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SIMILAR ACCELERATION/RETARDATION RESPONSE OF EXCISED *CATHARANTHUS ROSEUS* PETAL SENESCENCE YET DIFFERENT *MODUS OPERANDI* OF GROWTH REGULATORS

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Catharanthus roseus L. G. Don flowers were excised at young (stage I) and fully mature exhibiting onset of senescence (stage IV) stages. The flowers were subjected to $1x10^{-3}M$, $1x10^{-6}M$ each of Kinetin, Spermine and Abscisic acid treatments besides distilled water as control. Interestingly enough at stage I accumulation of anthocyanins marked acceleration of senescence by $1x10^{-3}M$ concentration of studied growth regulators and a degradation of the same marked delay by $1x10^{-6}M$ concentration of the three growth regulators. Delay exhibited a slowing down of overall metabolism, yet Kinetin exceptionally induces accumulation of proteins and total nitrogen owing to probable protein synthetic activity. Excision at the onset of senescence reverses the pattern of accumulation/degradation of anthocyanins during acceleration/retardation of senescence. ABA clearly delays senescence by $1x10^{-6}M$ dose through retention of proteins and utilization of sugars for necessary synthetic activities whereas Kinetin utilizes its nitrogen pool retaining sugars, and spermine at the expense of sugars and total anthocyanin besides retention of protein and nitrogen.

Key Words: *Catharanthus roseus*, Abscisic acid (ABA), Spermine, Kinetin.

Senescence has been defined as the series of deteriorative processes that are natural cause of death (Medawar, 1957; Leopold, 1961). Transition to flowering in plants is usually associated with senescence and death (Monocarpic). A series of biochemical and physiological changes are reported to occur during petal senescence (Halevy and Mayak, 1981). Flower in most cases being the organ with shortest period of longevity is chosen as excellent model system for the fundamental studies on senescence process. The process involved in senescence and death have gained importance due to its commercial value too. The present study was carried out to move a step forward unfolding the mechanism of senescence through hormonal polyamine-mediation for regulation of the process.

Abscisic acid, spermine and kinetin are known to regulate growth in different ways e.g., abscisic acid accelerates senescence kinetin is known to retard and spermine, a polyamine, is also reported to retard senescence. Their senescence regulating mechanisms are hardly known well. The objective of the present study is to search for a common molecule which may gear on its own synthesis or induce suppression of synthesis for exhibition of the mentioned response.

MATERIALS AND METHODS

The experiment was so designed that all marked stages of growth and development of *C. roseus* flowers are studied for their response towards post excision treatment with $1x10^{-3}M$ and $1x10^{-6}M$ concentrations of ABA, Kinetin and spermine along with distilled water after screening. Thus sets of excised *C. roseus* young (just open stage I), fully mature flowers exhibiting onset of senescence (stage IV), were subjected to the mentioned treatments in glass vials. Specific activity of amylase (Filner and Varner, 1967), protease (modified Green and Neurath, 1954), and peroxidase (Maehly and Chance, 1967), total protein (Lowry *et al.*, 1951), total sugars (Nelson, 1944), total nitrogen (Snell and Snell, 1967) and total anthocyanin (Sharma, 1981; Fuleki and Francis, 1968) were analysed in three experiments done in triplicates in all the sets, at the time of senescence in one of the mentioned treatments.

RESULTS AND DISCUSSION

The behaviour of same concentration of the growth regulator was found to be different at different developmental stages, suggestion an age dependent variation in avilability of receptors for a particular growth regulator manifesting varied response.

Thus at stage I, $1x10^{-3}M$ ABA induces increased amylase activity declining carbohydrates but accumulating anthocyanins during acceleration of senescence, whereas $1x10^{-6}M$ concentration of the same

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Table 1. Changes in biochemical parameters in excised stage I and stage IV *C. roseus* flowers treated with $1x10^{-3}$M and $1x10^{-6}$M concentrations of Abscisic acid with respect to distilled water (DW) controls (in terms of percentage).

Parameters	Stage I		Stage IV	
% over/below	Molar conc. of Abscisic acid			
DW	$1x10^{-3}$M	$1x10^{-6}$M	$1x10^{-3}$M	$1x10^{-6}$M
Specific activity of enzymes, mg protein^{-1}				
Amylase	2.5*	22.5*	33.33*	4.12*
Protease	26.2*	29.08*	4.44*	7.41
Peroxidase	179*	-	78.48	38.03
Total proteins, mg gdw^{-1}	0.112*	38.15*	8.05	15.33*
Total nitrogen, mg N gdw^{-1}	21.77	37.2*	25.47	15.68
Total sugars, mg glucose equivalents gdw^{-1}	8.95	11.4	1.77*	4.00*
Total Anthocyanins mg gdw^{-1}	37.45*	3.40*	8.15	7.80

* - % increase.
- Samples with no activity.

Table 2. Changes in biochemical parameters in excised stage I and stage IV. *C. roseus* flowers treated with $1x10^{-3}$M and $1x10^{-6}$M concentrations of spermine with respect ot distilled water (DW) controls (in terms of percentage).

Parameters	Stage I		Stage IV	
% over/below	Molar concentrations of Spermine			
D.W.	$1x10^{-3}$	$1x10^{-6}$M	$1x10^{-3}$M	$1x10^{-6}$M
Specific activity of enzymes, mg protein^{-1}				
Amylase	188.75*	32.50	15.62	6.05
Protease	147.50*	20.39	14.28	4.44*
Peroxidase	629.90*	85.06	64.37	0.00
Total protein, mg gdw^{-1}	48.60	16.00	43.08	26.70*
Total Nitrogen, mg N gdw^{-1}	15.10*	30.20	52.97*	22.28*
Total sugars, mg glucose equivalent g dwt^{-1}	2.98	12.87	16.76*	46.60
Total anthocyanins mg gdwt^{-1}	24.97	61.35	0.78	14.65

*-% increase.

Table 3. Changes in biochemical parameters in excised stage I and stage IV *C. roseus* flowers treated with $1x10^{-3}$M and $1x10^{-6}$M concentrations of Kinetin with respect to distilled water (DW) controls (in terms of percentage).

Parameters	Stage I		Stage IV	
% over/below	Molar concentrations of Kinetin			
DW	$1x10^{-3}$M	$1x10^{-6}$M	$1x10^{-3}$M	$1x10^{-6}$M
Specific activity of enzymes mg protein^{-1}				
Amylase	12.5	27.5*	8.69*	8.69
Protease	93.58	8.78*	6.56	94.83
Peroxidase	27.53	84.06	43.25	47.68*
Total protein, mg gdw^{-1}	0.82	0.61	20.65	9.83*
Total Nitrogen mg N gdw^{-1}	7.85*	25.2*	9.64*	20.70*
Total Sugars mg glucose equivalent gdw^{-1}	4.12	45.45*	64.2*	19.54*
Total Anthocyanins, mg gdw^{-1}	13.29*	58.23*	9.84	4.92*

* -% increase.

growth regulator brings about decline in anthocyanin and accumulation of total carbohydrates with similar increase in amylase activity yet retarding visible senescence. It therefore, seems to accelerate/retard senescence at stage I through regulation of sugar metabolism. This can also be related with other effects of ABA in which it causes dormancy or closes stomata by regulation of sugar metabolism (Table I).

Interestingly enough at stage IV a reversal in this pattern of total sugars and anthocyanins by both the concentrations of ABA points towards less availbaility of endogenous ABA and its active receptors at stage I than at stage IV causing only slight delay of senescence. Most of exogenous ABA accumulates at the base of petal tube making it swell and get hardened in the form of a ring. An accumulation of total proteins and total nitrogen in $1x10^{-6}$M ABA treatment in flowers of both the stages,with delay of senescence suggests triggering of shikimate pathway accumulating bound form of nitrogen to withstand stress within physiological limits. $1x10^{-3}$M ABA, as is imperative is a non-physiological shock/stress concentration depleting nitrogen reserves (Table 1).

Spermine behaves as ABA st stage I but st stage IV unlike ABA or kinetin, it exhibits accumulation of anthocyanin by $1x10^{-3}$M and decline by $1x10^{-6}$M treatment. Spermine treatment too induces decline in total sugars during delay of senescence (by $1x10^{-6}$M treatment) at both the stages. Total nitrogen content accumulates more in treatment with higher concentration of spermine and even that of kinetin compared to treatment with lower concentration (1x

10^{-6}M). This may be due to their being nitrogenous in nature too (Table 2).

Kinetin exhibits a decline in the activities of all the studied enzymes in both ($1x10^{-3}$M and 10^{-6}M) concentrations yet the increase in protein is marked in stage I with $1x10^{-6}$M Kinetin whereas, in stage IV an increase in total sugars with accumulation of anthocyanins marks delay of senescence. Thus it indicates shift of metabolic control by Kinetin during different developmental stages (Table 3).

In ABA and spermine treatments a climacteric rise in peroxidase activity in stage I marks acceleration of senescence by $1x10^{-3}$M whereas lesser rise or decline in peroxidase activity marks delay in senescence. In stage IV compared to controls the peroxidase activity declines highly in $1x10^{-3}$M of all growth regulators whereas, $1x10^{-6}$M treatment only, exhibits increase in peroxidase activity at the expense of probably total proteins in contrast to stage I. Thus, ABA clearly delays senescence in $1x10^{-6}$ M dose through retention of proteins (no synthesis) and utilization of sugars for necessary synthetic activities, whereas Kinetin utilizes its nitrogen pool retaining sugars and total anthocyanin besides retention of proteins and nitrogen for the same.

Therefore, tissue/organ-age dependent variations in response to different concentrations of growth regulators have been evidently noted to exist. Thus $1x10^{-3}$M concentration of ABA, kinetin and spermine tend to accumulate total anthocyanins with decline in sugars in stage I *C. roseus* excised flowers at the time of acceleration of senescence. $1x10^{-6}$M co ncentration of all the mentioned growth regulators induces decline in total anthocyanins while retarding senescence.

The pathways followed for retardation of senescence by $1x10^{-6}$M concentrations of all the growth regulators were however, different indicating the differences in mechanism of action. Not only that, the same growth hormone acts differently during different developmental stages of the flowers. Thus from the results it can be concluded that for retarding senescence at stage IV ABA might be acting through retention of proteins (no fresh synthesis) and utilization of sugars for necessary synthetic processes: Kinetin through utilization of nitrogen pool and to retain sugars, and spermine via retention of protein and nitrogen, utilizing sugars and total anthocyanin.

For retardation of senescence at stage I ABA probably retains sugars, protein and nitrogen. Kinetin accumulates very high amount of protein (probably synthesis). Spermine slows down the metabolic rate.

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