VARIATION IN LEAF TISSUE MEMBRANE THERMOSTABILITY AMONG CLONES OF *HEVEA BRASILIENSIS*

Environmental stresses often cause damage to cellular membranes (Levitt, 1980). High temperature injury to the leaf tissues of *Hevea brasiliensis* is observed in non-traditional areas where rubber cultivation is now being extended to. Drying of leaf margins, whole leaves, twigs etc. are the common symptoms. Even in the traditional tract the same symptoms are observed in years of unusual drought. Injury can be caused by the direct effect of high ambient temperature and by the elevated leaf temperature caused by soil water deficit. Variation in drought resistance among *Hevea* clones is already known (Raghavendra *et al*, 1984; Vijayakumar *et al*, 1988). However, clonal variation in heat tolerrance in *Hevea* has not been studied. Establishment of such genetic variation and evolving screening methods will be useful to identify genotypes with better heat tolerance. Sullivan (1971, 1972) and Martineau *et al*, (1979) have described a technique, employing leaf discs, for evaluating tolerance to high temperature in terms of membrane thermostability in annual crops. This test is based on the observation that when leaf tissue is injured by exposure to high temperature, cellular membrane permeability is altered resulting in diffusion of electrolytes and other soluble substances. Using this method, genotypes of *Sorghum* and soybean have been categorised as heat tolerant and susceptible respectively (Sullivan, 1971, 1972; Martineau *et al*, 1979). In this paper we report variation in leaf tissue membrane thermostability in a few clones of *Hevea brasiliensis*.

Eight clones with known drought tolerance and susceptibility in terms of latex yield during summer (Gururaja Rao *et al*, 1988; Vijayakumar *et al*, 1988) were used for the study. Clone RRII 118, though susceptible to drought in terms of yield (Devakumar *et al*, 1988) was found to be drought tolerant in terms of growth in many trials. The other clones studied were Gl 1, RRII 308, RRIM 600, RRII 105, RRIM 623, RRII 43 and Tjir 1.

Leaf samples were collected during the dry season of 1987 from three year-old plants, planted in completely randomised design on a single-tree single-plot basis. Five plants were selected at random from each clone. Five fully mature leaves were collected from each plant (15 leaflets). From each leaflet, seven discs of one centimeter diameter were removed with a cork borer. Fifteen leaf discs, one from each leaflet, constituted one sample. Thus seven samples including control, were obtained from each plant for giving the thermal shock treatments at different temperatures. Thermal injuries were estimated following the method given by Martineau *et al*, (1979) with a slight modification that the leaf discs were de-aerated by keeping in water under vacuum and by giving thermal shocks by keeping in water at different temperatures (Vijayakumar *et al*, 1982). The temperature shock treatments ranged between 40–65°C with 5°C intervals and the duration of shock was 15 min. The controls were maintained at 25°C. The electrolytes leached out were estimated in terms of electrical conductivity using a Systronics 305 Conductivity bridge.

The percentage of injury was calculated as described by Martineau *et al* (1979). Thermal injury curves were prepared for each clone using the mean values obtained. Temperature causing 50 per cent injury in different clones was worked out using these curves. Data were statistically analysed.

The electrolyte-release curves obtained for two typical clones, RRII 308 and RRIM 623 are shown in Fig. 1. Using such curves, the temperature causing 50 per cent injury in different clones was worked out and is presented in Table 1. The data indicate that thermal stability is the highest in clone RRII 308 and the lowest in RRIM 623. In the former, a thermal shock of 15 min duration at 58°C is required for causing 50 per cent injury, whereas in the latter the temperature required for causing the same level of injury is 52.25°C. Variation in leachates at isothermal shocks can be ascribed to changes in lipo-protein composition of the cell membranes (Levitt, 1980).

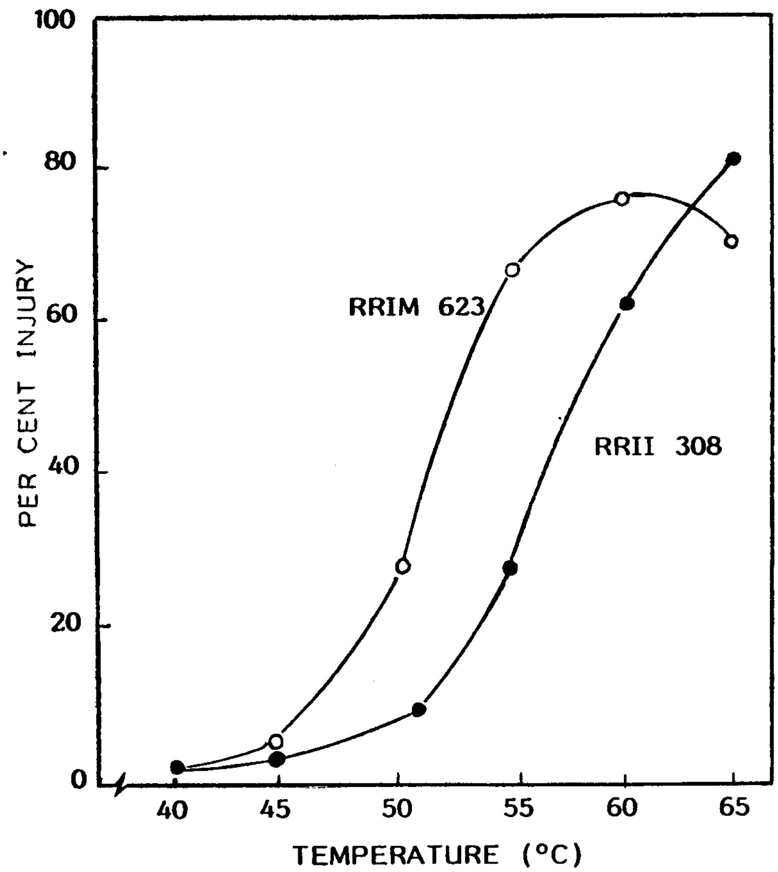

Fig. 1. Thermal injury curves of RRII 308 (tolerant) and RRIM 623 (susceptible) clones of *Hevea*.

Table 1. Temperature of 15 min thermal shocks required for 50 per cent leaching of electrolytes and the percentage of electrolytes leached at 55°C.

CLONE	LD_{50}+(°C)*	Per cent injury at 55°C **
RRII 308	58.00	27.10
RRII 118	57.50	31.40
GI 1	55.37	46.14
RRII 105	54.75	53.05
Tjir 1	54.62	57.88
RRIM 600	54.00	58.26
RRII 43	53.12	60.19
RRIM 623	52.25	67.37
C. D. 0.05	1.43	10.06

* Significant at 5% error

** Significant at 1% error

\+ LD_{50} — temperature for 50 per cent injury

The clonal differences were statistically siginficant at 5% error. Ranking of the clones in terms of heat tolerance, as observed by the present method, is given in Table 1. The relative ranking of clones RRII 118, RRII 105 and Tjir 1 indicates that heat tolerance of leaf tissues may not be associated with drought tolerance in terms of yield reduction. The mean temperatures for causing 50 per cent injury in various clones studied was found to be 55°C. When the percentage injury caused by shocks of 55°C in different clones was considered, the same ranking, as given in Table 1, could be obtained. Clonal differences were significant at 1% error. Hence, it is suggested that a thermal shock of 15 min duration at 55°C can be considered as standard procedure for mass screening of clones.

Ranking of clones on the basis of heat tolerance using electrolyte leaching method needs to be correlated with the data on growth and visual scorings for injuries under field conditions caused by high ambient temperatures or with the plants grown in controlled environmental conditions with elevated temperatures. Such studies are in progress.

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CYTOMIXIS IN *HEVEA BRASILIENSIS* (WILLD. EX ADR. DE JUSS.) MUELL. ARG.

Transmission of chromatin materials from one cell to another through cytoplasmic connections was first observed by Koernicke (1901) in *Crocus*. Gates (1911) studied this phenomenon in *Oenothera gigas* and *Oenothera bienis* and coined the term 'cytomixis'. Since then cytomixis has been recorded in a very wide range of taxa (Omara, 1976; Narain, 1979; Siddiqui *et al*, 1979; Bauchan et al, 1987; Jayabalan and Rao. (1987). However, this phenomenon has not so far been reported in *Hevea*. This communication reports the occurrence of cytomixis in the pollen mother cells of *Hevea brasiliensis* (Willd. ex Adr. de Juss.) Muell. Arg. which was observed during cytological analysis of male sterile clones.

The plant was a resultant of seed irradiation (3000 r). After the treatment, the treated seeds were germinated in the normal way and the sprouted ones were raised in ground nurseries. Those seedlings which showed morphological variations were propagated vegetatively through budgrafting and the budgrafts were planted in the field. Young floral buds of appropriate stages were fixed in modified Carnoy's fluid (ethyl alcohol: glacial acetic acid : chloroform, 3: 1: 1, by volume). Anther columns were dissected out and kept in 1 per cent acetocarmine. Microscopic preparations were made following the usual techniques. A total of 500 pollen mother cells (PMCs) were observed. In addition, fresh materials at the appropriate stage were also observed.

Cytological analysis of the PMCs revealed cytomixis in 30 per cent cells. Cytomixis was observed in the PMCs of both fresh and fixed anthers suggesting that it was not an artifact. Cytomixis was observed at telophase II (Fig. 1). Cytoplasmic bridges were clearly visible in all cases, which, however, were not seen in normal cells

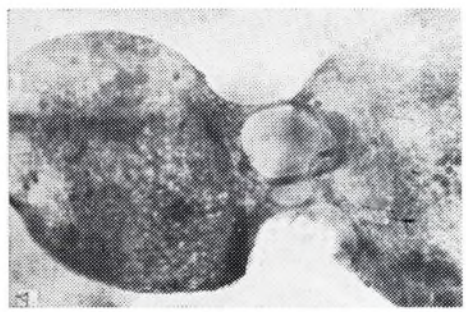

Fig. 1. Telophase II showing cytoplasmic connections x 3000