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Synergistic effect of heat and osmotic stress in causing membrane injury in *Hevea brasiliensis*

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ABSTRACT

Heat stress, especially in conjunction with soil moisture stress, is the most common environmental factor that limits the growth and productivity of *Hevea brasiliensis* in many non-traditional areas of rubber cultivation in India. It is an established fact that cellular membrane stability assesment using the electrolyte leakage method can be used to assess the tolerance of crop plants to different stresses. In nature, high temperature often interacts with water deficit. In order to quantify the interaction of two stresses in causing membrane injury, an experiment was conducted where osmotic stress (polyethylene glycol, PEG 6000) followed by high temperature stress was applied to leaf segments in a temperature-controlled water bath. When high temperature was superimposed on water stress, the stability of cellular membranes decreased drastically resulting in high leakage of electrolytes from the tissue. This synergistic response indicates that combined stress or multiple stress is more injurious than stress due to a single environmental factor. This method appears to be useful for evaluation of a large number of *Hevea* genotypes for tolerance to a combination of heat and water stress.

Introduction

Recently, scientists from all over the world have predicted global environmental change in the next century. Obviously, water and temperature will be major abiotic stresses which strongly limit plant growth and productivity. It is a known fact that the membrane systems in plant cells are sensitive to water and temperature stresses. At the cellular level, membrane stability has been recognised as an important attribute to stress tolerance in crop plants. Conductimetric measurement of electrolyte leakage has been used as a test for estimating membrane stability[1]. Since the amount of electrolyte leakage is a function of membrane permeability, the cell membrane stability of different genotypes can be evaluated in terms of electrical conductance.

In the non-traditional areas of rubber cultivation, more than one environmental stress is usually experienced. In the summer months, plants are exposed to high temperatures and severe drought. The result is a low growth rate, increased thermal injury and decreased crop productivity[2]. Success in expansion of rubber cultivation into non-traditional areas lies in selecting promising clones that are tolerant to both water and high temperature stresses. Rajagopal *et al*[3] observed significant clonal variations in membrane thermo-stability for heat. The purpose of this study was to determine how various clones respond to both stresses together using a simple laboratory technique for measuring cellular membrane stability. By employing multiple stress treatments, an attempt has been made to characterize clones for their tolerance to both water and high temperature stresses.

Materials and methods

The method used for the measurement of cell membrane stability of *Hevea brasiliensis* was that of Sullivan[4]. Heat stress was applied to leaf discs using a temperature-controlled water bath. Osmotic stress was applied using a known osmotic agent, polyethylene glycol (PEG 6000). Five-year-old *Hevea* clones (RRII 105, RRIM 600, SCATC 88/113, Haiken 1 and PB 260) randomly selected from the experimental field of the Rubber Research Instute of India were used for the study. Fully expanded leaves with similar stages of physiological maturity were collected and washed in deionised water before punching the middle of the leaflets (leaf disc diameter = 1cm). Eight discs each were collected from either side of lamina and used for treatments (T) and controls (C) separately.

Each sample consisted of eighty leaf discs collected from the middle of the leaflets, and from this twenty each were used for heat, PEG, PEG + heat treatment and the control. For clonal comparison similar samples were collected for five replications. All of these samples were washed thoroughly with distilled water in order to remove exogenous and endogenous electrolytes leached out due to cutting of the tissues. The sample without any treatment served as the control. The following treatments were carried out:

PEG treatment

Each sample consisted of three treatments and one control tube. Polyethylene glycol solution was prepared by dissolving PEG in distilled water. 20ml PEG solution was added to each tube and kept for 24 hours. The samples were washed three times with distilled water and the volume was then made up to 30ml. The tubes were incubated at 10°C for 18 hours before reading the initial conductance using a conductivity bridge.

Heat treatment

The treatment tubes were placed in a controlled water bath at 45°C for 1 hour. The volume was made up to 30ml and incubated at 10°C for 18 hours. This was to allow diffusion of electrolytes from the leaf tissue before reading the initial conductance.

PEG plus heat treatment

The sample tubes were first incubated in PEG solution for 24 hours, washed repeatedly with distilled water and kept in a temperature-controlled water bath at 45°C for 1 hour. The volume was made up to 30ml and incubated at 10°C for 18 hours for the diffusion of electrolytes. The sample tubes were brought to room temperature and the initial conductance was then read.

Upon the completion of measurements of initial conductance the control and treatment tubes were wrapped in Aluminium foil and autoclaved at 1.4 kg/sq cm for 15 minutes to completely kill the leaf tissue. The tubes were then brought to room temperature, the contents were thoroughly mixed and the final conductance read. The degree of injury to the cell membranes was calculated as follows:

$$\% \text{ Injury} = \left[1 - \frac{(1 - T1/T2)}{(1 - C1/C2)}\right] \times 100$$

where T1 and T2 are the initial and final conductances of the treatments and C1 and C2 are the initial and final conductance of the controls, respectively. T1/T2, the ratio of initial conductance, is a relative measure of the amount of electrolyte leakage induced by the treatments and is assumed to be proportional to the amount of injury induced in cellular membranes.

Results

In *Hevea brasiliensis*, the polyethylene glycol treatment enhanced the high temperature induced leakage of electrolytes from the leaf tissue. There was concentration dependent membrane injury in clone RRII 105. When the stressed leaf discs were subjected to superimposed high temperature, there was an appreciable increase in levels of injury as expressed by the increased leakage of electrolytes from the leaf tissue. The effectiveness of PEG induced water stress in promoting heat injury of *Hevea* leaf discs was even more apparent at 60, 70 and 80% concentrations of PEG and 45°C treatment for 1 hour (Figure1).

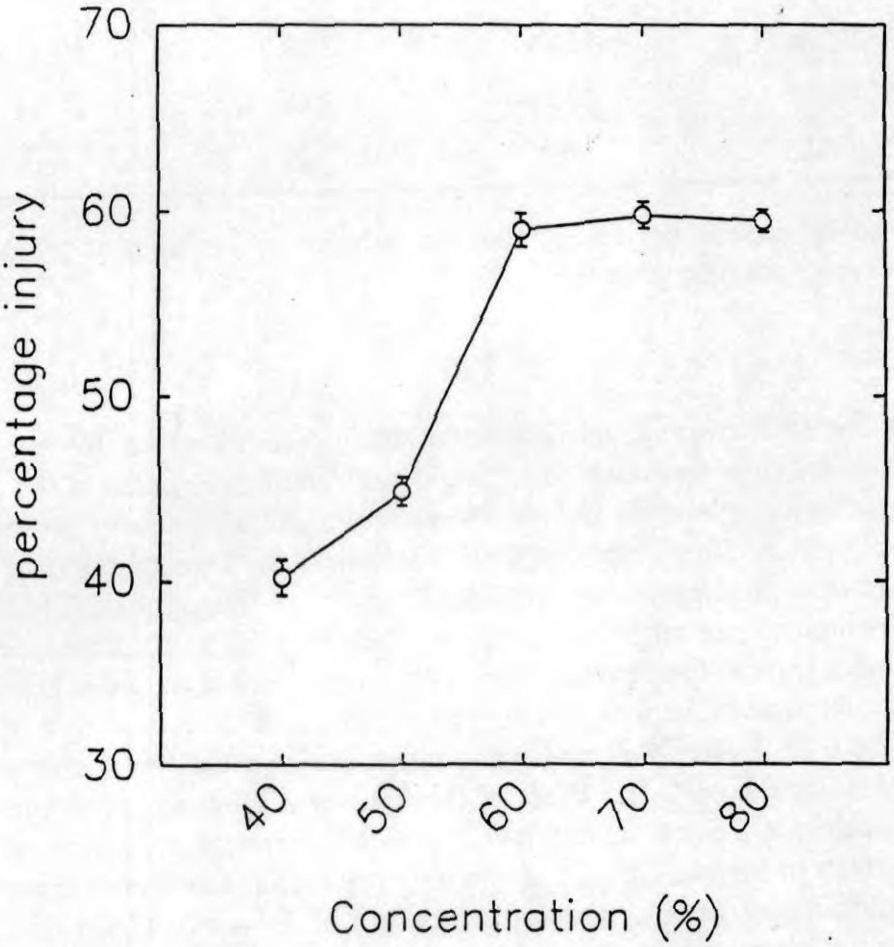

Figure 1 ***Effect of pre-treatments with different levels of osmotic stress (polyethylene glycol) on subsequent induction of heat injury at 45°C in leaf tissue***

The combination of treatments at which about 50% injury was induced has been suggested as a suitable means for the estimation of the stress tolerance of *Hevea* to water and high temperature stresses. The clones were screened for multiple stress tolerance using this combination of treatments. Significant clonal variations in membrane injury for osmotic and heat stresses were observed (Table 1). Different clones expressed similar trends in membrane injury for various treatments. The leakage of electrolytes from the leaf tissue was always higher in multiple stress treatments. Comparatively, clones SCATC 88/113, RRII 105 emerged as susceptible and clones RRIM 600, Haiken 1 and PB 260 emerged as tolerant to both stresses.

Table 1 *Clonal variations in cell membrane injury*

Sl. No.	Clone	Percentage injury		
		Heat	PEG	PEG + Heat
1.	SCATC 88/113	34.0a	26.2	69.7a
2.	RRII 105	35.3a	23.3	62.3b
3.	RRIM 600	21.5b	20.8	49.6c
4.	Haiken 1	18.6b	21.9	47.2c
5.	PB 260	20.2b	20.4	37.2d
	CD at 0.05	3.05	ns	4.47

Note: Means not followed by same letters are significantly different at 5% level of probability, analysed according to Duncan's multiple range test.

Discussion

Laboratory screening of *Hevea brasiliensis* for both water and high temperature stresses using this technique has advantages over screening for a single environmental stress. In the field, plants are seldom exposed to a single stress and are often exposed simultaneously to multiple stresses. In nature, temperature often interacts with water deficit. Thus, screening of a genotype either for water or high temperature stress alone will have low applicability in the field since *Hevea* is a perennial tree crop. This crop experiences a high air temperature in summer in the non-traditional areas. Temperatures above 45°C have been recorded at Dapchari in the summer months. The atmospheric stress or vapour pressure deficit at mid-day at Dapchari is reported to be 6KPa[5]. Here, soil moisture content is also a limiting factor which often remains below the wilting point[6]. The effect of these adverse climatic conditions is a decreased growth rate, drying of the leaf margin and low yield[2]. Extreme symptoms of leaf injury such as partial burning of lamina, whole leaf and twig drying have also been reported[7]. The impact of major environmental constraints on the growth of *Hevea* has long been the subject of study[8,9]. In the traditional rubber growing belt an unusual drought was experienced in the year 1986-87 and it was found that the soil water potential decreased below the wilting point for several days. The clone RRII 105 showed drying of the lower branches which reflected its susceptibility to severe drought (unpublished data). Chandrasekhar *et al*[2] characterized the clone RRIM 600 as a drought tolerant clone in comparison with other modern clones studied under the extreme conditions prevailing in the Konkon region of Maharashtra. Therefore, selection based on their tolerance to more than one stress will improve the strategy for survival of *Hevea* in adverse climatic conditions. It is also necessary to categorize modern clones on the basis of their tolerance to water and high temperature stresses in order to explore the possibilities of extending rubber cultivation to less congenial but potential areas in non-traditional rubber growing regions as suggested by Sethuraj *et al*[9]. Further studies are in progress to evaluate more *Hevea* clones for multiple stress tolerance.

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