RESEARCH ARTICLE

STUDY OF PATTERNS OF SENESCENCE IN LEAFLETS OF *TECOMA STANS* (LINN.) H.B. & K.

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Abstract: Senescence is the last stage in the development of leaf, it involves both leaf decay and a removal of the nutrients that are stored in the leaves to other parts of the plant. *Tecoma stans* has compound leaves which are oppositely arranged. Biochemical analysis was carried out for Total N, Total P, Total Chl. and some enzymes i.e. Protease, Amylase, IAA oxidase and RNase. Before biochemical analysis visual observations were carried out in different excised leaves of *Tecoma stans*, petioles were dipped in distilled water and dark incubated to study the pattern of senescence. According to visual observations leaflets of young leaf senescence a bit faster than leaflets of mature leaves. Pattern of changes of levels of constituents indicates that Total Chl., Total N, and Total P increases up to mature stage and then declines. Amylase, IAA oxidase, Protease and RNase increases up to presenescent stage in the leaflets.

Keywords: Senescence, Leaflets, Biochemical changes, Visual observations, *Tecoma stans*

INTRODUCTION

Senescence is the last phase of development of an organism. Leaves have been extensively used to understand the process of senescence. Besides, regulation by phytohormones etc., senescence is established to be a genetically programmed phenomenon. It is of interest to distinguish between the terms ageing and senescence. All organisms from the beginning of their life cycle undergo ageing which culminates in the final phase of senescence leading to death. The process leading to onset of senescence and accompanying it and modification of senescence have been of major interest. It was, therefore, of interest to extend such studies and in this paper results of certain visual observations and biochemical analysis of the leaves of *Tecoma stans* are presented. The system *Tecoma stans* have compound leaves which are oppositely arranged. The interesting feature of this system is that it shows polarity which can be visually observed. The leaves which are towards the earth are somewhat larger than their opposite leaves. Further this distinction is also clear in the opposite leaflets.

In this communication those leaflets were selected from a single leaf and visual observations and biochemical studies were carried out. The terminal leaflets were also taken for comparison.

MATERIAL AND METHOD

Leaves of *Tecoma stans* were collected from C.C.S. University, Meerut Campus. (Plate-1). Leaflets were selected from single leaf and sets were prepared. Visual observations were carried out. The terminal leaflets were also taken into comparison. Young growing leaves and fully expanded mature leaves were excised. They were surfaced sterilized in mercuric chloride solution. After washing in distilled water, excised leaves were kept with petiole dipping in distilled water in vials. Incubation was done in dark and visual changes were recorded till completion of senescence. Experiment was repeated at least thrice in triplicate.

Biochemical analysis were carried out for total nitrogen, total phosphate, chlorophylls and some enzymes i.e. protease, amylase, IAA Oxidase & RNase in *Tecoma stans*. All the data are averages of at least four experiments, each done in triplicate.

Total Nitrogen : For estimation of nitrogen, digestion was done according to Snell and Snell (1954) and the digest was estimated by colorimetric method.

Total Phosphate : Total phosphate was estimated after Allen (1940) using metol reagent.

Total Chlorophyll : For the estimation of total chlorophyll leaf sample of known weight was homogenized with 80% acetone with a pinch of sodium bicarbonate. The amount of chlorophyll a and Chlorophyll b were calculated according to the following formulae (Arnon, 1949).

Chl. a (mg/l) = $12.72\ A_{665} - 2.28\ A_{645}$

Chl. b (mg/l) = $22.87\ A_{648} - 4.67\ A_{663}$

Enzymes : A common Tris – maleate-NaOH buffer pH 6.8 (Vimala, Y, 1983) was used as the extraction cum assays medium for amylase, protease, IAA oxidase and RNase activity.

Amylase : It was estimated by the method given by Filner and Varner, (1967) with iodine reagent.

Protease : It was estimated with sulphate reagent and Pholin phenol reagent (Yamo & Varner, 1973).

IAA oxidase : Gordon & Weber (1951) with Salkowaski reagent.

RNase : Citrate phosphate buffer pH 5.0 used as a extraction medium. Method of Anfinsen et al. (1954) was used for enzyme estimation.

OBSERVATIONS AND CONCLUSIONS

Table 1. shows visual changes accompanying the senescence of young and mature excised leaves of *Tecoma stans* incubated in dark.

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The young as well as mature leaves were green initially. No change was observed in any of the stage till 4th day. By the 6th days tips of young leaves were started curling. Browning of the young leaves started from the margin on the 8th day and by 14th day whole leaf turned brown showing complete senescence. In mature leaves browning started as brown spots at the base of leaf on 10th day and complete senescence was observed by the 18th day. As far as different positions of leaflets in young and mature leaves were concerned there was no significant difference observed. The observations, thus, showed that the young leaves senescence a bit faster than the mature leaves. The colour changes may be due to involvement of phenol oxidases besides chlorophyllases.

Table 2. shows changes in fresh weight, dry weight, pigment levels, some chemical constituents and activities of some enzymes accompanying compound leaf development and senescence in *Tecoma stans* on per leaf basis.

According to table 2 fresh weight increased upto mature stage in terminal leaflets and then declined. The right and left leaflets showed increase upto presenescent stages. Same case was noted in case of dry weight. Total chlorophyll rise upto the mature stage and then decline. Total Chlorophyll was maximum in terminal leaflets in young presenescent leaves. Total chlorophyll was showing no significant differences in left and right leaflets of young, mature and presenescent leaves. While total nitrogen and total phosphate increased upto mature stage followed by decline. Total N and Total P was more in terminal leaflets and showed no significant difference in left and right leaflets.

Amylase activity increased continuously upto presenescent stage. Activity was more in left & right leaflets in case of mature and presenescent leaves.

IAA-oxidase activity increased continuously upto presenescent stage. Activity was more in terminal leaflets in case of mature and presenescent leaves than the sides leaflets.

Protease activity increased upto presenescent stage in all the three types of leaflets.

RNase activity showed rise upto presenescent stage. The level was more in side leaflets (left & right) upto presenescent stage.

In this system leaf as a whole shows normal pattern of senescence (e.g. chlorophyll wise). There are, however, difference in levels of chemical constituents and enzyme activities in different leaflets of the same leaf. Thus, the position of leaflet in a compound leaf is important as the position of leaf on a node.

The value of the present work is that, it indicates the importance of studying various patterns of senescence which further shows that generalizations with only standardized model systems may not necessarily lead to a unified concept. These studies form the basis for future indepth studies.

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Table 1. Visual changes accompanying senescence of different excised leaf lets of young and mature compound leaves of *Tecoma stans* during incubation in dark.

Position of Leaflet	Days of Incubation	Colour etc. changes in leaf stages	
		Young	Mature
Terminal	0	Light Green	Green
	2	Light Green	Green
	4	Light Green	Green
	6	Leaf Tip Curl	Leaf Tip Curl
	8	Brown Spots on Margin	Leaf Tip Curl
	10	Half Leaf Brown	Brown Spot at base
	12	More than Half Leaf Brown	Brown Spot at base
	14	Whole Leaf Brown	Half Leaf Brown
	16	-	Half Leaf Brown
	18	-	Whole leaf Brown

Position of Leaflet	Days of Incubation	Colour etc. changes in leaf stages	
		Young	Mature
Left/Right	0	Light Green	Green
	2	Light Green	Green
	4	Leaf Tip Curl	Leaf Tip Curl
	6	Leaf Wrinkled	Margin Brown
	8	Margins Brown	Margin Brown
	10	Half Leaf Brown	Margin Brown
	12	Whole Leaf Brown	Half Leaf Brown
	14	Whole Leaf Brown	Half Leaf Brown
	16	Whole Leaf Brown	More than Half Leaf Brown
	18	Whole Leaf Brown	Whole leaf Brown

Table 2. Changes in Pigment level, some biochemical components and enzyme activities in different leaflets Terminal (T), Left (L) and Right (R) of Young, mature and presenescent intact compound leaves of *Tecoma stans* (per organ basis)

Parameter	Position of Leaflet	Stages of Leaf		
		Young	Mature	Presenescent
Fresh weight (mg) ±SD	T	44.66 ± 2.06	209.16 ± 6.04	152.5 ± 1.80
	L	29.5 ± 1.04	106.66 ± 3.55	11.75 ± 1.60
	R	30.5 ± 1.87	116.83 ± 4.87	122.5 ± 1.62
Dry weight (mg) ±SD	T	6.60 ± 0.63	74.16 ± 2.13	60.5 ± 0.92
	L	4.82 ± 0.16	26.2 ± 1.13	50.5 ± 1.20
	R	5.57 ± 1.78	33.96 ± 1.09	52.9 ± 1.80
Chlorophyll a (mg/leaf) ±SD	T	0.028 ± 0.004	0.076 ± 0.007	0.054 ± 0.002
	L	0.009 ± 0.000	0.088 ± 0.023	0.055 ± 0.001
	R	0.014 ± 0.005	0.120 ± 0.001	0.064 ± 0.005
Chlorophyll b (mg/leaf) ±SD	T	0.020 ± 0.001	0.064 ± 0.001	0.051 ± 0.007
	L	0.003 ± 0.002	0.074 ± 0.001	0.036 ± 0.002
	R	0.027 ± 0.006	0.082 ± 0.001	0.036 ± 0.002
Total Chlorophyll (mg/leaf) ±SD	T	0.049 ± 0.001	0.136 ± 0.002	0.113 ± 0.004
	L	0.014 ± 0.002	0.162 ± 0.056	0.102 ± 0.003
	R	0.027 ± 0.006	0.194 ± 0.001	0.100 ± 0.001
Total Nitrogen (mg/leaf) ±SD	T	0.035 ± 0.005	0.094 ± 0.003	0.031 ± 0.001
	L	0.021 ± 0.001	0.082 ± 0.007	0.032 ± 0.001
	R	0.022 ± 0.001	0.093 ± 0.001	0.021 ± 0.003
Total Phosphate (mg/leaf) ±SD	T	0.039 ± 0.008	0.051 ± 0.001	0.043 ± 0.004
	L	0.009 ± 0.004	0.015 ± 0.002	0.025 ± 0.002
	R	0.088 ± 0.003	0.044 ± 0.001	0.029 ± 0.002
Amylase (µg starch degraded min^{-1} $leaf^{1}$±SD	T	0.077 ± 0.007	0.155 ± 0.008	0.196 ± 0.004
	L	0.053 ± 0.001	0.183 ± 0.001	0.232 ± 0.003
	R	0.091 ± 0.002	0.230 ± 0.001	0.250 ± 0.004
IAA Oxidase (µg IAA degraded min^{-1} $leaf^{1}$±SD	T	0.015 ± 0.003	0.056 ± 0.001	0.072 ± 0.003
	L	0.045 ± 0.001	0.013 ± 0.000	0.069 ± 0.009
	R	0.050 ± 0.001	0.019 ± 0.004	0.067 ± 0.012
Protease (µg aa released min^{-1} $leaf^{1}$)±SD	T	0.851 ± 0.004	2.657 ± 0.001	3.899 ± 0.083
	L	1.046 ± 0.016	2.254 ± 0.010	3.815 ± 0.002
	R	1.098 ± 0.019	2.259 ± 0.040	3.195 ± 0.012
RNase (µg RNA degraded hr^{-1} $leaf^{1}$)±SD	T	0.427 ± 0.003	0.953 ± 0.002	1.242 ± 0.004
	L	0.685 ± 0.004	0.898 ± 0.001	1.192 ± 0.007
	R	0.536 ± 0.001	1.012 ± 0.001	1.145 ± 0.008

Shrub of *Tecoma stans Stans* having terminal leaflet & right & left leaflets

Compound Leaf of *Tecoma*

Plate -1

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