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MEMBRANE STABILITY AS MEASURED BY ELECTROLYTE LEAKAGE: A TOOL FOR SCREENING *HEVEA* CLONES FOR COLD TOLERANCE

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Using the modified Sullivan (1972) method 13 *Hevea* clones were screened for their tolerance to cold temperature treatment in the laboratory. Leaf discs prepared from a composite leaf sample of each clone were subjected to low temperature (2° C) and electrolyte leakage from treated leaf discs was compared with control samples which were maintained at room temperature (25°C). The membrane injury in cold treated discs relative to control leaves was computed and expressed in percentage. Cold treatment of leaf discs led to appreciable loss of membrane stability which ranged from 5 to 50 per cent showing marked variability among the clones of *Hevea*. This method will be a promising experimental tool for screening clones for cold tolerance if the results ally with the performance of the clones under cold conditions in the field.

INTRODUCTION

Many plants of tropical and subtropical origin sustain damage when exposed to temperatures below a critical threshold temperature of 10-12° C and evokes multiple and complex symptoms. Chilling sensitive plants exposed to low temperatures become injured (Lyons, 1973). Studies on chilling injury in rubber becomes essential as rubber cultivation is now being extended to non traditional low temperature prone areas like Assam, Tripura, Meghalaya, Nagaland, Mizoram, etc. In these regions the temperature goes down occasionally to around 5° C and usually below 10° C at night during winter. Cold damage to rubber trees is a complicated genetical, physiological and ecological phenomenon. This is because responses to low temperature vary with clones, age of the plant and vigour status of the plant. Because of such a complicated relationship between rubber and its ecology, selecting a physiological parameter to screen the clones for cold tolerance becomes difficult and mostly unreliable.

Among the many physiological and biochemical parameters such as photosynthetic rate, respiration, membrane stability, lipid metabolism , chlorophyll fluorescence etc., electrolyte leakage measurement to study the stability of membrane is easy and economic. The permeability of the plasma membrane is very sensitive to low temperature. Low temperature influenced membrane injury leads to increased cell membrane permeability and electrolyte leakage (He and China, 1986). There is a negative correlation between temperature and electrolyte leakage in *Hevea*. It has been confirmed that cold tolerant varieties of *Hevea* showed less electrolyte leakage during extreme cold conditions. Low temperature intensity has more impact on membrane permeability of *Hevea* than the duration of exposure.

In the present study, leaf discs from *Hevea* clones were subjected to cold stress in the lab conditions and their membrane stability was studied with an objective to investigate if there is significant clonal variation in maintenance of membrane stability during cold stress.

MATERIALS AND METHODS

In this study 13 clones of *Hevea* were selected randomly viz. RRII 5, RRII 208, SCATC 88-13, Haiken 1, RRII 308, RRII 118, RRIM 600, RRII 300, RRIM 703, SCATC 93-114, RRII 105, PCK 2, and PCK 1 from a field of Rubber Research Institute of India Experimental Farm, planted in 1989. Membrane stability was studied using a modified electrolyte leakage technique (Sullivan, 1972). Leaf discs of 1 cm^2 size were prepared from a composite pool of physiologically matured leaves that were collected from five trees of each clone. Leaf discs were washed with distilled water four times to remove any excess electrolytes that were present in the cut ends of the leaf discs. After washing, 20 leaf discs each were transferred to 12 boiling tubes. Leaf discs in six boiling tubes were subjected to low temperature treatment (2° C for 16 h). The other six boiling tubes were maintained at 25° C for 16 h (control). Both the cold treated and controls samples were kept in dark and care was taken to prevent drying of leaf discs. At the end of 16 h both the treated and control samples were added with 30 ml of distilled water and were incubated in dark for 18 h at 25° C for allowing the electrolytes to leak into the water. After the incubation the electrical conductivity of the water (initial) was measured using a conductivity bridge at 25° C. Then the samples were autoclaved at 15 lb pressure for 20 min and cooled to 25° C. The volume of water was made up to 30 ml using distilled water and the electrical conductivity (final) was measured once again. The membrane stability was expressed as Relative Injury (RI) using the following formula:

$$RI\ \% = 1 - [(1-T_1/T_2)/(1-C_1/C_2)] \times 100$$

where T is electrical conductivity of treatment, C is electrical conductivity of control and 1 and 2 refer to the initial and final conductivity readings *i.e.* before and after autoclaving respectively.

RESULTS AND DISCUSSION

The temperature and duration of cold treatment were first standardized using RRII 105 leaf discs (Fig. 1). Membrane electrolyte leakage increased with decreasing temperature and at a given temperature, leakage increased with the duration of exposure. Treating leaf discs with 2°C was found to induce lethal injury (50 % electrolyte leakage) which was later adopted for this study.

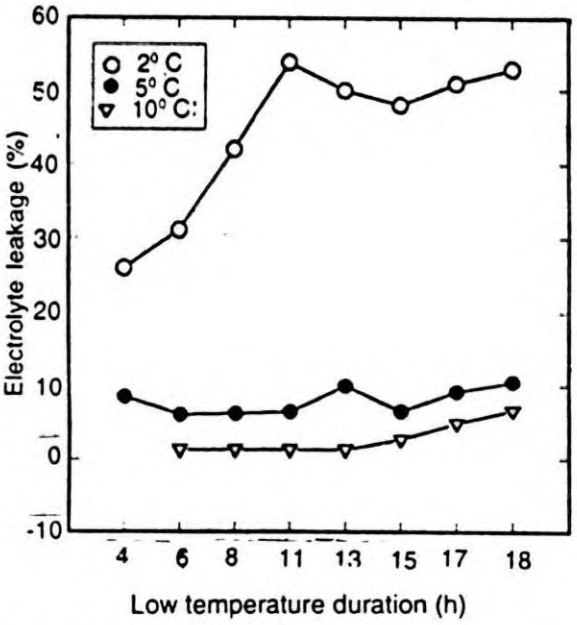

Fig.1. Electrolyte leakage pattern of RRII 105 leaves to varying levels and duration of low temperature

In the 13 clones studied here, the loss of membrane stability due to cold treatment ranged between 5 to 50 per cent (Fig. 2). Clones such as RRII 5, RRII 208, SCATC 88-13 and Haiken1 were found more stable to cold temperature in terms of their membrane damage (6 - 9 % electrolyte leakage). Clones such as RRII 308, RRIM 600, RRII 300 and RRIM 703 exhibited a moderate level of membrane injury (28 - 39 % of electrolyte leakage). Higher rates of electro-

lyte leakage were found in SCATC 93-114, RRII 105, PCK 2 and PCK 1.

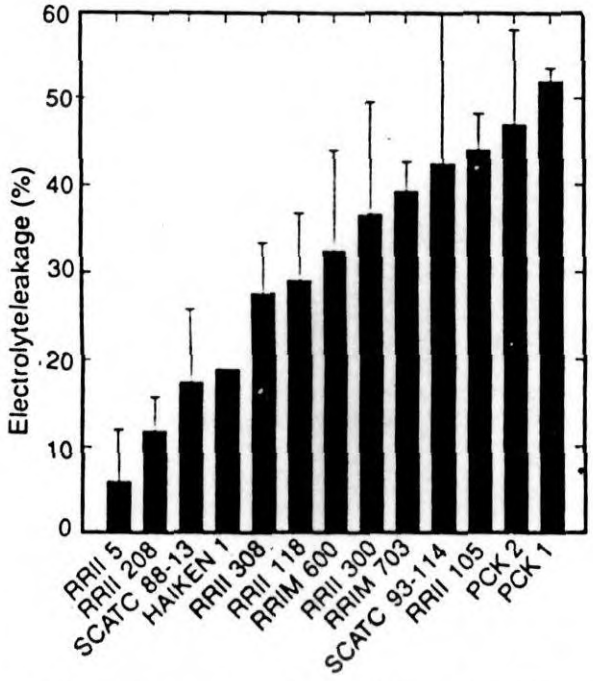

Fig. 2. Low temperature induced loss of membrane stability in 13 *Hevea* clones

The results of the present experiment show that a marked variability exists in loss of membrane stability at low temperature among the clones tested. It has been confirmed earlier that the low temperature induced ion leakage would be more in chilling sensitive plants and would be less in chilling tolerant plants (Collins *et al.*, 1993, 1995) which have some sort of adaptive or resistant mechanism of protecting the membrane structure so as to prevent leakage of electrolytes. The *Hevea* clones which showed minimum relative injury at low temperature may therefore be expected to tolerate chilling temperature better in field conditions also. If so proved, this technique can be used to screen germplasm materials for cold tolerant varieties.

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