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Role of Antioxidants on the Maintenance of *Phaseolus coccineus* Lam. Seeds under Adverse Storage Environment

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ABSTRACT

Pretreatment of Phaseolus coccineus *seeds with antioxidants viz. ascorbic acid, melatonin (N-acetyl-5-methoxy tryptamine) and green tea extract 12 hours before accelerated ageing treatment significantly arrested the leakage of soluble carbohydrates, amino acids and electrolytes and such treatments checked the loss of vital cellular components viz. proteins, carbohydrates, DNA, RNA etc.*

INTRODUCTION

The deterioration of seeds is the major problem in Darjeeling Hills for its high annual rainfall and a very high relative humidity (RH) which causes the loss of viability of seeds (Basu, 1976). One common facet of deteriorating seeds is the increased leakage of cellular constituents due to cell membrane disruptions associated with the loss of membrane phospholipids. Phospholipid decline has been reported in deteriorating tomato (Francis and Coolbear, 1984) and sunflower (Halder *et al.*, 1983) seeds. Free radicals react with protein and lipid components of membrane causing deterioration (Bhattacharjee and Mukherjee, 1997).

Previous observations showed that antioxidants have a role in delaying senescence under storage (Dey and Jana, 1988). The role of ascorbic acid in the retardation of seed senescence has already been reported (Chhetri *et al.*, 1993). Melatonin (N-acetyl-methoxy tryptamine) has well-demonstrated anti-oxidant functions (Reiter, 1998) and the efficacy of green tea extract against the lipid peroxidative damage of cell membrane is known (Ramanathan *et al.*, 1995). Therefore, an attempt has been made in the present investigation to enhance the storage potential of *Phaseolus coccineus* seeds using these chemicals as the manipulative agents.

MATERIALS AND METHODS

Experiments were carried out with freely harvested seeds of *Phaseolus coccineus* Lam. obtained from the office of the principal agricultural officer,

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Darjeeling. All experiments were carried out following the methods of Halder and Gupta (1980). The seeds were surface sterilized with 0.1% $HgCl_2$ for 90 seconds and all the seed lots were separately presoaked in an aqueous solution of ascorbic acid (100, 300 and 500 µg/ml), melatonin (100, 300 and 500 µg/ml) and green tea extract (100, 200 and 500 µg/ml v/v) prepared by boiling 15 gms of green tea obtained from *Camellia sinensis* in 300 ml of distilled water for 10 minutes, cooling the same in a closed container for 15 minutes followed by filtration. The pretreatment was carried out for 12 hours and then the seed lots were dried back to their original weight. A control set was prepared in similar manner by soaking the seeds in distilled water. Subsequently the pretreated seed lots were taken in separate cloth bags and stored in a desiccator in which an environment of 98.2% RH was imposed by keeping 250 ml of 5.96% H_2SO_4 at the base of the desiccator. The experimental set was kept at room temperature (20±2°C), allowing the seeds to experience the accelerated ageing treatment. The H_2SO_4 was changed periodically by quickly replacing the acid to maintain the desired RH within the desiccator for 2 weeks. From the pretreated and control seed lots, analysis were made at 7-day intervals i.e., after 0, 7 and 14 days of accelerated ageing.

For the analysis of germination percentage the pretreated seed lots (50 each) were transferred to separate petridishes containing filter paper moistened with 10 ml of distilled water. The seeds were allowed to germinate for 7 days as per the international rules for seed testing (1976) before calculating the percentage germination. Samples of protein estimation were taken from dehusked seeds. Extraction of protein was made following the method of Kar and Mishra (1976) and the estimation was done using folinciocalteau reagent (Lowry *et al.*, 1951). Free amino acid was measured from the pooled seed leachate after immersing 1 g of seed sample in 20 ml of distilled water for 24 hours. The estimation was done with ninhydrin following the method of Sadasivam and Manickam (1996). The soluble carbohydrate level was determined from the seed leachate after immersing 1 g of seed sample in 20 ml of deionised distilled water for 16 hrs. The estimation was done following the method of Mc Cready *et al.* (1950). Extraction of RNA was made from seed kernels following the method described by Cherry (1962) and the quantitative estimation of the same was done as per the method of Markham (1955) modified by Choudhury and Chatterjee (1970). Extraction of DNA was made from the seed kernels in the same way as that of RNA and the estimation was done as per Plummer (1979). To analyse electrical conductivity 5 gm of seed lots of each treatment were immersed in 25 ml. of deionised distilled water for 16 hours at room temperature. Leakage of electrolytes was then measured from the pooled seed leachate of the control and treated samples by a direct reading conductivity meter.

RESULTS AND DISCUSSION

The germination rate of *Phaseolus coccineus* seeds steadily declined with increased duration of accelerated ageing (Fig 2). However, the rate of this decline occurred at a slower rate for seed treated with ascorbic acid, melatonin and green tea extract. In this regard the effect of seed pretreatment with melatonin was found to be the most significant. The decline in the vigor of *Phaseolus coccineus* seeds during accelerated ageing was due to attack by seed microflora and the degradation of cell membrane and macromolecules by the activated oxygen species which were evaluated by biochemical and physiological parameters.

The protein content in the seeds of *Phaseolus coccineus* remarkably declined in the control samples after 14 days of accelerated ageing treatment (Table 1). Hydrolysis of protein to amino acids and polypeptides takes place by protease and peptidase action during seed deterioration (Pernolett, 1978; Basha and Cherry, 1978). In addition there is a depletion of some enzymes (Cherry *et al.*, 1976), which are the causes of protein depletion. However, the magnitude of the decline was substantially controlled in the antioxidant pre-treated seed samples. Of the chemical treatment melatonin was found to be most effective.

Table 1. Effect of pretreatment with ascorbic acid (AA 100, 300 and 500 µg/ml) melatonin (MT 100, 300 and 500 µg/ml) and green tea extract (GT 100, 300 and 500 µg/ml v/v) on protein content (mg./gm) and leaching of free amino acids (mg/gm/20 ml) from *Phaseolus coccineus* seeds under accelerated ageing conditions. (Values are mean ± SE of 3 replicates)

Treatments	Protein content			Leaching of amino acids		
	Days after accelerated ageing			Days after accelerated ageing		
	0	7	14	0	7	14
Control	42.7±0.40	31.4±0.41	27.1±0.11	3.9±0.82	9.2±0.30	13.8±0.53
AA 100	41.6±0.61	36.6±0.34	32.5±0.28	2.9±0.15	5.4±0.23	9.2±0.44
AA 300	42.7±1.53	39.2±1.14	34.3±0.20	3.8±0.27	6.6±0.05	9.4±0.37
AA 500	40.0±0.41	37.1±0.34	30.4±0.20	2.8±0.23	5.8±0.15	10.5±0.28
MT 100	43.3±0.34	39.6±0.44	38.6±0.30	3.7±0.24	4.6±0.33	7.3±0.64
MT 300	43.1±0.26	40.2±0.30	38.3±0.17	3.1±0.24	5.1±0.17	6.8±0.30
MT 500	41.6±0.29	38.4±0.23	36.2±0.20	3.6±0.30	6.4±0.41	7.8±0.60
GT 100	43.9±0.47	34.1±0.05	28.2±0.23	3.4±0.10	8.0±0.11	11.5±0.15
GT 300	40.8±0.59	29.4±0.11	25.4±0.38	3.9±0.30	9.2±0.20	12.5±0.15
GT 500	43.4±0.30	32.6±0.18	28.5±0.15	4.0±0.14	8.7±0.10	11.8±0.11

A large increase in the soluble carbohydrate content (Table 2) occurred from all seed lots and the magnitude of the sugar content showing positive correlation with the duration of accelerated ageing. However, the level of the increase was found to be remarkably low in seed lots treated with different concentrations of ascorbic acid, melatonin and green tea extract. Amino acid content also showed a progressive increase with advancing period of accelerated ageing following the trend of soluble carbohydrate content. However, the level of increase was much lower in the anti-oxidant pre treated seeds (Table 1). In this case the pre treatment of seeds with melatonin was found to be more effective than the other two chemicals used in controlling the increase of amino acid level. A large increase in the electrical conductivity was observed in the pre-treated as well as the control seeds after two weeks of accelerated ageing. However, the magnitude of the increase was considerably slowed down in case of antioxidant pretreated seeds (Fig 1).

Regarding the changes in RNA level, it followed the declining trend identical to those of protein content (Table 3). The present study clearly showed that the declining drift in RNA content was significantly slowed down in seed samples subjected to melatonin pretreatment, though the immediate effect of the pretreating chemical seemed insignificant. In the deteriorated seeds the spoolable DNA content showed a negative correlation with the advancement of ageing (Table 3). It cannot be pointed out how the declining rate of such DNA level is related to the progressive deterioration of *Phaseolus coccineus* seeds. However, in melatonin, green tea extract and ascorbic acid pretreated seeds the deterioration is largely checked. May be, these pretreating substances stimulate the activity of free radical scavenging enzymes. Melatonin, is a novel chemical, used for the seed deterioration studies, which acts both as a radical scavenging agent and

Table 2. Effect of pretreatment with different concentrations of ascorbic acid (AA 100, 300 and 500 µg/ml), melatonin (MT 100, 300 and 500 µg/ml) and green-tea extract (GT 100, 300 and 500 µg/ml v/v) on leaching of soluble carbohydrates (mg/g/20 ml) from Phaseolus coccineus seeds under accelerated ageing conditions. (Values are mean ± SE of 3 replicates)

Treatments	Days after accelerated ageing		
	0	7	14
Control	3.3±0.12	10.6±0.20	14.1±0.15
AA 100	3.1±0.26	8.3±0.17	12.1±0.05
AA 300	2.9±0.20	8.6±0.11	10.9±0.10
AA 500	3.2±0.24	8.2±0.05	10.3±0.25
MT 100	3.2±0.20	7.8±0.10	11.0±0.26
MT 300	3.0±0.11	6.5±0.15	9.8±0.82
MT 500	2.8±0.15	6.0±0.62	8.7±0.46
GT 100	2.9±0.20	8.7±0.70	12.4±0.15
GT 300	2.9±0.05	8.9±0.38	10.6±0.22
GT 500	3.4±0.56	9.1±0.10	12.0±0.26

Table 3. Effect of accelerated ageing and pretreatment with ascorbic acid (AA 100, 300 and 500 µg/ml), melatonin (MT 100, 300 and 500 µg/ml) and green-tea extract (GT 100, 300 and 500 µg/ml v/v) on RNA content (mg/g fresh wt) and spoolable DNA content (mg/g fresh wt.) of Phaseolus coccineus seeds. (Values are mean ± SE of 3 replicates).

Treatment	RNA content			DNA content		
	Days after accelerated ageing			Days after accelerated ageing		
	0	7	14	0	7	14
Control	995±35.02	646±109.58	423±1.22	18.3±0.20	14.1±0.67	9.6±0.36
AA 100	969±64.23	717±19.48	573±80.50	19.4±0.15	15.3±0.15	13.7±0.61
AA 300	953±41.09	633±62.02	589±71.41	21.5±1.51	18.4±0.53	13.2±0.20
AA 500	930±140.68	680±20.61	530±1.22	20.7±0.53	14.3±0.21	11.4±0.11
MT 100	973±56.75	729±23.21	638±16.71	19.7±0.70	17.2±0.41	14.1±0.14
MT 300	968±67.58	711±40.74	601±52.74	18.6±0.25	15.3±0.30	12.9±0.21
MT 500	943±18.63	683±50.35	564±67.41	17.9±0.05	14.6±0.74	13.0±0.18
GT 100	964±27.54	703±17.63	572±20.92	18.3±0.65	13.1±0.21	9.8±0.30
GT 300	941±37.65	647±20.11	551±26.25	17.2±0.21	13.5±0.28	10.9±0.25
GT 500	879±52.81	630±27.32	426±45.53	18.4±0.20	14.2±0.25	11.4±0.15

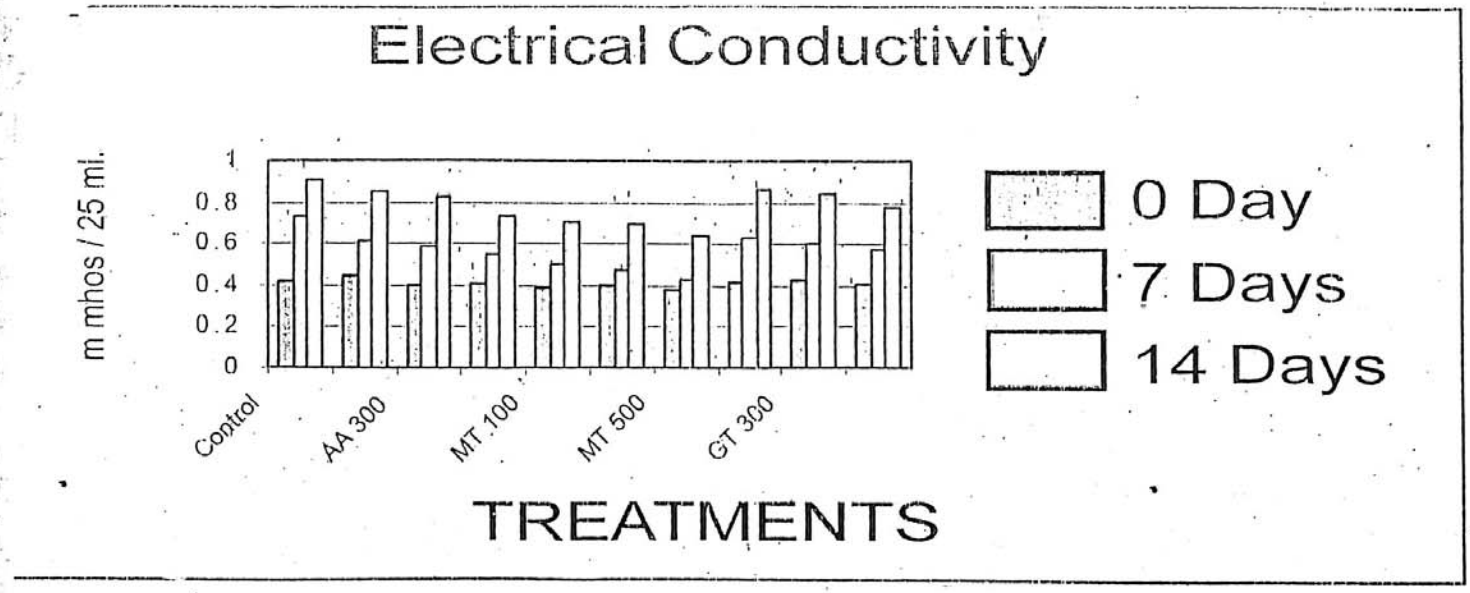

Fig. 1 Effect of pretreatment with ascorbic acid (AA 100, 300, and 500 ug/ml), melatonin (MT 100, 300 and 500 ug/ml) and green-tea extract (GT 100,300, and 500 ul/ml v/v) on electrical conductivity(m mhos / 25 ml.) of pooled leachate from *Phaseolus coccineus* seeds under accelerated ageing conditions.

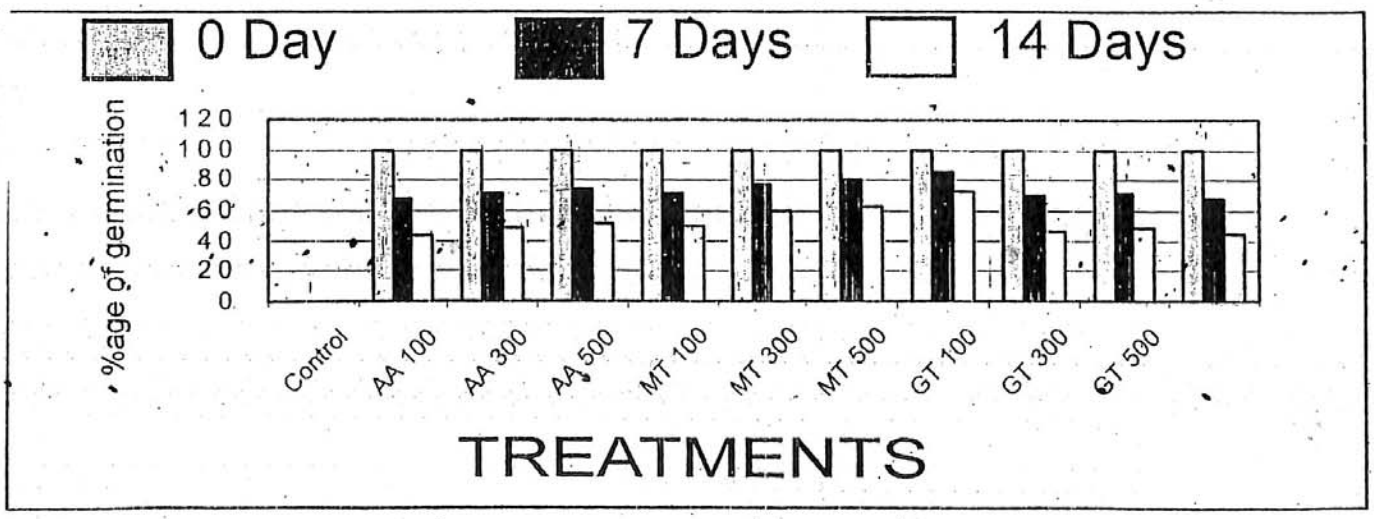

Fig. 2 Effect of accelerated ageing and pretreatment with ascorbic acid(AA 100, 300, and 500 ug/ml), melatonin (MT 100, 300 and 500 ug/ml) and green-tea extract (GT 100,300, and 500 ul/ml v/v) on percentage of germination of *Phaseolus coccineus* seeds.

a preventive antioxidant (Reiter, 1998). Preventive antioxidants arrest the formation of free radicals by enzymatic decomposition of their precursor molecules. Besides, melatonin reduces the activity of pro-oxidant enzymes like nitric oxide synthetase (NOS), which is implicated in the ageing process.

Despite several biochemical pointers indicating the status of seed deterioration, the mechanism of deterioration is still unclear. The action of melatonin treatment strongly supports the view that it protects DNA molecules, lipids and proteins from oxidative attack (Srinivasan, 1999). Another pretreating agent green tea extract is known for its superoxide radical and hydroxide radical scavenging properties. The components of green tea mainly catechines like (-) epicatechin gallate and (-) epigallocatechin gallate produced the strongest protection against lipid peroxidation (Ramanathan *et al.*, 1995).

The present study showed that high RH treatment rapidly accelerated the ageing of *Phaseolus coccineus* seeds even under the short-term ageing period of two weeks. The results indicate the fact that accelerated ageing damages the membrane, which consequently resulted in higher leakage of soluble substances, and the pretreating chemicals alleviated this condition to a great extent. The data presented also revealed that the level of protein, DNA and RNA gradually declined in control samples with the duration of ageing and the seed pretreating chemicals considerably slowed down this declining trend. From this we can conclude that though deterioration is a common phenomenon both in the treated and the control samples, the catabolic processes remained suppressed in the treated seeds making them tolerant to the adverse environmental conditions. Whatever may be the mechanism of preventing the deterioration, the pretreating chemicals were effective in slowing down the ageing of *Phaseolus coccineus* seeds. These chemicals may have slowed down the catabolic processes within the seeds and also checked the membrane damage by free radicals. If the chemicals controlled the growth of seed microflora, the mechanism is not known. This study showed that ascorbic acid, green tea extract and melatonin were mildly, moderately and substantially effective respectively as a pretreating chemical for seed preservation.

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