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ARTICLE

Variability in Cold Tolerance in *Hevea brasiliensis* Seedlings: Plausible Source for Crop Improvement

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Hevea brasiliensis, a highly cross-pollinated tree species, produces seeds of diverse genetic constituents. To exploit the readily available genetic variability, seedlings were grown at experimental station in Tripura. Two-month-old seedlings showed distinct variability in growth parameters such as plant height, shoot diameter and leaf expansion rate during winter season. On the basis of these parameters, a few seedlings with variable growth parameters were selected for further studies to evaluate their intrinsic capability to withstand low temperature. High vigor (HV) seedlings recorded significantly higher mean plant height, shoot diameter, leaf expansion rate compared to low vigor (LV) seedlings. Further, the CO_2 assimilation rate and stomatal conductance recorded in LV seedlings were 65% and 15% less respectively, compared to HV seedlings. In a separate experiment _in vitro_, the degradation of chlorophyll was 7% over control in HV types whereas 33% lower in LV types when the excised leaves were exposed to low temperature of 10°C for two days. Low temperature of the same severity and duration had caused 57% and 41% membrane injury in LV and HV plants respectively. In the same experiment, LV seedlings had recorded 54% more anthocyanin accumulation compared to other types.

Keywords: *Hevea brasiliensis*, low temperature tolerance, photosynthetic rate, chlorophyll degradation, membrane damage, anthocyanin accumulation.

Introduction

Hevea brasiliensis, the natural rubber, is grown in various distinct agro-climatic zones in India. One of the major areas of cultivation of *H. brasiliensis* is in the Northeast region of India, where it encounters low temperature stress for its normal growth and development during winter season. To combat this abiotic stress, efforts have been made in different directions either to develop resistant genotypes or to minimize the adverse impact of low temperature in susceptible ones. One of the directions has led us to examine the polyclonal seedlings of *H. brasiliensis*, as the species is highly cross-pollinated.

In the present study, we made an effort to assess the variability in low temperature tolerance among the polyclonal seedling population. We identified two groups of plants contrasting in their stress adaptation. Further, gas exchange studies and other biochemical analysis, viz. chlorophyll degradation and membrane leakage studies were also conducted to characterize these two groups of seedlings.

Materials and methods

Environmental condition and plant materials

The soil (0–30 cm) of the experimental site contains 21% clay, 25% silt and 54% sand. The organic carbon content was 1% with pH of 4.6. The mean monthly maximum temperature recorded ranged from 25.8°C to 29.0°C and the minimum temperature varied from 7.8°C to 12.9°C during the winter period. Within the period, the RH fluctuated from 66 to 69% with an average sunshine hour of 8.2. Under this agro-climatic condition, polyclonal seeds of *H. brasiliensis* collected from multi-clonal orchard were sown in 0.6 m × 0.6 m spacing in seedbed at Taranagar Farm, RRS, Agartala (longitude 91°15′E, latitude 23°53′N and 20 m MSL). Two hundred and thirty five seedlings of one-month age were monitored during the winter season of 2000. This study was continued until 2002.

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Growth parameters. The height of plants (cm) from shoot apex to ground level was measured once in a week. Shoot diameter (mm) at 10 cm height was measured at monthly intervals by screw gauge. Leaf expansion rate ($cm^2\ day^{-1}$) was estimated by measuring initial leaf area (LA_1) as multiplied by length and breadth of the leaf *in situ*. After a week of the initial observation, the final leaf area (LA_2) was recorded. The leaf expansion rate was calculated by using the formula $(LA_1–LA_2)/7$.

Gas exchange studies: Net photosynthetic rate (A) and other related parameters were recorded on uniformly matured leaves using a portable photosynthesis system (Licor 6200, Lincoln, NE, USA). The measurements were taken from 8 am to 10 am under saturated photon flux density (~ 1500 μmole $m^{-2}\ s^{-1}$).

In vitro studies: The effect of low temperature on chlorophyll degradation and membrane damage was studied by exposing uniformly matured leaflets to low temperature (10°C) for 48 h in a BOD incubator (Caltan DT 909) in presence of light.

Chlorophyll degradation

Leaves of uniform maturity from individual plants were exposed to low temperature to study the stress-induced chlorophyll degradation. One set of leaves was kept in ambient condition as control. After 48 h of exposure to low temperature five leaf discs of 1 cm diameter were incubated in 80% acetone : DMSO (1 : 1) mixture overnight. The absorbance of decanted solution at 546 nm, 652 nm and 663 nm wavelengths was recorded. The chlorophyll content was then calculated by using the formula of Arnon (1949).

Membrane damage

Leaf discs taken from both treated and control leaves were incubated in 7 ml of sterile water in a test tube overnight. The water was decanted and absorbance at 273 nm (D_1) was recorded both in treated and control samples using a UV spectrometer. The absorbance at 273 nm was again recorded (D_2) for the decanted water after autoclaving the same test tubes at high pressure. The membrane damage was calculated using the formula: $[(D_2–D_1)/D_2] \times 100$ (He Jing, 1982).

Anthocyanin content

Young bud tissue (1 g) was taken from each sample and homogenized in 5 ml of methanol containing 1.0 N HCl maintained at 4°C for four hours (Christe *et al.*, 1994). The particulates were removed by centrifugation of the homogenate at 10,000 *g* for 30 min. The absorbance of the clear supernatant at 530 nm per gram of tissue serves as a measure of anthocyanin content as previously described by Kho *et al.* (1977).

Results and discussion

Growth analysis

Preliminary growth of the seedlings during low temperature season reveals that there is a difference among the seedlings in terms of their interaction with low temperature (Table 1). The mean increase in plant height of seedlings was 16.31 cm ranging between 3.0 cm and 36.3 cm during the 120 days period in winter season. The rate of increase in other parameters namely, shoot diameter and leaf expan-

Table 1. Mean and range of different growth characteristics of polyclonal seedlings.

Increment in	Mean	Range	SE
Plant height (cm)	16.31	3.0–36.3	± 0.47
Shoot diameter (mm)	0.98	0.26–2.18	± 0.036
Leaf expansion rate ($cm^2\ day^{-1}$)	1.33	0.12–5.15	± 0.033

Table 2. Growth characteristics of contrasting *Hevea* seedlings in winter season.

Seedling no.	Plant height (cm)	Shoot diameter (mm)	Leaf expansion rate ($cm^2\ day^{-1}$)
HV 11	36.7	2.01	1.91
HV 45	28.8	2.21	1.17
HV 98	33.5	2.18	1.32
HV 106	27.1	1.99	2.48
HV 110	36.3	1.92	2.47
Mean	30.4	2.06	1.87
LV 170	3.0	0.26	0.21
LV 212	7.0	0.41	0.45
LV 230	5.0	0.36	0.19
Mean	5.0	0.34	0.28

HV: High vigor; LV: Low vigor.

Table 3. Assimilation rate (A), stomatal conductance (g_s), intercellular CO_2 (Ci) and an estimate of mesophyll efficiency (Ci/g_s) of selected seedlings grown in winter season.

Category	A	g_s	Ci	Ci/g_s
HV	8.30	0.26	281.3	1065
	(± 0.15)	(± 0.03)	(± 1.38)	(± 3.3)
LV	2.90	0.22	300.7	1363
	(± 0.083)	(± 0.009)	(± 1.27)	(± 3.6)
CD ($P = 0.05$)	0.72	0.02	5.37	–

HV: High vigor; LV: Low vigor.
Values in parentheses indicate the SE of the data ($n = 15$).

sion was 0.26 to 2.18 mm and 0.12 to 5.15 cm^2 day^{-1} respectively. Based on the increment in plant height and shoot diameter, a few seedlings were grouped into two distinct categories, viz. high vigor (HV) and low vigor (LV) seedlings.

The HV seedlings recorded average plant height of 30.4 cm, shoot diameter of 2.06 mm and leaf expansion rate of 1.87 cm^2 day^{-1}, whereas LV types showed 5.0 cm and 0.34 mm and 0.28 cm^2 day^{-1} respectively (Table 2).

Gas exchange studies

A significant variability was observed in net photosynthesis rate (A) and other related parameters such as stomatal conductance (g_s), intercellular CO_2 (Ci) and the *in vivo* estimated mesophyll efficiency (Ci/g_s) between the HV and LV seedlings. Similar variability was also reported by Samsuddin and Impens (1979), Dey *et al.* (1995) and Nataraja and Jacob (1999). The HV seedlings showed an average assimilation rate of 8.3 µmole CO_2 m^{-2} s^{-1} whereas the LV types recorded only 2.9 µmole CO_2 m^{-2} s^{-1} (Table 3). The ratio of Ci to g_s is an estimate of mesophyll efficiency (Krishnaprasad *et al.*, 1996). The HV types recorded the Ci/g_s of 1065 in comparison with 1363 in case of LV types, which reveals better mesophyll activity in HV types in relation to the other one.

Chlorophyll degradation

Degradation of pigments is a reflection of stressful environment such as low temperature (Stamp *et al.*, 1983; Stamp, 1984; Baker and Nie, 1994). In the present study, the degradation of chlorophyll after low temperature exposure is 7% in HV types, whereas the LV type showed 33% (Figure 1). This parameter is used as an indication of low temperature impact as chlorophyll is more liable to oxidative damage under this adverse condition (Mac William and Naylor, 1967).

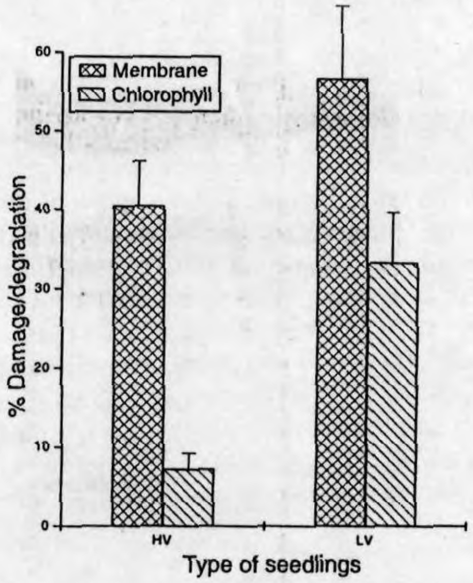

Figure 1. *In vitro* membrane damage and chlorophyll degradation in high vigor and low vigor seedlings.

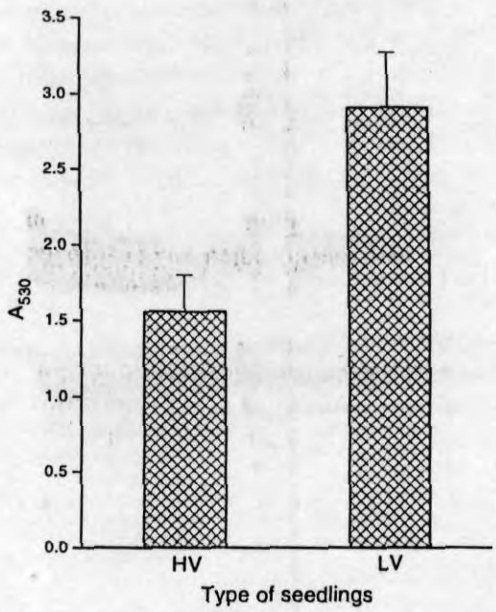

Figure 2. Anthocyanin accumulation in high vigor and low vigor *Hevea* seedlings under *in vitro* condition.

Membrane damage

The membrane damage due to low temperature exposure was significantly different between HV and LV seedlings. The HV seedlings showed 41% injury compared to 57% in LV types (Figure 2). It was demonstrated that low temperature caused increased membrane permeability that resulted by membrane injury (Hu Deyou, 1984; He Ruiyan and China Zhenfei, 1986).

Anthocyanin accumulation

The two types of seedlings differ significantly in the accumulation of anthocyanin in young bud tissue. The HV types recorded less amount of anthocyanin content of 1.56A_{530}/g bud tissue fresh weight compared to LV seedlings (2.91 A_{530}/g bud tissue fr wt). It has been reported that the flavonoid-like anthocyanin is induced by low temperature stress in plant species (Hajime *et al.*, 2001). The low accumulation of anthocyanin in HV types indicates the lesser extent of stress experienced by the seedlings as they have a better mechanism to withstand the low temperature stress compared to other type of seedlings (Figure 2).

The results of this experiment show that the HV seedlings are less susceptible to low temperature stress compared to LV types, as HV seedlings could maintain better structural stability under stress condition. Empirical growth analysis of these polyclonal seedlings would provide greater source of variability in cold tolerance trait for crop improvement. These selected high vigor seedlings can be used as parental material for further breeding programmes for improving cold tolerance characteristics of *Hevea* clones.

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