RESEARCH PAPER Asian Journal of Bio Science, Vol. 4 Issue 2 : 195-198 (October, 2009 to March, 2010)

Biochemical and molecular markers of auxin induced senescence in mustard seedlings

K. TULASI RAMA KRISHNAN[1] AND V. SIVAKUMARI[2]
[1]Department of Biochemistry, Marudu Pandiyar College, THANJAVUR (T.N.) INDIA
[2]Department of Environmental and Herbal Sciences, Tamil University, THANJAVUR (T.N.) INDIA

(Accepted : July, 2009)

The objective of the present study was identification and characterization of the biochemical and molecular events during senescence. Senescence is known to be an active developmental process and not a passive degradative process. Accordingly a set of biomolecules, namely phtohormones (IAA), ethylene and abscisi acid (ABA), generation of signaling molecules and induction of enzyme, for e.g. catalase, constitute the components of active process of senescence. 2, 4-D induced senescence pathway was the focus of the present study. Native auxin (IAA) and synthetic auxin (2, 4-D) were employed in the present study. It is known that besides 2, 4-D two other hormones namely, ethylene and ABA are involved in this process. Silver ions in the from of $AgNO_3$ was provided to the young seedling of black mustard to interfere in the ethylene biosynthesis which is the midpoint in induced senescence.

Key words : Senescence, Phtohormones, Auxin and mustard

INTRODUCTION

Senescence in plant is an anabolic process and termed as negative aspect of growth. This means senescence in plants is not a passive process. In contrast it is a programmed morphogenesis and termed as programmed cell death (PCD) (Lohman *et al.*, 1994). PCD occurs even in the normal course of the plant development for example, even during the embryogenesis in immature seeds there is PCD in relation to the formation of water conducting elements, namely, xylem formation.

Control and regulation of senescence in plants have biotechnological prospective in terms of regulating developmental process such as, fruit ripening, regulation of ageing, compression of life cycle or life span in *in vitro* plants, optimal storage of seeds and tuber, shelf life of agricultural commodities such as, fruits, seeds and grains, germinability of seeds subsequent to storage etc (Salisbury and Ross, 1991).

The present study was undertaken to characterize the senescence markers in the 2, 4-D induced senescence pathway. Effect of 2, 4-D + Ag NO_3 a few selected inhibitors of ethylene biosynthesis and action were studied to mark the senescence stages. The study focused on the identification of biomolecules or bioprocesses during senescence as possible markers (Mittler and Lan, 1995). Pigment degradation, catalase activity and nuclear DNA degradation were considered as prominent senescence markers. These markers differed from the signal molecules because of the fact; these are consequences or responses and manifestations of senescence. Signal molecules on the contrary mean those bimolecules which induce and regulate senescence. Thus, the focus of the present study was to sequence the senescence markers in the young seedling of black mustard (Lohman *et al.*, 1994).

MATERIALS AND METHODS

Seeds of black mustard (*Brassica nigra* L.) was allowed to germinate at room temperature 35 ± 2°C on a thin layer of cotton. ABA, 2, 4 – D + 0.4% 1-Butanol, 2,4-D + Na- tungstate was added on the 3rd, 5th and 7th day similarly on 0´ d, 1´ d, 3´ d and 4´ d. The root and shoot of the plants were measured in cm and the samples were collected from the above days.

Determination of root-shoot length:

One week old mustard seedling were uprooted and the roots and shoots were stretched with the help of a scale and root and shoot lengths were measured for every treatment in 3rd, 5th and 7th day similarly on 0´ d, 1´ d, 3´ d and 4´ d. Chlorophyll (Arnon, 1949), Carotenoids (Mackinneys, 1941), lipid (Chapman, 1998), Thin layer chromatography (Manoharan, 1981), Phospholipids (Wagner *et al.*, 1962), galactolipids, Genomic DNA from plant tissue by the method of CTAB, Agarose Gel Electrophoresis and catalase activity were analysed.

RESULTS AND DISCUSSION

The results obtained from the present investigation are summarized below:

Effect of ABA on the growth of mustard seedling:

The objective of the present study is to understand the hormonal interplay on the regulation of senescence. Auxin induced senescence via ethylene of ABA is the focus of the study. Accordingly ABA effect on mustard seedling was undertaken. ABA at 25 µm concentration was worked out. Among the parameters determined in the young seedling shoot weight, specific shoot growth and the content of chlorophyll pigments showed decrease due to ABA.

Acetone positive control:

Since the 2, 4-D and IAA for various experimental solutions were prepared in 1% acetone, its effect was studied in the young mustard seedling during the experimental period. Among the parameters determined in the young seedlings there was no difference in the growth of seedling.

Effect of cobalt ions on auxin induced senescence (AIS):

A set of experiments was undertaken to evaluate the effect of an ethylene biosynthesis inhibitor namely, $CoCl_2$ on the AIS. On the basis of shoot length, it could be seen that the inhibitory effect of $CoCl_2$ was not observed in the seedlings. However, there was a stimulated growth of seedling height. These observations when taken together with specific shoot growth revealed that there was inhibition of ethylene production in the seedlings by ethylene inhibitor. There was an increase in the shoot weight of the seedlings due to cobalt ions. It is relavent to mention that ethylene response is related to radial growth of the seedlings in a typical triple response phenotypic effect (TRP).

Effect of supplementation of 2, 4-D and $AgNO_3$ on the PL composition of mustard seedlings:

Membrane lipids are known to be the target of degradative enzymes during senescence. In order to characterize the senescence associated changes in the membrane lipids the following analysis was carried out. Control on the 0' day and 2' day were included in the experiment. These 2 controls revealed any possible change due to ageing under experimental conditions. 2, 4-D and $AgNO_3$ supplemented seedlings were compared with grown seedlings (control).

The results showed that the total content of PL did not show significant change due to 2, 4-D and $AgNO_3$ for the two days duration. Among the PLs, 6 molecular species were identified by the TLC system employed in the present study. Accordlingly the following are the PL species in the control, 2, 4-D and $AgNO_3$ treated seedlings: LPC, PC, PI, PS, PE, PA.

Effect of 2, 4 –D on seedlings the galactolipids composition of mustard:

A set of experiments was undertaken to find out the change in chloroplast specific galactolipid due to 2, 4 –D induced senescence. It was the aim of the experiment to mark the appearance of death signals and markers of chloroplast degradation during senescence. The results showed that 2'd 2, 4 –D treated seedlings did not show any significant change in the total amount of galactolipids. There was also no significant change in the molecular species of galactolipids, namely: MGDG, DGDG and SL. The galactolipid composition of 2, 4-D + $AgNO_3$ treated was comparable to that of 2'd control.

Effect of 2, 4-D and 2, 4-D + $AgNo_3$ on the MGDG: DGDG ratio of mustard seedlings

An analysis was carried out to determine the MGDG: DGDG ratio in lipid samples prepared from the shoot tissue. MGDG and DGDG are the specific chloroplast lipids localized in the internal (grana and stroma membrane) and external (chloroplast envelope membrane), respectively. MGDG: DGDG ratio would provide insight into the intactness / degradation of the chloroplast membrane. Accordingly, 0'd and 2'd control and 2, 4-D supplemented samples were analysed for these parameters.

The results showed that there was no significant change in the MGDG: DGDG ratio of the 2'd, 2, 4-D treated plants as compared to the control. This would reflect the intactness of the chloroplasts in situ and no degradation of the chloroplast membrane biomolecules due to 2, 4-D on the 2'd (Table 1). Even the marginal increase observed in the 2'd seedling due to 2, 4-D was reversed in the 2'd, 2, 4-D + $AgNO_3$ treated plants. This showed the reversal of 2, 4-D response by the ethylene biosynthesis inhibitor namely $AgNO_3$.

Table 1 : Effect of 2, 4-D + $AgNO_3$ on the MGDG: MGDG ratio of 2'd mustard seedlings

Sr. No.	Sample	MGDG / DGDG
1.	0'day control	2.22
2.	2'day control	2.50
3.	2' day 2, 4-D+ $AgNO_3$	2.30

Catalase activity and its relationship to senescence:

In the present study catalase activity was determined in the shoot tissue of mustard seedlings which were subjected to auxin induced senescence. Catalase, an enzyme involved the ROS scavenging is positively correlated with senescence. Experiments were undertaken to study catalase activity to enzyme content and H_2O_2 concentration in 4'd seedlings.

Catalase activity in relation to senescence of seedlings:

One of the ROS scavenging enzymes, namely catalase was assayed in the ultimate stage in the present experimental conditions of auxin induced senescence (AIS) in mustard seedlings. 2, 4 – D supplemented to 4'd old seedlings for the subsequent 4'd with 500 µm 2, 4-D was the test system of the present study. Catalase activity was calculated on the basis of depletion of H_2O_2 in the assay mixture and its unit of expression was on the basis of specific activity of catalase. The results showed that whereas 2, 4-D treated seedlings have stimulated catalase activity to the tune of 4 fold increase, the supplementation of silver ions along with 2, 4-D resulted in the minimal activity of catalase in the treated sample in comparison to control.

Effect of 2, 4-D and 2, 4-D+ $AgNO_3$ on the induction and reversal of nucleases activity and associated pattern of nDNA degradation during senescence:

A set of experiments was undertaken to characterize the nucleolytic changes in relation to 2', 4-D supplementation. Nucleases activity was visualized in the form of nDNA degradation by observing the DNA fragments in 0.75% agarose gels. The pattern of nDNA fragments on 3' and 4'd to 2, 4-D + $AgNO_3$ were compared with their respective controls. 0'd, 3'd and 4'd control and 3' and 4'd 500mm 2, 4-D + 0.1mm $AgNO_3$ seedlings were compared for the pattern of nDNA fragmentation. 2, 4-D as an inducer of senescence and $AgNO_3$ as an inhibitor of 2, 4-D induced senescence pathway were employed in the present study. The nDNA in 3'd, 2, 4-D + $AgNO_3$ treated seedlings showed the nucleases activities and appearance of nDNA fragments in a wide range of sizes, as compared to 3'd control. When 0'd and 4'd controls were compared there was no aging related nDNA fragmentation under the experimental conditions of the present study, except in one of the samples. The RNA present in the samples got to the front of the gel. The 4'd control however did not have significant RNA contamination.

The nuclear DNA in the samples has a moleculars size of 23 kb as compared to the Hind III digested ëDNA. This 23kb nDNA appeared as a discrete band in all the samples, which represented the intact and under graded genomic DNA of mustard. The 4'd 2, 4-D treated plants also showed distinct pattern of nDNA degradation which fell in the range of 23 downwards to about 9kb as compared to the marker DNA. However, the intensity of the nDNA fragments was comparatively weak in this preparation which indicates the efficacy of $AgNO_3$ in reversing the 2, 4-D induced senescence pathway.

When a comparison was made between the nDNA fragments of the 4'd and 3'd 2, 4-D + $AgNO_3$ treated samples, there was extensive nDNA degradation in both the cases as compared to the respective controls. This showed that the reversal of 2, 4-D induced degradation varied in different experimental samples possibly due to weak inhibition by $AgNO_3$ on the intermediary ethylene biosynthesis in the senescence pathway.

Abbreviation:

2, 4-D: 2, 4-dichlorophenoxy acetic acid; ABA: Abscisic acid; ACC: Amino cyclo propane carboxylic acid; $AgNO_3$: silver nitrate; HRS: Hyper sensitive response; IAA: Indole 3 acetic acid; MGDG: Mono Galactosyl Di Glycerite; PA: Phosphatidic acid; PCD: Programmed Cell Death; PE: Phosphatidyl ethanolamine; PLD: Phospholipase D; PS: Phosphatidyl Serine; PC: Phosphatidyl Colline; ROS: Reactive oxygen species; SAGs: Senescence associated Genes; SOD: Superoxide dismutase.

REFERENCES

Arnon, D.I. (1949). Copper enzyme in isolated chloroplasts: Polyphenoloxidase in Beta vulgaris. *Plant Phyiol.,* **24**: 1-15.

Chapman, R. (1998). Phospholipase activity during plant growth and development and in response to environmental stress. *Trends in Plant Sci.,* **11**: 419-426.

Lohman, K.N., Gan, S., John, M.C. and Amasino, R.M. (1994). Molecular analysis of natural leaf senescence in *Arabidopsis thaliana*. *Plant Physiol.,* **92** : 322-328.

Mackinneys, R. (1941). Separation of plant phospholipids and glycolipids. In: James, A.T and Mossus, L.J (eds.), *New biochemical separatists*, Van Nostrand, Princeton. pp. 321-337.

Manoharan, K. (1981). Chromatographic characterization of polar lipids of Pisum sativum L. var Bonneille. M. Phil. Dissertation, Jawaharlal Nehru University, New Delhi.

Mittler, R. and Lan, E. (1995). *In situ* detection of nDNA fragmentation during the differentiation of treacheary elements in higher plants. *Plant Physiol.,* **108**: 489-493.

Salisbury, F.B. and Ross, C.W. (1991). Plant Physiology. IV edition. Wardsworth publishing Co., Belmont, California, USA. 401-407.

Wagner, H., Lissan, A. Holzi, Z. and Horhammer, L. (1962). Estimation of Inorganic phosphate in perchloric acid digests of phospholipids. *J. Lipid Res.,* **3**: 177-180.