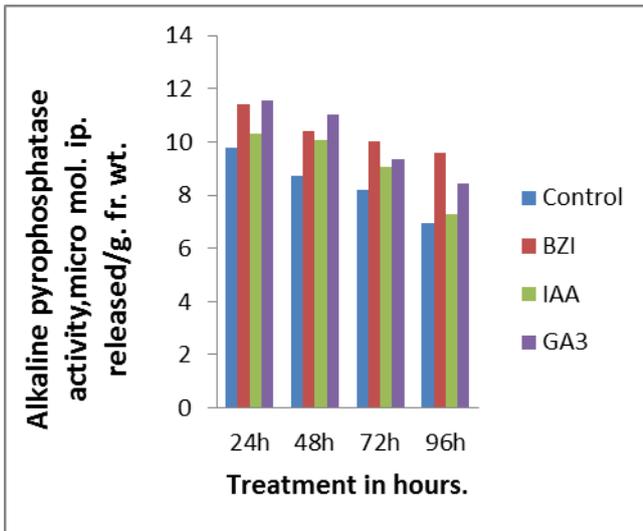


Fig. 23: Effect of Growth Regulators on Changes in Alkaline Pyro Phosphatase Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

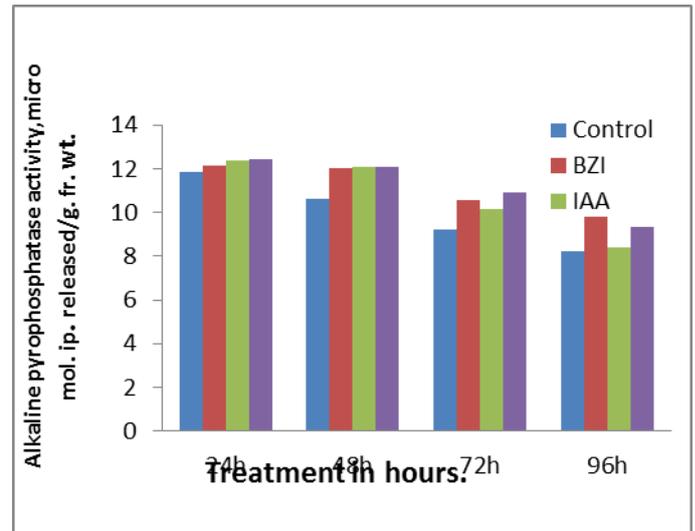


Fig. 24: Effect of Growth Regulators on Changes in Alkaline Pyro Phosphatase Activity in Excised *Setaria italica* L. Leaves During Senescence in Light.

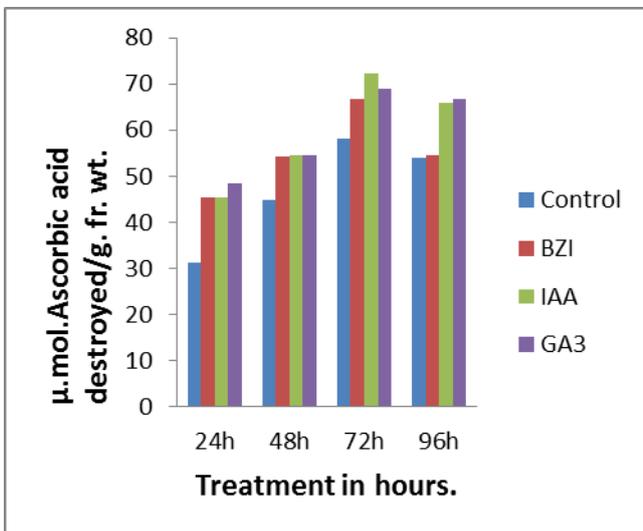


Fig. 25: Effect of Growth Regulators on Changes in Ascorbic Acid Oxidase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Dark.

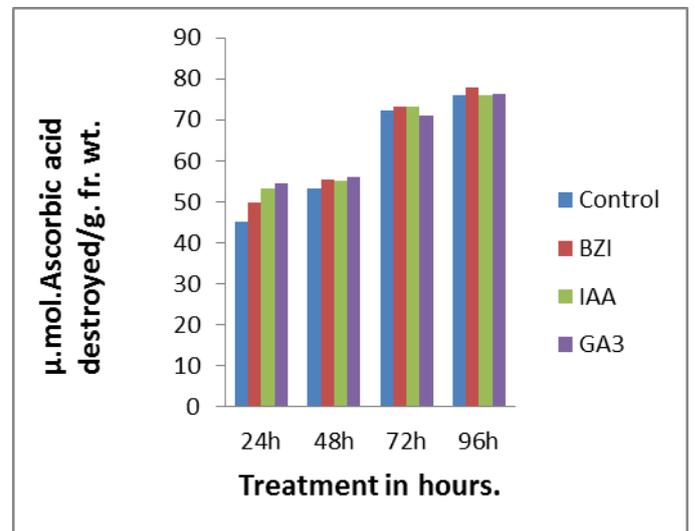


Fig. 26: Effect of Growth Regulators on Changes in Ascorbic Acid Oxidase Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Light.

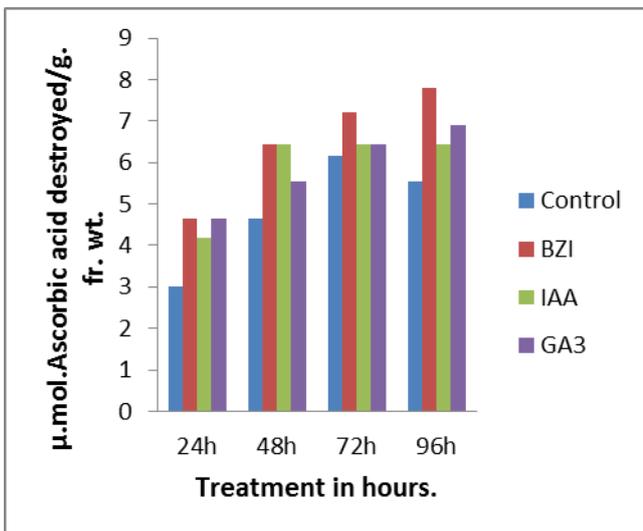


Fig. 27: Effect of Growth Regulators on Changes in Ascorbic Acid Oxidase Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

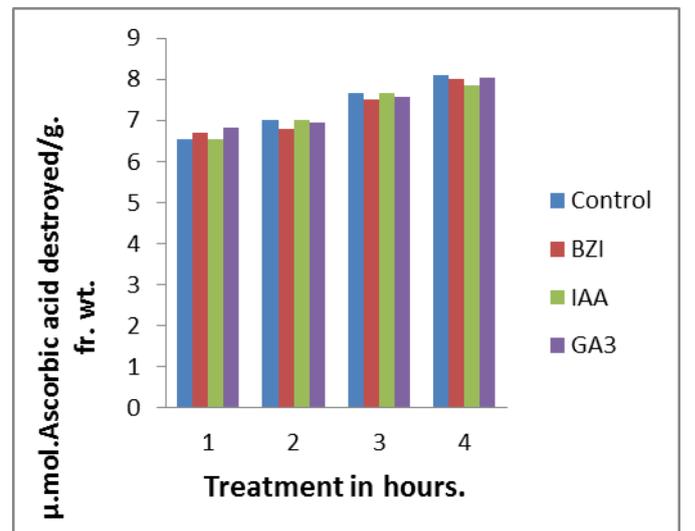


Fig. 28: Effect of Growth Regulators on Changes in Ascorbic Acid Oxidase Activity in Excised *Setaria italica* L. Leaves During Senescence in Light.

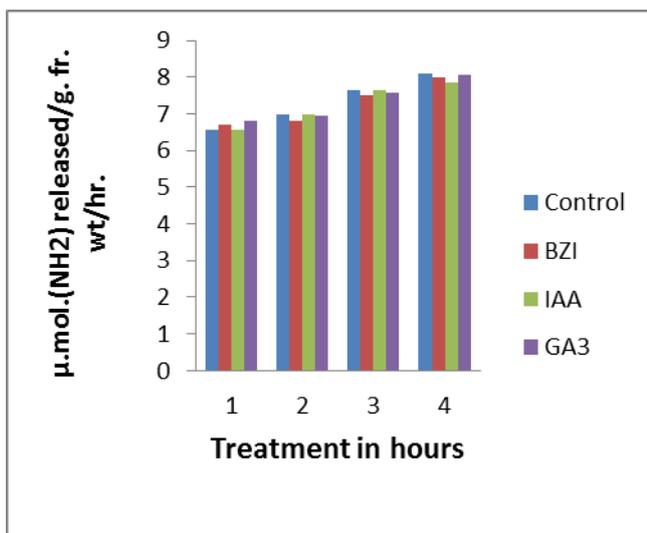


Fig. 29: Effect of Growth Regulators on Changes in Acid Protease Activity in Excised *Setaria italica* L. Leaves During Senescence Light.

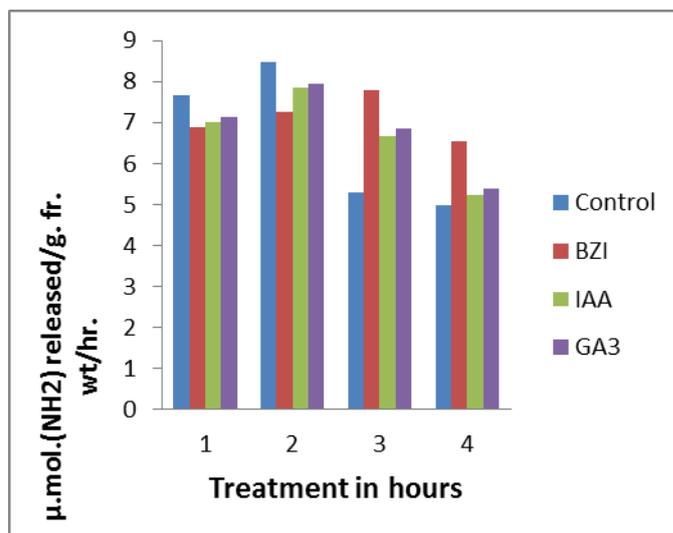


Fig. 30: Effect of Growth Regulators on Changes in Acid Protease Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

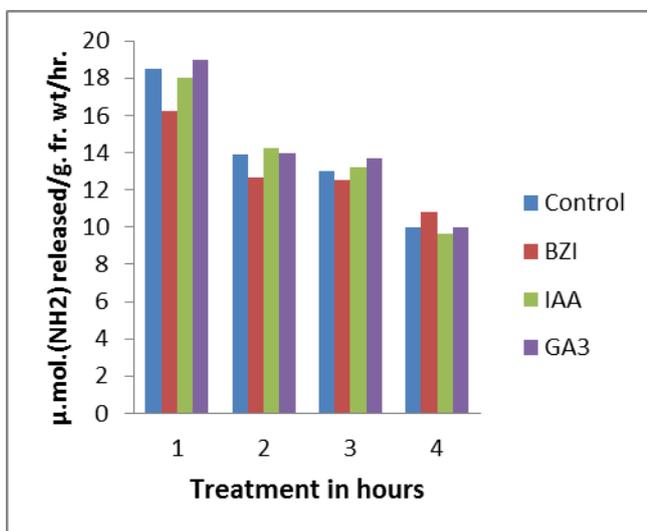


Fig. 31: Effect of Growth Regulators on Changes in Acid Protease Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Light.

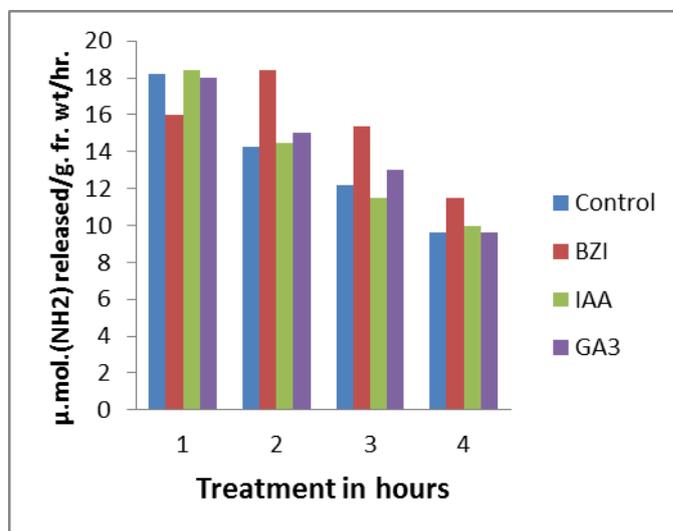


Fig. 32: Effect of Growth Regulators on Changes in Acid Protease Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Dark.

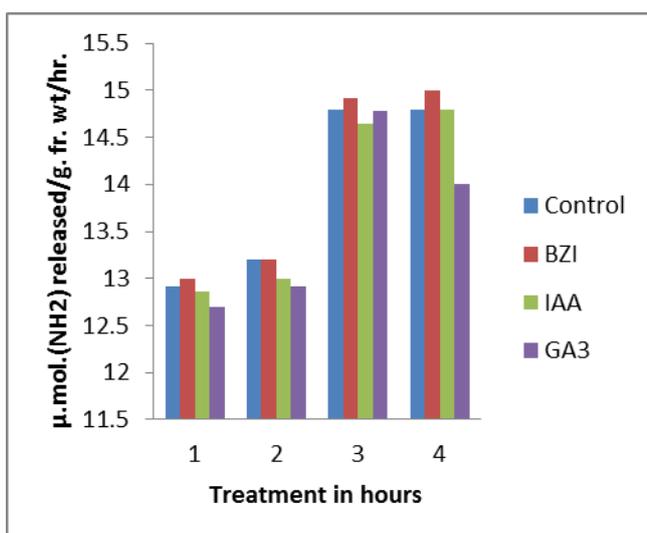


Fig. 33: Effect of Growth Regulators on Changes in Alkaline Protease Activity in Excised *Setaria italica* L. Leaves During Senescence in Light.

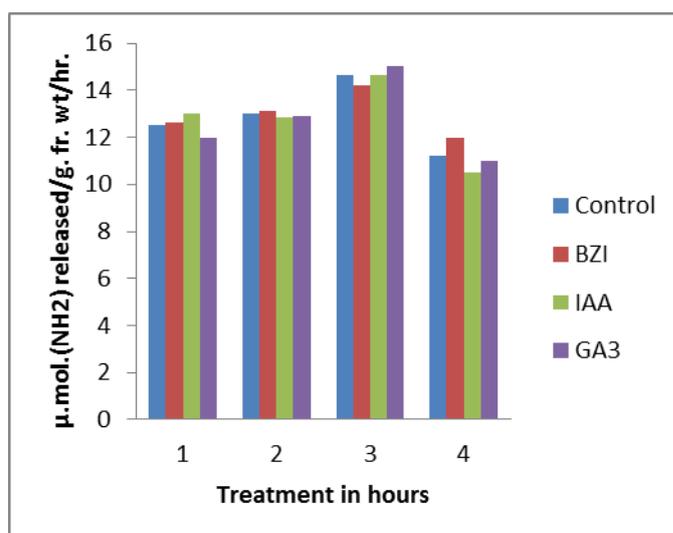


Fig. 34: Effect of Growth Regulators on Changes in Alkaline Protease Activity in Excised *Setaria italica* L. Leaves During Senescence in Dark.

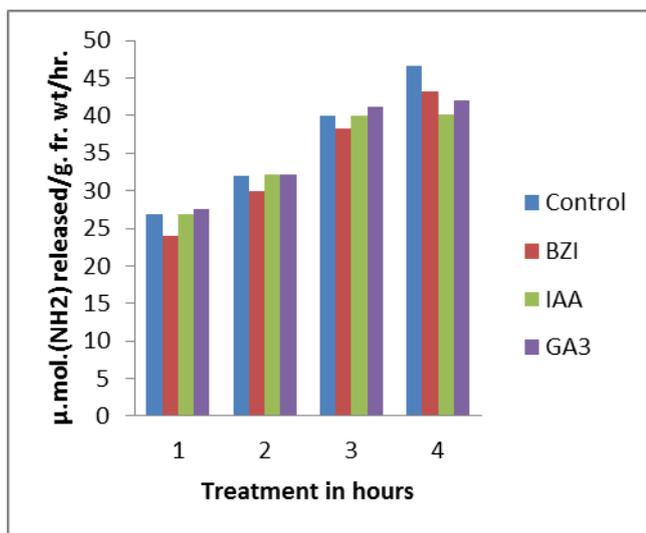


Fig. 35: Effect of Growth Regulators on Changes in Alkaline Protease Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Light

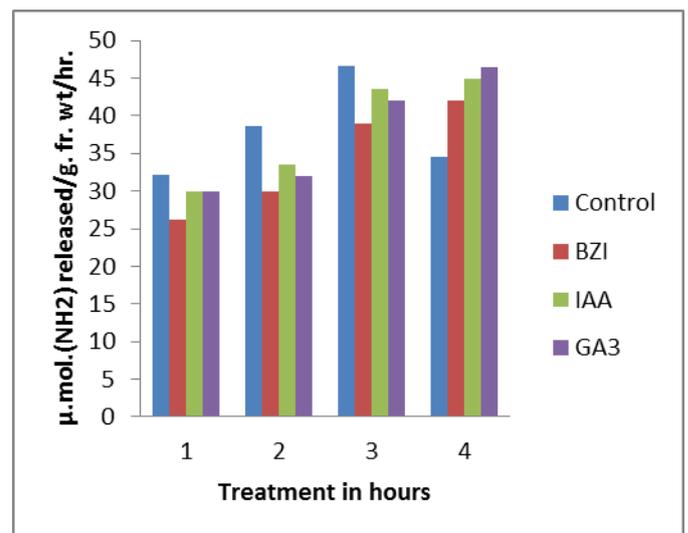


Fig. 36: Effect of Growth Regulators on Changes in Alkaline Protease Activity in Excised *Pennisetum typhoides* Burm. Leaves During Senescence in Dark.

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References

- Amir, D., Goldschmidt, E.E. and Altman, A. (1987) Chlorophyll Catabolism in senescing plant tissues: in vivo break down intermediates suggest different degradative pathways for citrus -fruit and parsley leaves- *Proc. Natl. Aca. Sci., USA.* 1901-1905. <http://dx.doi.org/10.1073/pnas.84.7.1901>.
- Anderson, J.W. and Rowan, K.S. (1966) the effect of 6-furfuryl aminopurine on senescence in tobacco leaf tissue after harvest *Biochem. J.*98: 401-404. <http://dx.doi.org/10.1042/bj0980401>.
- Bagi, G. and Farkas, G.L. (1967) on the nature of increase in ribonuclease activity in mechanically damaged tobacco leaf tissue. - *phytochemistry*.6: 161-169. [http://dx.doi.org/10.1016/S0031-9422\(00\)82759-4](http://dx.doi.org/10.1016/S0031-9422(00)82759-4).
- Balz, H.P. (1966) Inter cellular likalization and Funktion van hydrolytischen Enzymen bei Tabak. - *planta* 70: 207-236. <http://dx.doi.org/10.1007/BF00396489>.
- Beevers, L., (1968) Growth regulator control of senescence in leaf discs of *Nasturtium CTropaeolum majus*. In: *biochemistry and physiology of plant growth substances.* (F. Wightman, G. Setterfield, eds).pp.1417-1434. Runge Press, Ottawa.
- Beevers, L. (1976) Senescence. In: *plant Biochemistry* (J. Bonner J.E. Varner, Eds:) pp. 771-94. Academic Press, New York.
- Blank, A. and Thomas T.A. Mc Keon (1991) Three RNase in senescent and non-senescent wheat leaves. *Plant physiol* 97, 1402-1408. <http://dx.doi.org/10.1104/pp.97.4.1402>.
- Brady, C.J. (1988) Nucleic acid and protein synthesis. In *Senescence and Aging in plants.* (L.D. Nooden and A.C. Leopold eds.)Pp.147-171. Academic press, San Diego. <http://dx.doi.org/10.1016/b978-0-12-520920-5.50011-0>.
- De jong, D.W. (1972) Detergent extraction of enzymes from tobacco Greaves varying in maturity. - *Plant physiol.*50:733-37. <http://dx.doi.org/10.1104/pp.50.6.733>.
- De Leo, P. and Sacher, J.A. (1970) Control of ribonuclease and acid phosphatase by auxin and abscisic acid during senescence of Rhoec leaf sections. - *Plant physiol.*46:806-11. <http://dx.doi.org/10.1104/pp.46.6.806>.
- Drazkiewicz, Maria. And Zbigniew, Krupa. (1992) the participation of in chlorophyll metabolism. *Acta. Soc. Bot. Pol.* 60(1-2) 139-154.
- Garcia, A.L. and Galindo, L. (1991) Chlorophyllase activity as biochemical indicator of manganese and iron deficiencies in *Citrus*. *Photosynthetica* 25(3): 351-357.
- Ellsworth, R.K., Tsuk, R.M. and St. Pierre, L.A. (1976) studies on Chlorophyllase IV Attribution of hydrolytic and esterifying chlorophyllase activities observed in vitro to two enzymes. *photosynthetica* 10: 312-323.
- Hodge, E.T. and Sacher, J.A. (1975) Effects of kinetin auxin and abscisic acid on ribonuclease and acidphosphatase during senescence of leaf tissues. - *Biochem. Physiol.* Pflanzen. 168: 433-441.
- Holden, N. (1974) Chlorophyll degradation products in leaf protein preparations. -*J. Sci. Food Agric.*25: 1427-32. Huang, Y. and Kao, C.H. (1991) Senescence of rice leaves: XXV changes of acid phosphatase activity *Bot. Bull. Acad. Sin.* (Taipei) 32: 31-42.
- Kar, M. and Feirabend, J. (1984) Metabolism of activated oxygen in detached wheat and rye leaves and its relevance to the initiation of senescence. - *Planta* - 160:385-391. <http://dx.doi.org/10.1007/BF00429753>.
- Kar, M. and Mishra, D. (1975) Inorganic pyrophosphatase activity during leaf senescence. - *Gen. J. Bot.* 53: 503-511. <http://dx.doi.org/10.1139/b75-061>.
- Kar, M. and Mishra, D. (1976) Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. - *Plant physiol.* 57: 315-318. <http://dx.doi.org/10.1104/pp.57.2.315>.
- Kenis, J; D. and Trippi, V.S. (1982) Envejeimiento. Y abscission en *Phaseolus Vulgaris* l. Actividad de fosfatase acida: en relacion con la edad y su regulacion por calidad de ja luz. Azucares Y cicloheximida. - *Phyton.* 42:9-16.
- Kumar, K.B. and Khan, P.A. (1984) Levels of deoxyribonucleic acid and of deoxyribonucleolytic activity in Ragi leaves during senescence. - *Biol. Plant.* 26:174-180. <http://dx.doi.org/10.1007/BF02895044>.
- Lewington, R.J., Talbot, M. and Sinson, E.W. (1967) the yellowing of attached and detached cucumber cotyledons. - *J. Exp. Bot.* 18: 526-634. <http://dx.doi.org/10.1093/jxb/18.3.526>.
- Mahapatra, M.(1992): Study of the exogenous hormonal regulation of leaf senescence in two millets, *Setaria italica, L. and Pennisetum typhoides, Burm* Ph.D thesis, Berhampur University, Berhampur, Odisha
- Mazumdar, Sonali. Ghosh, Sibdas. Glide, R. Bernard and Dumdroff B. Erwin (1991) Activities of chlorophyllase, Polyphenol pyruvate Carboxylase and ribulose 1-5-diphosphate carboxylase on the primary leaves of soybean during senescence and drought. *Physiol. Plant* 81(4) 473-480. <http://dx.doi.org/10.1034/j.1399-3054.1991.810405.x>.
- Mc Hale, J.S. and Dove, L.D. (1968) Ribonuclease activity in tomato leaves as related to development and senescence. - *New Phytol.* 67:505-515. <http://dx.doi.org/10.1111/j.1469-8137.1968.tb05479.x>.
- Martin, C. and Thimann, K.V. (1972 b) the role of protein synthesis in the senescence of leaves II. The influence of amino acids on senescence. *Plant. Physiol.*50:432-437. <http://dx.doi.org/10.1104/pp.50.4.432>.
- Martin, C. and Thimann, K.V. (1972a) the role of protein synthesis in the senescence of leaves I. The formation protease. -*Plant Physiol.* 64-71. <http://dx.doi.org/10.1104/pp.49.1.64>.
- Meyer, R. and Wagner, K.G. (1986) *Physiol. Plant.* 67: 666. Moll, W.A., Wit, B. and Lutter, K. (1978) chlorophyllase activity in de-

- veloping leaves. - *Planta* 139:79-83.
<http://dx.doi.org/10.1038/174593a0>.
- [28] Naganna, B. and Sripathi, C.E. (1954) Pyrophosphatases in Plants during growth. - *Nature* 174:594-598.
- [29] Naganna, B., Vegugopal, B. and Sripathi, C.E. (1955b) Occurrence of alkaline pyrophosphatase in vegetable tissues. *Biochem. J.* 60:224-225. <http://dx.doi.org/10.1042/bj0600224>.
- [30] Parrida, R.K. and Mishra, D. (1980) Acid phosphatase and adenosine triphosphate activities during rice leaf development and senescences. - *Photosynthetica* 14:431-136.
- [31] Parida, R.K., Kar, M. and Mishra, D. (1981) Enzymatic change in gourd (*Cucurbita maxima* and bean (*Phaseolus vulgaris*) cotyledons during ageing and the effect of detopping. *Boil. Plant.* 23: 2937-2941.
- [32] Peoples, M.B. and Dalling, M.J. (1988) the interplay between proteolysis and amino acid metabolism during senescence and nitrogen reallocation. In: *Senescence and agein plants*. (L.D. Nooden and A.C. Leopold, Eds.) pp. 182-212. Academic press, New York.
- [34] Rudiger, W., Benz, J., Guthoff, C. (1980): Detection and partial characterization of activity of chlorophyll synthetase in etioplast membranes. - *Eur. J. Biochem.* 109: 193-200. <http://dx.doi.org/10.1111/j.1432-1033.1980.tb04784.x>.
- [35] Sabater B. and Rodriguez, M.T. (1978) Control of chlorophyll degradation in detached leaves of barley and oat through effect of kinetin on chlorophyllase levels. *Physiol. Plant.* 43:274-276. <http://dx.doi.org/10.1111/j.1399-3054.1978.tb02577.x>.
- [36] Simon, E.W. (1967) Types of leaf senescence. - In *Symp. Soc. Exp. Biol.*, pp. - 215-230. Cambridge Univ. Press, London.
- [37] Simons, S. and Butler, L.G. (1969) alkaline inorganic pyrophosphatase of maize leaves. *Biochem. Biophys. Acta.* 172: 150-157. [http://dx.doi.org/10.1016/0005-2728\(69\)90100-5](http://dx.doi.org/10.1016/0005-2728(69)90100-5).
- [38] Simpson, K.L., Lee, T.C., Rodriguez, D.B. and chichester, C.O. (1976) Metabolism in senescent and stored tissues. In: *chemistry and Biochemistry of Plant Pigments*. Vol.I (T.W. Goodwin, Ed.) pp 779-842. Academic Press, New York.
- [39] Srivastava, B.I.S. and Ware, G. (1965) the effect of kinetin on nucleic acid and nucleases in excised barley leaves. *Plant Physiol.* 40:62-64. <http://dx.doi.org/10.1104/pp.40.1.62>.
- [40] Sodek, L. and Wright, S.T.C. (1969) Effect of Kinetin on ribonuclease, acid phosphatase, Lipase and esterase levels in detached wheat leaves. *Photochemistry* 8: 1629-1640. [http://dx.doi.org/10.1016/S0031-9422\(00\)85947-6](http://dx.doi.org/10.1016/S0031-9422(00)85947-6).
- [41] Sudhakar, C. Reddy, P.S. and Veeranjanejulu, K. (1991) change in respiration, its allied enzymes, pigment composition, chlorophyllase and Hill reaction activity of horsegram seedlings under salt stress. *Indian J. Plant. Physiol.* 34(2)171-177
- [42] Thomas, H. (1987) Sid. a Mendelian locus controlling thylakoid membrane disassembly in senescing leaves *Fe stucapratensis Theoreticallapp*.
- [43] Thomas, H., Bortelik, K., Rentsch, D., Schellenlurg, M. and Matile, P. (1989) Catabolism of chlorophyll *in vivo*: significance of polar chlorophyll catabolites in a non-yellowing senescence mutant of, *Festuca Pratensis* Huds. *New Phytol.* 111: 3-8. <http://dx.doi.org/10.1111/j.1469-8137.1989.tb04211.x>.
- [44] Thompson, J.C., Legge, R.L. and Braber, R.F. (1987) the roles of free radicals in senescence and wounding. *New phytol.* 105: 317-344. <http://dx.doi.org/10.1111/j.1469-8137.1987.tb00871.x>.
- [45] Udvardy, J., Farkas, G.L., Marre, E. and Forti, G. (1967) the effects of sucrose and light on the level of soluble and particle bound ribonuclease activities in excised *Avena leaves*- *physiol. Plant.* 20: 781 - 788.
- [46] Truelsen, T.A. (1967) Indole acetic acid - induced decreases of the ribonuclease activity *in vivo* *Physiol. Plant.* 20: 1112-1119.
- [47] Upadhyaya, A., Davies, T.D., Sankla, D., Sankla, N. and azmith, B.N. (1985) Effects of padobutrazol on the activities of proteolytic enzyme and RNase. In *Soybean leaves during senescence*.-*Comp. Physiol. Ecol.* 10: 49-54.
- [48] Van der Valk, Hendry, C.P.M. and Van Loon, L.C (1988) subcellular localisation of proteases in developing leaves of oats. (*Avena sativa*) *Plant Physiol.* 87:536-541. <http://dx.doi.org/10.1104/pp.87.2.536>.
- [49] Woolhouse, H.W. (1978) Senescence process in the life cycle of flowering plants. - *Biosciences* 28: 25-31. <http://dx.doi.org/10.2307/1307483>.