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Some Aspects of Metabolism of *Vigna* Leaves during Senescence and Water Stress

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Changes in chlorophyll, protein, RNA and proline contents and activities of RNase and protease were studied in excised leaves of *Vigna catjang* Endl. cv Pusa Barsati during dark-induced senescence and polyethyleneglycol (PEG 6,000)-induced water-stress. There was a gradual decline in chlorophyll, protein and RNA, a significant accumulation of proline and an increased activity of RNase and protease with increasing dark incubation. Increasing magnitudes of water stress (−500, −1,000 and −1,500 KPa) further accentuated the above changes, except in RNA content and RNase activity. Unlike senescence, there was a significant increase in RNA but RNase activity remained unaffected due to water stress treatment. Benzyladenine (BA, 10^{-4} and 10^{-5} M) treatment significantly inhibited the ageing induced decline of the above macromolecules and rise of enzyme activity but the proline content remained unaffected. Though BA-treatment increased the chlorophyll and protein contents and decreased protease activity in water-stressed tissues, it could not affect the high RNA content and low RNase activity in such tissues. It was concluded that proline accumulation was not a true index of senescence since it was not affected by BA and that RNA metabolism was also distinctly different in senescent and water-stressed leaf tissue of *Vigna*.

Key Words: Senescence, Water-stress, PEG-6000, RNA metabolism, Benzyladenine

Introduction

Water-stress is known to accelerate the degradation of different cellular macromolecules like chlorophyll, protein and nucleic acids (Dwivedi et al. 1980, Mukherjee & Choudhuri 1981, Levitt 1972) with concomitant increase in the activities of many hydrolytic and oxidative enzymes (Hsiao 1973, Mukherjee & Choudhuri 1981). Proline accumulation is also a characteristic phenomenon associated with water-stress (Mali & Mehta 1978). Leaf senescence is characterised by the decline in chlorophyll, protein and rise of protease and RNase activities (Woolhouse 1967, Martin & Thimann 1972). Proline accumulation

has also been implicated in ageing of *Vigna* seedlings (Prabha & Bharti 1980). All these results indicate that similar cellular changes may occur during water-stress and senescence. But the relationship between water-stress-induced and senescence-induced changes is still not clear. If senescence is accelerated due to water stress, then the variables associated with senescence would also be expected to show comparable changes during water-stress. Hence it will be worthwhile to study the changes in chlorophyll, protein, RNA, proline contents and activities of RNase and protease in isolated leaf discs during water-stress and senescence. The present study was undertaken to throw light on the relationship between senescence-induced water-stress and induced-changes in isolated leaf of *Vigna* treated with cytokinin.

Materials and Methods

The primary leaf of *Vigna* seedlings (*Vigna catjang* Endl. cv Pusa Barsati) grown for 15 days under cool white fluorescent light (1500 lux at the base of the plants) was used. The experiments were performed in the following ways.

1. The senescence rate in excised leaves incubated in 5 ml of distilled water in the dark for different durations (0, 2, 4 and 6 days) was studied on the basis of changes in chlorophyll, protein, RNA and proline content and protease and RNase activity. Effects of three magnitudes of water-stress viz.,—500 —1,000 and—1,500 KPa (19.6%, 20% and 36% of polyethyleneglycol 6,000 solution), were examined on the above-mentioned changes in excised leaves of *Vigna* after incubation for 4 days in the dark in 5 ml of these solutions.

2. Effects of two concentrations (10^{-4} and 10^{-5}M) of benzyladenine (BA) were examined on the above-mentioned changes in excised leaves of *Vigna* after 4 days of dark incubation. In addition, BA effect was also analysed in excised *Vigna* leaf discs subjected to water stress (—1000 KPa) in the presence of BA for the same duration in the dark.

In all cases, the leaf discs (pre-weight) were incubated in Petri dishes lined with Whatman filter paper No. 1 and contained requisite test solution as well as distilled water (control) containing 25 μg Na-penicillate and streptomycin sulphate per ml of the solution to check microbial growth. All the sets were maintained in the dark for durations as desired and at a temperature of 24±2°C.

Chlorophyll was estimated from 100 mg leaf tissue following Arnon (1949). Protein was extracted from the residue with 2 ml of 1N NaOH at 80°C for 1 hr (Mukherjee & Choudhuri 1981) and estimated according to Lowry et al. (1951). RNA extracted from 100 mg leaf tissue (Cherry 1962) and estimated by orcinol reagent adopting the modifications of Choudhuri and Chatterjee (1970). Extraction of proline was done from 500 mg leaf samples with 3% aqueous sulphosalicyclic acid. The supernatant collected after centrifugation at 5000× g for 15 min was used for the estimation of proline (Bates et al. 1973). RNase and protease were extracted and estimated by the method of Biswas and Choudhuri (1978). One g of excised leaves homogenized in 5 ml of 0.1. M phosphate buffer (*p*H 6.5) at 0°C was centrifuged at 10,000× g for 20 min. The procedure was repeated thrice for complete extraction and the supernatants were pooled together. The reaction mixture for protease consisted of 1 ml of enzyme extract, 0.1 ml $MgSO_4 . 7H_2O$ (0.1M) and 1 ml of bovine serum albumin (BSA, 0.5 mg/ml). After 1 hr of incubation at 37°C, the reaction was stopped by 50% TCA (1 ml)

and the residual protein was measured by Folin phenol reagent (Lowry et al. 1951). RNase was measured by incubating 1 ml of enzyme extract with 1 ml yeast RNA (4 mg/ml in 0.1M phosphate buffer, *p*H 5.7) for 30 min at 37°C. The reaction was stopped by 0.2 ml perchloric acid (70%); after centrifugation the supernatant was mixed with BSA (0.5 mg/ml of acetate buffer, *p*H 4.0) and the turbidity was measured at 420 nm.

In each enzyme assay, zero time control was taken as blank and the activity of each enzyme was expressed as $[(\Delta A \times TV)/t \times v]$, where ΔA is the absorbance of the sample after incubation minus the absorbance at zero time control, TV is the total volume of the filtrate, t is the time (min) of incubation with substrate and v is the volume of filtrate taken for incubation (Fick & Qualset 1975).

Each experiment was repeated at least 6 times with 2 replications for each set. The results in between treatments were statistically analysed and critical difference (CD) at 95% confidence limit was determined.

Results

There was a gradual decline in the contents of chlorophyll, protein and RNA and a significant proline accumulation with increasing dark incubation of leaf discs (table 1). The activities of RNase and protease also showed an increase with increasing incubation time. With the imposition of different magnitudes (−500, −1,000 and −1,500 KPa) of water stress for 4 days in the dark, the decline of chlorophyll and protein was further accentuated and a significant increase of proline and RNA and a higher activity of protease were noted in leaves

Table 1 *Changes in chlorophyll, protein, RNA and proline content and activities of RNase and protease of excised* Vigna *leaves, during ageing (0, 2,.4 and 6 days in the dark) and water stress*

Treatments	Chlorophyll content mg/100 mg dry wt	Protein content mg/100 mg dry wt	RNA content mg/100 mg dry wt	Proline content μ mole 100 mg dry wt min	RNase activity enzyme unit* 100 mg dry wt min	Protease activity enzyme unit 100 mg dry wt min
Control (initial)	2.17	55.30	3.17	9.09	0.021	.012
Ageing (2 days)	1.94	44.78	2.52	9.22	0.025	.014
Ageing (4 days)	1.82	39.65	2.07	15.88	0.036	.020
Ageing (6 days)	1.15	20.56	1.42	22.14	0.043	.029
Ageing (4 days) + Water stress (−500 KPa)	1.52	32.98	3.60	26.34	0.034	.031
Ageing (4 days) + Water stress (−1,000 KPa)	1.31	30.18	4.63	35.24	0.035	.041
Ageing (4 days) + Water stress (−1,500 KPa)	1.26	26.31	5.37	46.60	0.032	.048
CD at 5% level	0.14	3.01	0.59	3.57	.006	.003

* Enzyme unit is expressed as $[(\Delta A \times TV)/t \times v]$; ΔA, absorbance of the sample after incubation minus the absorbance at zero time control; TV, total volume of the filtrate; t, time (min) of incubation with substrate; v, volume of filtrate taken for incubation

subjected to water stress of higher magnitude (table 1). RNase activity remained unaffected due to water stress treatment.

BA at higher concentration (10^{-4}M) was responsible for significant decrease in chlorophyll, protein and RNA contents of leaves incubated in the dark for 4 days (table 2). But proline accumulation was not significantly reduced by BA treatment. The activities of protease and RNase were also significantly reduced by BA (10^{-4}M) treatment. BA (10^{-4}M) treatment of excised leaves of *Vigna* undergoing water stress (—1,000 KPa) for 4 days in the dark, significantly increased the content of chlorophyll and protein and protease activity (table 2). RNA and RNase activity were not altered significantly from stress control by this treatment (table 2). Also, proline accumulation was not significantly changed by BA treatment.

Discussion

Senescence in detached isolated leaves of *Vigna* is related to a decline in the levels of chlorophyll, protein and RNA (Mukherjee & Choudhuri 1981) which is either due to higher activities of protease and RNase or reduced synthesis of these molecules during senescence (Woolhouse 1967, Thimann 1980). It has been shown by Prabha and Bharti (1980) in *Vigna* and Wang et al. (1982) in rice that ageing is also associated with proline accumulation. Our data also support that above changes are occurring in isolated leaves of *Vigna* during ageing in the dark.

The present study also demonstrates that the loss in chlorophyll and protein due to ageing is further accentuated by water stress except RNA, which rather increases. Such a decline in chlorophyll and protein content due to water-stress

Table 2 *Effect of BA (10^{-4} & 10^{-5}M) on chlorophyll, protein, RNA and proline content and activities of RNase and protease of excised* Vigna *leaves during ageing (4 days in dark) and water stress*

Treatments	Chlorophyll content mg/100 mg dry wt	Protein content mg/100 mg dry wt	RNA content mg/100 dry wt	Proline content μ mole/100 mg dry wt min	RNase activity Enzyme Unit* 100 mg dry wt/min	Protease activity Enzyme unit/100 mg dry wt/min
Control (initial)	2.17	55.30	3.17	9.09	0.021	0.012
Ageing (dark)						
Ageing (4 days)	1.82	39.65	2.07	15.88	0.036	0.020
10^{-4}M BA+Ageing (4 days)	2.13	47.72	2.54	14.68	0.024	0.016
10^{-5}M BA+Ageing (4 days)	2.04	44.90	2.27	15.29	0.030	0.017
Ageing+Stress (dark)						
Ageing (4 days)+Stress (—1,000 KPa)	1.31	30.18	4.63	35.24	0.035	0.041
10^{-4}M BA+Ageing (4 days)+Stress (—1,000 KPa)	1.65	36.95	4.39	32.91	0.035	0.033
10^{-5}M BA+Ageing (4 days)+Stress (—1,000 KPa)	1.60	36.70	4.56	34.07	0.035	0.036
CD at 5% level	0.16	3.33	0.41	2.60	0.006	0.003

* Enzyme unit is expressed as in table 1

is quite consistent with the observations of Dwivedi et al. (1979) and Mukherjee and Choudhuri (1981). The decline in protein content may be correlated with the higher protease activity during water stress, as was also observed during senescence. But, interestingly, the RNase activity, instead of an increase (Levitt 1972), remained unaffected due to water stress. It can, therefore, be argued that water stress does not induce identical changes in all cellular activities in *Vigna* leaves as are generally associated with leaf senescence.

There are many studies which reveal that cytokinin is most effective in inhibiting leaf senescence. BA (10^{-4} and 10^{-5}M) treatment shows better retention of chlorophyll, protein and RNA contents than control in senescing tissues. These are also in conformity with the observations of Richmond and Lang (1957), Thimann (1980). But, interestingly, proline accumulation is not significantly arrested in BA treated senescing tissues suggesting that the proline accumulation may not be a true index of senescence.

It has also been reported that the adverse effects of water stress could be ameliorated by kinetin treatment (Levitt 1972). In water-stressed tissues of *Vigna*, BA treatment increases the chlorophyll and protein contents as compared to control by checking their decline and the water-stress-induced higher activity of protease is also inhibited by BA. As in senescing tissues, water-stress-induced proline accumulation is not significantly affected by BA treatment. This finds support from the observations of Palfi (1968), who has demonstrated that kinetin-treatment cannot affect the proline accumulation. Interestingly, unlike senescence, water-stress-induced RNA level and RNase activity in isolated leaf discs of *Vigna* remain unaffected by BA treatment.

The present results probably suggest that although many identical cellular changes are occurring during senescence and water stress, there are at least some metabolic aspects (e.g. RNA metabolism) which do not show similar changes, in *Vigna* leaf discs.

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