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Delayed fluorescence as an in situ probe of fluidity changes in maize photosynthetic membranes

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Since its discovery by Streheler and Arnold *(11)* delayed fluorescence has attracted attention as a useful tool in studies of the photosynthetic process, electron transport and associated reactions. It is closely linked to the photosystem-2 protein-pigment complex (PS_{II}) embedded in the thylakoid membranes and apparently results from a charge recombination in the form of a backward photosynthetic reaction. Absorbed light, already converted to electrochemical energy, is then re-emitted *(3)*. Delayed fluoresence exhibits a strong temperature dependence and a number of studies of this phenomenon at different levels of organization of the photosynthetic apparatus have been published *(2, 9, 11, 12)*. Our previous studies demonstrated the complex nature of delayed fluorescence measurements performed on leaf segments, when different responses can be obtained depending on pre-treatment and the condition of the leaf *(12)*. Since any factor affecting electron transport or subsequent biochemical reactions could also affect delayed fluorescence, it obviously is difficult to get specific data when working with a complex system such as a leaf. Our results have shown that the intensity of delayed fluorescence emitted from a leaf segment increases in certain cases at temperatures below ~ 15 °C. This could be explained by the occurrence of a phase transition of the lipid bilayer, as proposed by Lyons and Raison *(4)*.

Since the existence of phase transitions in membranes could have important implications in plant breeding of resistant varieties to chilling temperatures, we attempted to use delayed fluorescence of leaf segments in studies of fluidity of photosynthetic membranes without disrupting the system. To prevent the influence of subsequent photochemical and biochemical reactions, the leaf samples were, prior to measurement, infiltrated with 3–(3,4 dichlorphenyl)–1,1-dimethylurea (DCMU), a well known *(1)* inhibitor of electron flow from the primary acceptor Q to other components of the electron transport chain.

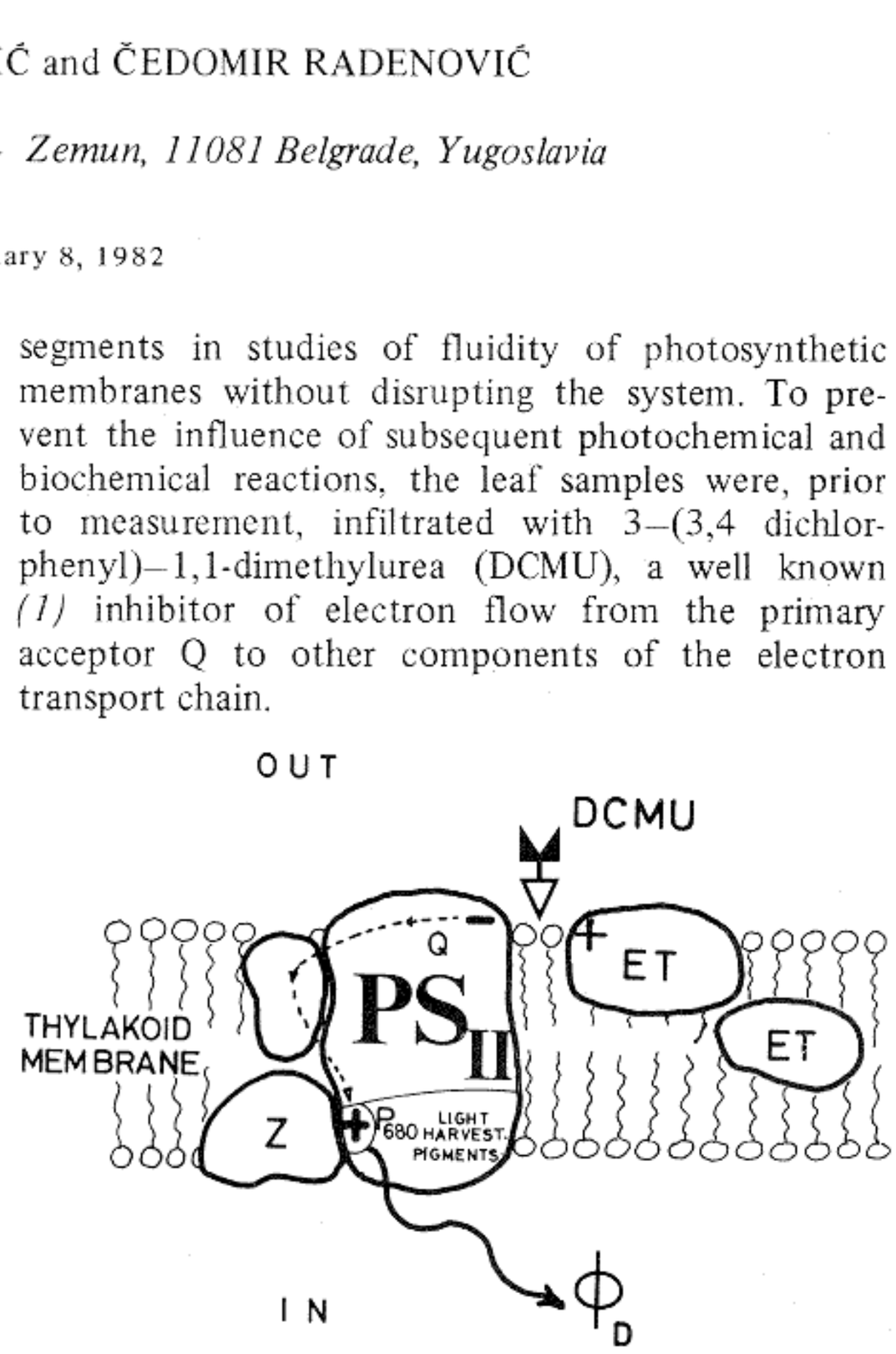

FIGURE 1. *Diagram showing the origin of delayed fluorescence (ϕ_D) in the thylakoid membrane. PS_{II} – photosystem 2 protein-pigment complex; Q – primary electron acceptor; P_{680} – reaction center chlorophyll$_a$, primary electron donor; Z – water splitting enzyme; ET – electron transport components, IN – internal thylakoid space; OUT – chloroplast stroma.*

We used three-to four-week old maize plants (inbred line Polj. 17) grown in water cultures and controlled environment (24/20 C, 12 hr day at 150 $\mu E \cdot s^{-1} \cdot m^{-2}$ light intensity and 85% relative humidity). Experimental plants were collected in the morning and kept in the laboratory till use. Leaf segments (2 cm^2) were cut under water, immersed in 10^{-4} M DCMU and soaked for 2 hrs. After infiltration, the segments were placed on a temperature regulated plate, equilibrated and adapted to the temperature of the plate (0 or 40 °C) and darkness, and delayed fluorescence was monitored. Light emission was measured in the dark period of intermittently illuminated leaves, using a Becquerel phosphoroscope and a 150 W quarzhalogen lamp. One cycle consisted of 2 ms light and 10 ms dark, delayed fluorescence being registered from 3.–7. ms of darkness using a water cooled photomultiplier. When light emission reached a steady state, temperature of the plate and leaf on it was gradually increased or decreased from the lower or upper limiting temperature (0 and 40 °C) respectively, the rate of temperature change being 1–2 C. min^{-1}. The intensity of light emission was registered simultaneously with the temperature of the leaf surface (using a thermocouple) on a two-channel strip chart recorder.

In Figure 2, we present a typical temperature response of delayed fluorescence of a control leaf segment and DCMU infiltrated sample. In the control sample, the intensity of delayed fluorescence increases with decreasing temperature from approximately 15 °C. In the region from 15–30 °C, the increase is slight in delayed fluorescence but above 30 °C, it is sharper. Above ~ 40 °C, a sharp increase is followed by an irreversible decline to zero, and in our experiments, 40 °C was the upper limiting temperature of the applied temperature gradient ramp. Leaves infiltrated with DCMU had a steady state level lower than that of normal samples in the temperature region from 0 to 20 °C, as well as above ~ 35 °C. From 20–35 °C, frequently, but not always, the intensity of delayed fluorescence of DCMU treated samples can be greater than that of control samples.

An Arrhenius plot of delayed fluorescence intensity of DCMU infiltrated leaf segment is shown in Figure 3. The results are not linear but can be fitted with four straight line segments with intersecting points occurring at ~ 12, 20 and 30 °C. (Straight lines were fitted to the experimental data by a computer program using the least squares method). Frequently at temperatures above 30 °C, instead of a rise in fluorescence intensity, there was a gradual decline, and this varied from sample to sample.

In this work we adopted a procedure similar to Murata and Fork *(6)* who studied prompt fluorescence of DCMU inhibited algae and isolated chloroplasts. Their results show maxima in fluorescence, which they correlated with phase transitions in membranes. However, they did not observe with their method transitions or maxima in prompt fluorescence in chloroplasts from higher plants. Ono and Murata *(9)* used delayed fluorescence to study the temperature dependence of different types of algae and also could detect changes which they associated with phase transitions in membranes. However, the form of delayed fluorescence with temperature that they obtained differed from ours for all the algae studied except that of *Chlorella pyrenoidosa,* where a similar form to Figure 1A was reported. Jursinic and Govindjee *(2)* in their study of short liver delayed fluorescence (120-340 μs) of isolated chloroplasts

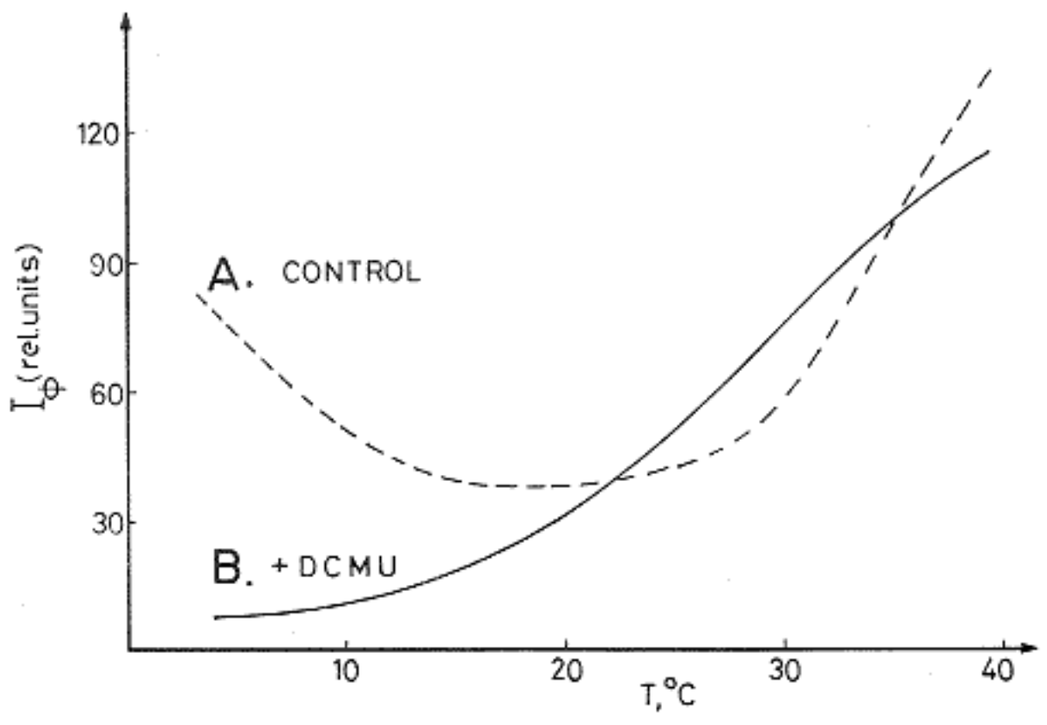

FIGURE 2. *Temperature dependence of delayed fluorescence of maize leaf segments. A (---) control plants; B (——) DCMU infiltrated plants; I_ϕ – intensity of fluorescence.*

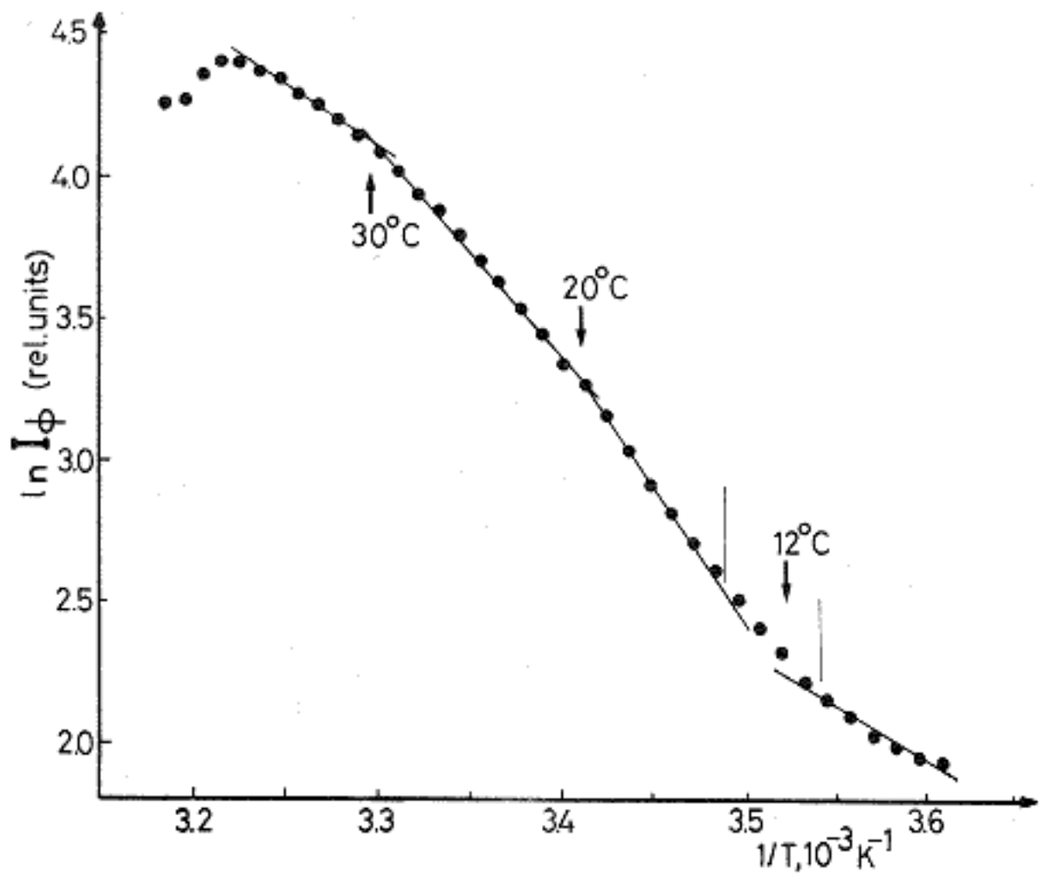

FIGURE 3. *Arrhenius plot of delayed fluorescence intensity (I_ϕ) of a DCMU infiltrated leaf segment.*

from a number of different species, showed the existence of discontinuities in the Arrhenius plots of decay constants in the 12–20 °C temperature region. They excited their chloroplast suspensions with a single flash of short duration (20 ns) and monitored the decay of light emission in the dark. Breaks in Arrhenius plots were registered only in chilling sensitive and not in chilling resistant plants. These breaks were correlated with lipid fluidity changes obtained by measuring the motion of spin probes incorporated into thylakoid membranes, using EPR spectroscopy. In these experiments also, they obtained a break around 13 °C in chilling sensitive and not in chilling resistant species.

Similar breaks in Arrhenius plots of enzyme activity, fluidity of isolated membranes montiored by EPR spectroscopy of incorporated spin probes, the Hill activity of isolated chloroplasts, etc. (see *10*) in chilling sensitive and not in chilling resistant plants, supports Lyons and Raison's hypothesis. Their concept, however, has not met with unanimous approval *(5, 13)*. In chloroplast membranes, a serious objection was put forward by Nolan and Smillie *(8)* who showed that breaks in the Arrhenius plot of Hill activity could also be detected in chilling resistant species (barley). They obtained breaks at 9, 20 and 29 °C. However, they were unable to detect changes in the fluidity of chloroplast membranes at 20 °C, monitored by EPR spectroscopy of spin probe 16 NS. They did register small changes in slope at 10 and 28 °C. These results conflict with those of other researchers *(2, 11)*. Thus, published data seems to be contradictory and the entire issue is still under debate.

Our data differs from that previously published since we discerned three temperature regions in which breaks in the Arrhenius plot occur, (5–12, 20 and 30 °C) in a chilling sensitive plant. This would be closer to the results published by Nolan and Smillie. Our results on the fluidity of isolated thylakoids *(7)* also support our delayed fluorescence measurements. Using different spin probes we observed conformational changes in the fluidity of two temperature regions, 5–12 and 20 °C. Furthermore, both delayed fluorescence and EPR spectroscopy indicate that the 5–12 °C transition is gradual rather than a sudden change. This should be expected, considering that biological membranes are heterogeneous, which supports Wolfe's arguments *(13)*.

If fluidity changes of membranes prove to be responsible for the different survival capacity of various plant species and varieties at chilling temperatures, practical breeding programmes will require fast and convenient, nondestructive methods which enable breeders to obtain specific information on such parameters. Delayed fluorescence of intact leaves, capable of monitoring the fluidity of photosynthetic membranes *in situ* seems to fulfill these requirements. It would be interesting to apply in the future our method for studying the fluidity of species differing in resistance to chilling temperatures, and, varieties amongst a single species, which have different low temperature tolerance.

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