J Indian bot Soc Vol 76 (1997) 25-27

BIOCHEMICAL CHANGES ACCOMPANYING DEVELOPMENT AND SENESCENCE IN LEAFLETS OF *TECOMA STANS*

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(Received June, 1997)

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 presenescence stage in the leaflets.

Key Words : Biochemical changes, Senescence, *Tecoma stans*.

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 phenomena. 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 leaflet were also taken for comparison.

For visual observations young growing and fully expanded mature leaves were excised and after surface sterilization, kept with petiole dipping in distilled water in vials. Incubation was done in dark and visual changes recorded till completion of senescence. For biochemical studies young growing, fully expanded mature and presenescent leaves were taken. The experiments were repeated at least thrice in triplicate.

MATERIAL AND METHODS

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 atleast four experiments, each done in triplicate.

Total Nitrogen : For estimation of nitrogen, digestion was done according to Shell 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 homogenised 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).

$$\text{Chl. a (mg/l)} = 12.72\ A_{665} - 2.58\ A_{645}$$
$$\text{Chl. b (mg/l)} = 22.87\ A_{648} - 4.67\ A_{663}$$

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

Received March, 1996

Table 1. 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 ± 0.000	0.074 ± 0.001	0.036 ± 0.002
	R	0.012 ± 0.004	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 (ug 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	0.014 ± 0.004	0.067 ± 0.012
Protease (ug aa relased 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 ± 0.002
RNase (ug 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	1.146 ± 0.008

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

For visual observations young and mature leaves were excised and kept in dark.

The young as well as mature leaves were green initially. No change was observed in any of the stage till 4th day. By 6th day tip of the leaves started curling. Browning of the young leaves started from 8th day and by 14th day whole leaf turned brown showing complete senescence. In mature leaves browning started as small spots at base of the leaf on 10th day and complete senescence was observed by the 18th day.

Table 1, 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 1 fresh weight increased upto mature stage in terminal leaflets and than declined. The right and left leaflets showed increase upto presenescent stage. 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 leaflet 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 standarized model systems may not necessarily

lead to a unified concept. These studies form the basis for future indepth studies.

The authors acknowledge the valuable suggestions and help given by Prof. D. Banerji and Prof. C.M. Govil.

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