

A comparative study of the thermoluminescence glow curves recorded from varieties of *Vitis vinifera*, *V. labrusca* and *V. champini*

by

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S u m m a r y : Thermoluminescence (TL) glow curves were recorded from leaves of eight *Vitis* varieties grown in South-West India, viz. Thompson Seedless, Sonaka, Anab-E-Shahi, Kishmish Charni, Tas-A-Ganesh, Black Monuka, Bangalore Purple and American Dogridge belonging to the *Vitis vinifera*, *V. labrusca* and *V. champini* species. The TL peaks were analysed, using a computer-assisted model based on the general order kinetics theory, to determine thermodynamical parameters such as activation energy, entropy, frequency factor and free energy. It was observed that all six *V. vinifera* samples showed an entirely different TL pattern consisting of only one prominent and highly narrow peak at around -5 °C (peak II). The prominence of this peak attributed to $S_2/S_3 Q_a$ recombination suggests a modification at the plastoquinone electron acceptor sites. On the other hand, the remaining two varieties Bangalore Purple and American Dogridge showed broader TL spectra consisting of a higher number of peaks. This basic difference is attributed to the difference in species of the selected varieties. Moreover, the TL recorded from Bangalore Purple resembled the standard TL spectra of spinach. The study undertaken in this paper may be relevant in grape breeding programs.

Key words : thermoluminescence, electron transport, *V. vinifera*, *V. labrusca*, *V. champini*, thermodynamical parameters, grape breeding.

Introduction

Few reports related to the functioning of the photosynthetic apparatus in different grapevine varieties are available in literature. These include the determination of the chlorophyll content and photosynthetic activity studies within shoots of *Vitis vinifera* grapevines by KRIEDEMANN *et al.* (1971) and comparative ultrastructural and chlorophyll fluorescence studies of *V. vinifera* chloroplasts isolated from leaf tissues by IVANOV *et al.* (1990). Recently, LIMA DA SILVA *et al.* (1996) have evaluated the photoautotrophic ability of *Vitis* plantlets using a special open flow gas exchange system.

Among the various techniques that have so far been used to study the photosynthetic apparatus, thermoluminescence (TL) remains to be one of the important probes of the electron transfer mechanism in intact photosystem (PS) II (VIDYASAGAR *et al.* 1993). TL is the thermally stimulated luminescence sent out by low temperature-preilluminated photosynthetic material when warmed. It involves the radiative recombination of thermally released electrons from their traps, with the holes at the recombination centre. The participation of charge storage states of the water oxidation complex and the primary (Q_a) and secondary (Q_b) quinone acceptors in the generation of TL has been well established (SANE and RUTHERFORD 1986). Since TL characteristics are very sensitive to subtle changes in redox properties of the involved electron transport components, the TL technique has become a useful tool for probing a wide range of PSII characterization applications (INOUE and SHIBATA 1982; DEMETER and GOVINDJEE 1989). TL is generally observed in

the range -160 to 50 °C. The nomenclature for assigning the main TL glow peaks is as follows: peak I occurs at about -25 °C, peak II at about -10 °C, peak III at about +10 °C, peak IV at about +25 °C and peak V at about +50 °C (SANE and RUTHERFORD 1986).

It has been observed that though the overall properties of these bands remain the same from cyanobacteria to higher plants, a qualitative survey showed that their apparent characteristics, namely temperature at peak maxima (T_m), intensity, full width at half maxima (FWHM) and peak shape are dependent on a variety of factors such as species, growth conditions, pH of the medium, herbicide resistant mutation, and the chemical nature of any inhibitor affecting the electron transport (DUCRUET and MIRANDA 1992). Taking this point into account, it would be appropriate to compare the TL glow curves of grape varieties belonging to different species. The study would also help in standardization of TL spectra of grape leaves.

Material and methods

Grapevine materials: The commonly cultivated grape varieties in South-West India were selected for the present study. TL was recorded from mature leaves at around the 6th nodal position of the shoot. The varieties selected were Thompson Seedless, Sonaka, Anab-E-Shahi, Kishmish Charni, Tas-A-Ganesh, Black Monuka, Bangalore Purple and American Dogridge. While the first 6 varieties belong to the *V. vinifera* species, Bangalore Purple is a *V. labrusca* variety and American Dogridge a *V. champini* variety.

Thermoluminescence measurements: The samples were obtained from the Ganeshkhind garden (Mahatma Phule Agricultural University) located in the University of Pune campus. TL glow curves were recorded from intact leaf discs of 1 cm diameter, on a PC-based apparatus built in our laboratory (VIDYASAGAR *et al.* 1993; THOMAS *et al.* 1995). The leaf disc was illuminated at room temperature (R.T.) for 15 s and again at $-80\text{ }^{\circ}\text{C}$ for 5 s with a laser diode (wavelength: 670 nm and power: 3 mW). This particular illumination condition was used for reasons explained elsewhere in the text. A linear heating rate of $15\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ was obtained using a microprocessor-based temperature controller. TL was recorded in the temperature range of -80 to $+50\text{ }^{\circ}\text{C}$. The fitting and analysis of the glow curve was done using a model based on the general order kinetics theory (VIDYASAGAR *et al.* 1993). As per this model, the equation used to obtain a theoretical fit to the experimentally observed glow curve is given as

$$I = sn_0 \cdot \exp\left(\frac{-E}{k_B T}\right) \left\{ \left[b - 1 \right] \frac{s}{\beta} \int_{T_0}^T \exp\left(\frac{-E}{k_B T}\right) dT + 1 \right\}^{-b/(b-1)}$$

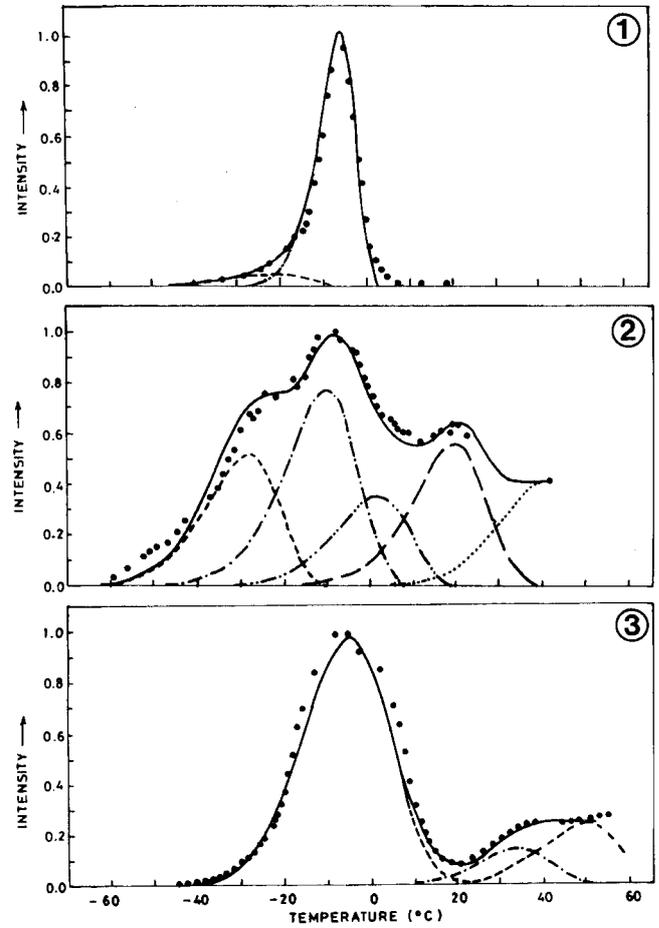
where s = pre-exponential factor; $s = s_0 \cdot T$ where s_0 = frequency factor; β = rate of heating, b = order of kinetics, n_0 = initial concentration of trapped charge carriers, k_B = Boltzmann constant; T = absolute temperature; T_0 = initial temperature; E = activation energy.

The expression for the various thermodynamical parameters, viz. activation energy (E), entropy (ΔS) and free energy (F.E.) can be obtained from our earlier paper (VIDYASAGAR *et al.* 1993). The entropy calculations are based on the absolute reaction rate theory (DE VAULT *et al.* 1983).

Results and Discussion

As observed from the fitting of the TL glow curves recorded from the grape varieties, all the *V. vinifera* varieties showed only one prominent TL peak at around $-5\text{ }^{\circ}\text{C}$, which is very narrow and highly intense. Fig. 1 shows the TL glow curve recorded from Thompson Seedless, the most commonly cultivated variety, which can be considered as a representative of the *V. vinifera* species. On the other hand, under the same experimental conditions, the TL glow curves recorded from Bangalore Purple (Fig. 2) and American Dogridge (Fig. 3) show much broader TL spectra consisting of a larger number of peaks. The TL pattern recorded for the first 6 varieties was indeed a new finding and to eliminate any doubt of it being just an artifact, the wavelength of this TL emission was checked using a filter (coming CS-2-64). The emission wavelength was found to be in the red region, thus eliminating the possibility of the peak originating from chemiluminescence.

Generally, in most TL recordings, the illumination is performed only at low temperatures (around $-80\text{ }^{\circ}\text{C}$). However, the use of this illumination condition for Thompson Seedless resulted in the much more intense low temperature peak at the same temperature ($-6\text{ }^{\circ}\text{C}$) as mentioned above. Since, from literature it is known that additional



Figs. 1-3: Thermoluminescence glow curves recorded from grapevine varieties Thompson Seedless (1, top), Bangalore Purple (2, center) and American Dogridge (3, bottom).

preillumination at a temperature above $0\text{ }^{\circ}\text{C}$ enhances the high temperature peaks (DESAI *et al.* 1975; INOUE 1996), we adopted such an illumination condition (two preilluminations, i.e. one at R.T. and another at $-80\text{ }^{\circ}\text{C}$). Surprisingly, even this procedure did not result in the significant emergence of the high temperature peak (at around R.T.) for the *V. vinifera* samples.

Computer-based fitting of the peaks using the general order kinetics model (VIDYASAGAR *et al.* 1993), indicated that the intense peak from the *V. vinifera* varieties is at around $-5\text{ }^{\circ}\text{C}$. As per TL nomenclature, this is referred to as peak II attributed to $S_2/S_3 Q_a^-$ recombination (SANE and RUTHERFORD 1986). Weak TL contribution to the other peaks was observed in some of the varieties including the Thompson Seedless, Tas-A-Ganesh and Black Monuka. On the other hand, the TL spectra recorded from Bangalore Purple could be decomposed into 5 peaks (Fig. 2 and Table), corresponding to peaks I, II, III, IV and V, while 3 broad peaks, viz. II, IV and V are observed in case of American Dogridge (Fig. 3 and Table). Thus it can be clearly seen that the TL recorded from varieties belonging to *V. vinifera* are considerably different when compared to the TL from the other species. Only in case of the *V. labrusca* variety the TL resembles the well studied TL from spinach leaves (DESAI *et al.* 1975).

At this stage, it would be appropriate to discuss the physical parameters associated with the detrapping kinet-

Table

Thermodynamical parameters of thermoluminescence glow curves recorded from *Vitis* (grapevine) varieties using the general order kinetics model

Variety	Peak	T _m (°C)	E (eV)	ΔS/k _B	F.E. (eV)	S ₀ (s ⁻¹)
Thompson Seedless	I	-21	0.677	-1.58	0.712	4.28x10 ⁹
	II	-6	1.55	35.34	0.739	4.66x10 ²⁵
Sonaka	II	-5	1.03	12.38	0.751	4.99x10 ¹⁵
Anab-E-Shahi	II	-2	0.957	8.31	0.763	8.51x10 ¹³
Kishmish Charni	II	-6	0.801	2.02	0.753	1.57x10 ¹¹
	II	-3	1.45	30.4	0.75	3.34x10 ²³
Tas-A-Ganesh	IV	25	1.12	10.96	0.84	1.20x10 ¹⁵
	II	0	1.004	10.06	0.767	4.88x10 ¹⁴
Black Monuka	V	38	1.241	13.49	0.879	1.52x10 ¹⁶
	I	-28.5	0.640	-2.38	0.690	1.91x10 ⁹
Bangalore Purple	II	-10	0.739	-0.205	0.744	1.69x10 ¹⁰
	III	2	0.81	1.297	0.779	7.63x10 ¹⁰
	IV	20	0.922	3.553	0.832	7.27x10 ¹¹
	V	40	1.05	6.063	0.89	8.96x10 ¹²
	II	-2	0.655	4.99	0.77	1.41x10 ⁸
American Dogridge	IV	35	1.02	5.436	0.87	4.78x10 ¹²
	V	50	1.12	7.32	0.919	3.15x10 ¹³

T_m = Temperature at peak maxima, E = Activation energy, ΔS = Entropy, k_B = Boltzmann constant, F.E. = Free energy, S₀ = Frequency factor.

ics, obtained from TL analysis. Since the prominent TL peak of the *V. vinifera* varieties, attributed to the Q_a⁻ recombination, is very intense and has a very small FWHM (less than 10 °C), it indicates that the distribution of energy levels corresponding to the Q_a traps is very narrow. Moreover, the absence of any significant peak attributed to Q_b⁻ recombinations show that under the present illumination conditions Q_b does not constitute a stable electron trap (VASS and GOVINDJEE 1996). Both these observations put together indicate that there are modifications at both, the primary and secondary plastoquinone acceptor sites (HORVATH 1986; KLERS *et al.* 1993). It is in fact known that the binding sites at the D1-D2 proteins influence the redox components Q_a and Q_b of PSII (DEMETER and GOVINDJEE 1989). The highly narrow and intense peak II in *V. vinifera* varieties also results in the extremely high values of the thermodynamical parameters, namely activation energy, frequency factor, entropy and free energy (Table). Since these values are much higher than the prescribed limits (DE VAULT *et al.* 1983), it seems that the general order kinetics theory may not be suitable for the fitting of this particular peak. At the other extreme, these values are considerably low in case of *V.*

champini cv. American Dogridge, due to its broad peak II which indicates a widely spread distribution of the traps. Such a behavior may also be indicative of some modification at the plastoquinone acceptor site. On the other hand, the TL profile and the thermodynamic peak parameters of *V. labrusca* cv. Bangalore Purple are comparable to standard spectra such as observed in spinach (DESAI *et al.* 1975; VIDYASAGAR *et al.* 1993). If one compares the E and S values within the *V. vinifera* varieties, it may be noted that Anab-E-Shahi, Kishmish Charni and Black Monuka which have a comparatively broader TL peak, have lower values of the activation energy and frequency factor.

In conclusion, our studies have demonstrated the application of the TL technique in differentiating between grapevine species. The TL pattern observed in case of *V. vinifera* varieties is drastically different from that of the other grape varieties, viz. Bangalore Purple and American Dogridge. It was only in case of Bangalore Purple that the pattern resembled the standard patterns reported for spinach, while American Dogridge showed a still different TL pattern. Differences were also observed in the detrapping kinetics as evident from the various thermodynamical parameters calculated from the TL glow curves of the 3 species. The different TL pattern in the *V. vinifera* varieties and American Dogridge have been suggested to be due to modifications at the primary and secondary plastoquinone electron acceptor sites.

The information obtained on the electron transport properties of the species examined would be of help in further definitive work on the breeding and genetic analysis of grapevines.

The observations are also very important as they form the basis for further studies related to possible modifications in the photosynthetic electron transport properties induced by pesticides, which are being extensively used to control various diseases.

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