a high degree of mitosis, followed by well-marked basophilia[8] of the cells (Figures 1 and 2).

Urethane, a well-known multipotential carcinogen in mice, can act as an initiator and promotor of carcinogenesis[11–13] and has been found to exhibit strong carcinogenic effect in the liver of this fish. Similarly, 2-AAF – the first pesticide synthesized, exhibiting carcinogenicity and initiation potential in mammals[14–16] – was found to initiate the formation of strong basophilia coupled with other cellular changes in the liver of this fish, thus extending its carcinogenic domain to fish as well. Similarly, carbaryl, which exhibits carcinogenicity in mammals[17], has been shown to cause cellular changes along with strong basophilia in the liver of *H. fossilis*.

It is thus evident that urethane, 2-AAF and carbaryl exhibit basophilia in the liver of this fish, indicating that liver cells undergo a tumourigenic process, leading eventually to hepatocellular carcinoma formation[7, 18].

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In vitro selection for high-temperature tolerance in cultured shoot explants of *Pinus caribaea* Morelet

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Selection of tolerant plants is the first step towards the production of plants adapted for growth in stressed environments. An attempt was made to select heat stress tolerant *Pinus caribaea* via tissue culture from explants originating from four provenances. The explant performance was evaluated using data on explant height and extent of leaf necrosis. The population structure of provenances was markedly altered by selection pressure imposed by high temperature. At 35°C, variations in growth were significant between explants within a provenance but not between provenances. At 37°C, variation in growth became significant between provenances also. This variation in performance of provenance explants may be attributed to the native temperature range experienced by a provenance. An improvement in explant performance at 37°C was also observed in those provenances which had been pre-conditioned at 35°C over unconditioned controls.

TEMPERATURE is an important environmental factor determining in part the geographical distribution of plants. The optimal temperature for growth and the range over which it occurs varies between species and genotypes of the same species. A plant's habitat is generally determined by temperature and when ambient temperature is higher than that of the plant's natural range, heat injury is more likely to occur[1].

Tissue culture, largely callus and cell suspension cultures, have been initiated in several angiosperm species for resistance against cold[2], minerals[3], frost[4], salt[5]. The use of tissue culture technique for high temperature tolerance[6, 7] is important because even marginally changed tolerance limits are likely to be reflected in an increased species range in managed ecosystems. Previously Bedi[8] established that 35°C was a supra-optimal temperature for the growth of *P. caribaea in vitro*. Explants grown at this temperature showed a poor rate of survival and those which survived showed a significantly lower growth rate than those grown at 25 or 30°C. This investigation seeks to determine the existing variation in tolerance to high temperature and to select tolerant material at the provenance and clonal level, depending upon the extent of diversity in the population.

Shoot explants were obtained from seedlings raised under glasshouse conditions. Seeds from five diverse

Table 1. Seed source of *Pinus caribaea**

Provenance number	Place of origin	Altitude (m)	Mean annual rainfall (mm)	Mean annual temperatue (°C)	Mean Maximum–Minimum temperature (°C)
PC 18/85	Los Limones	700	663	22 2	29.3 to 15.0
PC 20/80	La Mosquita	10 to 170	2859	27.0	30.8 to 24.7
PC 62/85	Queensland	10	1820	21 6	26.5 to 15.9
PC 1/83	Los Limones	700	663	22.2	29 8 to 15.0

*Greaves, 1978.

sites (Table 1) were obtained from Oxford Forestry Institute, Oxford, UK and raised following standard nursery procedures under conditions detailed by Bedi[9], Clones (seedlings) (19, 21, 22 and 18 in numbers respectively) were obtained from provenances PC 18/85, PC 20/80, PC 62/85 and PC 1/83 (Table 1)[10] from which eighteen replicates for each clone were established *in vitro* following the procedures as described by Bedi[9]. Plants and explants of uniform appearance, age and culture history were selected for each experiment. The explants were exposed to temperature at 35°C or 37°C for 4 weeks because in an earlier experiment an exposure to 40°C was too extreme, but 37°C appeared to be a suitable temperature to select tolerant variants[8].

The explant height, measured from the cut end to the growing tip using Vernier callipers to a resolution of 0.10 of a cm, was recorded at the beginning and after four weeks. During the course of the experiment, observations were recorded each week for the extent of leaf necrosis. Browning of the leaves was taken as a sign of leaf necrosis. The extent of leaf necrosis measures the extent of damage caused by temperature stress. The degree of leaf necrosis was recorded using a 5-point visual scale (0 to 4), where, 0 = no leaf necrosis; 1 = 1 to 40%; 2 = 0 to 60%; 3 = 60 to 80% and 4 = 80 to 100% of the total leaves browned. The score of leaf necrosis for a clone was mean value of 3 replicates within a particular clone.

The clones were placed at random among four different shelves in the incubator. The experiment was repeated three times and the mean values were analysed in hierarchical design using the GENSTAT 5 statistical package[11].

The hierarchical design was used because there is no correspondence between clones from different provenances or between replicates from different clones. The purpose of the analysis was to test whether differences between provenances are significantly larger than the differences between clones from the same provenance.

The significance of provenance differences was tested by dividing 'provenance mean square' by 'provenance · clone mean square'. The significance of difference between clones was tested by dividing 'provenance · clone mean square' by 'provenance · clone · replicate mean square'.

Table 2. Extent of leaf necrosis on a visual scale (0–4) of *Pinus caribaea* after various durations in culture at 35°C or 37°C (data are mean values of three replicates for each of the 80 clones)

	Extent of leaf necrosis			
Temperature	1 week	2.weeks	3 weeks	4 weeks
35°C	0	1	1	2
37°C	1	3	3	4

s.e.d. 35°C 0.1; 37°C 0.1.

Table 3. Analysis of variance on the extent of leaf necrosis in the shoot explants of *Pinus caribaea* grown at different temperatures and after four weeks in culture

		35°C		37°C	
Source of variation	d f.	m s.	F-ratio	m s.	F-ratio
Provenance	3	1.7108	1.51	10.4341	12.48*
Provenance Clone	76	1 332	2.98*	0.8358	6.22*
Provenance Clone · Replicates	160	0.3792		0 1344	
Total	239				

* Significant at 1% level of probability.
d.f.: degrees of freedom.

In general, clones showed poor survival rate and growth at 37°C and 35°C. The extent of leaf necrosis increased with the duration of exposure to a particular temperature.

The population structure of provenances was markedly altered by the selection pressure imposed by high temperature. At 35°C, there were significant differences in the extent of leaf necrosis ($p = 0.05$) among clones within a provenance while the differences in performances of provenances were not significant. At 37°C, the differences in performances of clones both inter and intra-provenance were significant (Tables 2 and 3). Clones which experience a wider range of temperature in their native provenance (Table 1) show greater survival (less leaf necrosis) at 37°C. This may be attributed to greater capacity for adaption to varying environments.

However, in terms of increase in explant height, differences among clones at 35°C were significant both

Table 4. Per cent increase in height in shoot explants of *Pinus caribaea* after four weeks in culture

	% Increase in explant height	
Provenance	35°C	37°C
PC 18 85	97 3	16.7
PC 20 80	38 3	16 2
PC 62 85	61 1	10 6
PC 1 83	48 8	10 0

within and between provenances. At 37°C, these differences were significant only at intra-provenance level (Tables 4 and 5). Such rank changes in field trials among *Pinus caribaea* from two different altitudes in Belize have also been observed[12]. At Mountain Pine Ridge (MPR, 530 m) site, provenance Alicamba and Poptun showed better height growth than the MPR provenance. But at Melinda site (10 m), the MPR provenance outperformed the other two provenances.

Provenance differences within species are well recognized[13], however, there is sufficient variation even among clones within a provenance and good genetic gains may be achieved by a breeding programme directed at these tree-to-tree differences[14].

When the extent of leaf necrosis was studied in individual clones both at 35°C and 37°C, at least 2.5% individuals were lower than 2 on the leaf necrosis scale at both temperatures. 81.3% of clones showed high leaf necrosis at 37°C and low necrosis at 35°C, while 16.3% showed a high leaf necrosis both at 35°C and 37°C (Figure 1). The explants that survive at 35°C but show high leaf necrosis at 37°C may not be suitable for areas with fluctuating temperatures. Explants showing high leaf necrosis at both 35°C and 37°C definitely need to be avoided for high temperature environments. One clone from provenance PC 1/83 showed low leaf necrosis (< 3) at both 35°C and 37°C.

A provenance/environment interaction may lead to the selection of a provenance only suitable for a particular site[15]. Therefore, it is important to define genotypes with little interaction so they can be used over a

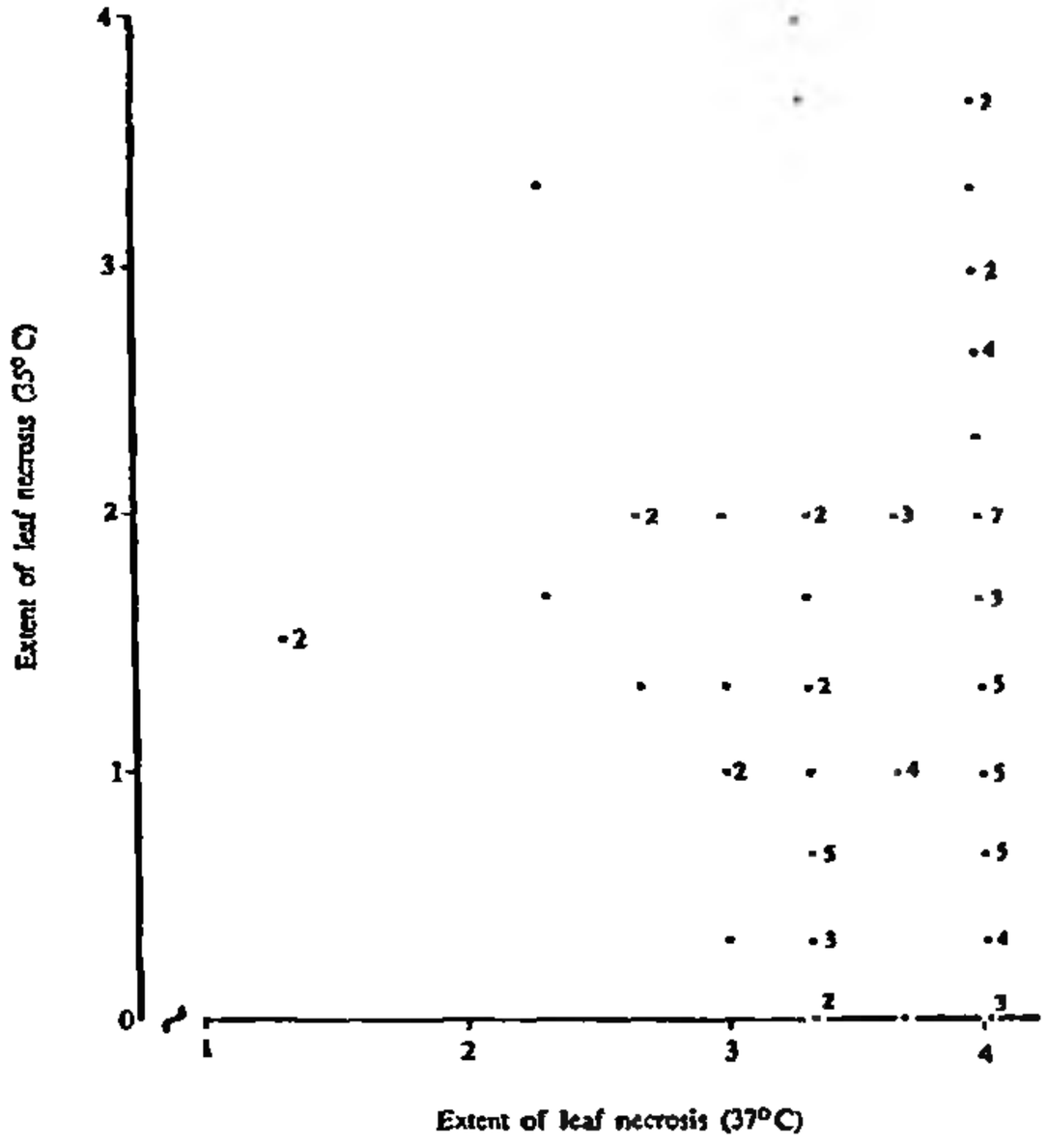

Figure 1. Frequency distribution of clones of *Pinus caribaea* showing leaf necrosis at the end of 4 weeks in culture at 35°C and 37°C (The numbers on the graph refer to number of observations at the associated point)

wider range of environments[16]. In *Pinus caribaea*, large differences have been seen in individuals from two different provenances with the same mean levels of tolerance[17]. By selection at the clonal level, the inferior clones in the best provenance can be avoided and superior clones in other provenances can be included[18].

Survival and growth of various genotypes over a range of temperature appear to be related to seasonal temperature ranges of their native habitat (Table 1) The performance of plants in a habitat is dependent on acclimatization of plants to daily temperature fluctu-

Table 5. Analysis of variance of increase in explant height in shoot explants of *Pinus caribaea* after four weeks in culture

		35°C		37°C	
Source of variation	d f	m s	F-ratio	m s	F-ratio
Provenance	3	30407.5	7 66*	981 20	2.27**
Provenance × Clone	76	3967 8	5 24*	431 65	7 69*
Provenance × Clone × Replicates	160	757 1		56 06	
Total	239				

*Significant at 1% level of probability
**Significant at 5% level of probability
d f : degrees of freedom
m s : mean sum of squares

Table 6. Effect of pre-conditioning at 35°C temperature on growth on shoot explants of *Pinus caribaea* after four weeks* in culture

Provenance	35°C	37°C	35°C–37°C (4 weeks)
PC 18/85	97.3 ± 11.0 (58.5)	16 7 ±1.6 (2.9)	11.5 ± 2 4 (44 4)
PC 20/80	38 3 ± 4 5 (62.0)	16 2 ± 9 9 (9.5)	9 5 ± 2 0 (47.6)
PC 62/85	61.1 ± 8.2 (56.8)	10.6 ± 1.8 (6.7)	10 67 ± 1 9 (40 9)
PC 1/83	48 8 ± 7.6 (65 8)	10 ± 0 (5.7)	20.4 ± 4 0 (38.9)

*Time period excludes pre-conditioning, where applicable, figures are mean values for % increase in explants height in provenance ± s.e.: Figures in parentheses are % clones survived after four weeks in culture.

ations[19]. A reliable ranking of genotypes for temperature tolerance requires pre-conditioning under a defined hardening treatment[20]. Explants that survived after four weeks in culture at 35°C were transferred to 37°C for a further four weeks and their performance compared with the clonal counterparts at 37°C which had not been pre-conditioned at 35°C. The pre-conditioned explants showed a significant increase in tolerance of 37°C compared with unconditioned controls after 4 weeks in culture. Growth in conditioned explants was either equal to or less than unconditioned explants (Table 6).

Selection of tolerant plants is the first step towards production of plants adapted for growth in stressed environments. Even marginally extended tolerance limits may help in expanding the silvicultural range of a species[21].

The *in vitro* system has the advantage of the short time required for genotype selection, with tests which may be performed on seedling-derived cultures. Moreover, the traits selected *in vitro* can be expressed in the regenerated plants and inherited by the progeny[22, 23]. Conifers appear to have the advantage of a greater genetic stability *in vitro* than angiosoperms[24].

The purpose of micropropagation techniques is not to replace conventional breeding but to complement it for obtaining greater efficiency in selection and to utilize the results of genetic progress rapidly. The use of clones gives a significant added power to progeny tests as several genetically identical propagules can be tested simultaneously and rapidly in different environments. Tree improvement programmes can be significantly accelerated by reliable methods of early selection of reliable growth traits[25].

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