

THE INFLUENCE OF WATER CRYSTALLIZATION AND ICE THAWING  
IN PLANT TISSUES ON ULTRAWEAK BIOCHEMILUMINESCENCE  
OF GREEN RAPE LEAVES

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Introduction

Investigation concerning the spontaneous and induced ultraweak biochemiluminescence /UBCL/ of biological objects allowed to ascertain is as one of the characteristic features of live matter /Mamiedov et al. /1969/, Grabikowski /1977/, Boveris et al. /1980/, Salin and Bridges /1981/, Sławiński et al. /1981/, its emission to be predominantly an effect of the processes of non-enzymatic free-radical oxidation of tissue lipids contained in the cell membranes and mitochondria /Tarusov /1968/, Vasiliev /1969/, Mamiedov et al. /1969/, and their spectral scope to spread between 200 nm and 800 nm /Mamiedov et al. /1969/, Grabikowski /1977/.

Processes of free-radical self-oxidation of lipids in live tissues are controlled by the system of tissue bio-oxidants /Veselovski /1982/ and therefore the UBCL intensity /I UBCL/ under normal conditions remains at a low stationary level. Whereas, under the influence of certain external physico-chemical factors it changes. For example, under the thermic influence, in a certain scope of temperature, both below and above the range of optimal temperature of plant growth, the I UBCL decreases and increases successively. Destructive impact of temperature below 0°C

is connected mainly with the crystallization of water in plant tissues. The scope of injuries and their final result depend above all on the spot in which water crystallizes.

Disturbance of the metabolic processes in cells occurs also during the ice thawing in plant tissues. Crucial role in this case is played by the speed of the phenomenon of ice to water phase transition in the intracellular spaces. Over-rapid process of ice thawing can lead to the permanent mechanical injuries in cell membranes caused by an instantaneous hydration of intracellular spaces /Kacper-ska-Palacz and Długokęcka /1971/, Bakradze et al. /1985/.

The phenomena presented above which occur under the influence of temperature are also reflected in the I UBCL change of plant tissues. Shapes of kinetic curves of the I UBCL dependence of plant tissues upon their temperature can be used in the estimation of their frost resistance.

#### Material and investigation methodology

The investigations comprised two varieties of winter rape ie. Górczański and Jupiter, exhibiting a similar resistance to the influence of low temperature /below 0°C/. Jupiter is a refined single-zero variety, whereas Górczański is an erucic one.

Rape seeds were sown into containers with soil at the end of the third decade of August 1986. The containers were dug into the ground on the experimental field. At the end of October a few containers were brought from the field and placed in the thermoluminostat at the temperature  $5 \pm 1/^{\circ}\text{C}$ , ie. at the air temperature on that particular day. Measurements of the I UBCL of leaves tissues of both varieties of rape were conducted on 3 discs /1 disc per leaf/ of 2 cm in diameter, cut out from the oldest healthy leaves. The discs were rinsed in distilled water, placed on the thermoelectric plate /Peltier's plate/ at the temperature  $20.0 \pm 0.5/^{\circ}\text{C}$  and covered they a plexiglass place of 0.4 cm thickness. After about 20 min,

ie. after the I UBCL settled, the surface temperature of the Peltier's plate was lowered at the speed of  $1^{\circ}\text{C}/\text{min}$  and the I UBCL was simultaneously recorded at each fixed temperature.

The same I UBCL measurements of discs of the investigated rape varieties were obtained after their hurdening and achieving frost resistance at the end of January 1987.

Detection of the I UBCL was achieved by means of a highly sensitive quantimetric apparatus consisting basically of: the M12FCQ51 photomultiplier of spectral sensitivity within range 160 nm - 800 nm, high and low voltage feeders, Peltier's thermobattery, thermoregulator and an electronic computer.

### Results and discussion

Results of the I UBCL measurements of the investigated rape varieties are presented on figures 1-3, by means of the kinetic curves of I UBCL of the discs of the unhardened rapes green leaves /figures 1, 2/ and the hardened ones /figures 3 A,B/.

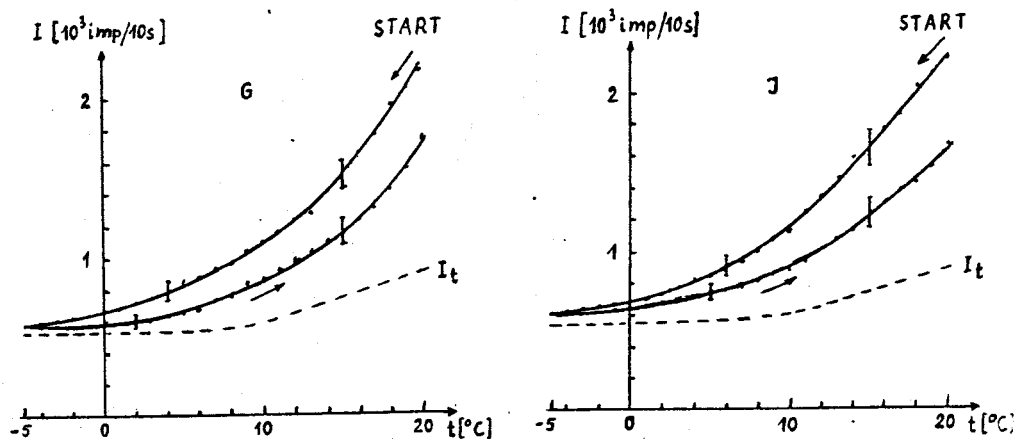


Fig. 1. Kinetic curves of the ultraweak biochemiluminescence intensity  $I$  of green tissues of leaves of two varieties within the range of temperature  $20^{\circ}\text{C} \rightarrow -5^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$ .  
G - Górczański, J - Jupiter,  $I_t$  - background.

Measurements of the I UBCL changes for the unhardened rapes Górczyński and Jupiter were taken within certain temperature ranges:  $20^{\circ}\text{C} \rightarrow 10^{\circ}\text{C} \rightarrow 30^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$ ,  $20^{\circ}\text{C} \rightarrow 0^{\circ}\text{C} \rightarrow 35^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$ ,  $20^{\circ}\text{C} \rightarrow -5^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$ , and  $20^{\circ}\text{C} \rightarrow -10^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$  while the temperature of plant tissues was being changed at the same speed of  $1^{\circ}\text{C}/\text{min}$ . Within the first three temperature ranges the obtained I UBCL changes were identical for both varieties. Exemplary course of the I UBCL changes for the range  $20^{\circ}\text{C} \rightarrow -5^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$  is shown on fig. 1. The characteristic feature for these temperature ranges is the occurrence of the curve of I UBCL increase, leaves being heated, below the curve of luminescence intensity decrease, temperature being lowered.

Whereas, in the case of the range  $20^{\circ}\text{C} \rightarrow -10^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$  /fig. 2/ a certain differentiation of the course of the I UBCL kinetic curves of the investigated rape varieties is observed, while an instantaneous increase of the I UBCL occurs in both cases when the leaves heated within the range  $-10^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$ .

Measurements of the I UBCL changes for the hardened rape varieties were taken within the temperature ranges:  $3^{\circ}\text{C} \rightarrow -10^{\circ}\text{C} \rightarrow 12^{\circ}\text{C}$  and  $3^{\circ}\text{C} \rightarrow -15^{\circ}\text{C} \rightarrow 12^{\circ}\text{C}$  / figures 3 A,B/.

Within the first range the kinetic curves of the I UBCL decrease and increase for both investigated rape varieties almost coincide. This testifies to the fact that the lowering of leaves temperature to  $-10^{\circ}\text{C}$  and the successive heating of them to  $12^{\circ}\text{C}$  did not cause any permanent frost injury of the cell membranes of plants.

Whereas, in the case of the temperature range  $3^{\circ}\text{C} \rightarrow -15^{\circ}\text{C} \rightarrow 12^{\circ}\text{C}$  /fig.3 B/ while the discs of rape leaves were being heated, an increase of the I UBCL occurs. However, the kinetic curves of the I UBCL increase starting with the  $-6^{\circ}\text{C}$  can be noticed to occur above the curves of the I UBCL decrease in the case of the temperature of leaves being lowered.

The obtained initial measurements imply that the unhardened rapes Górczyński and Jupiter exhibit a characte-

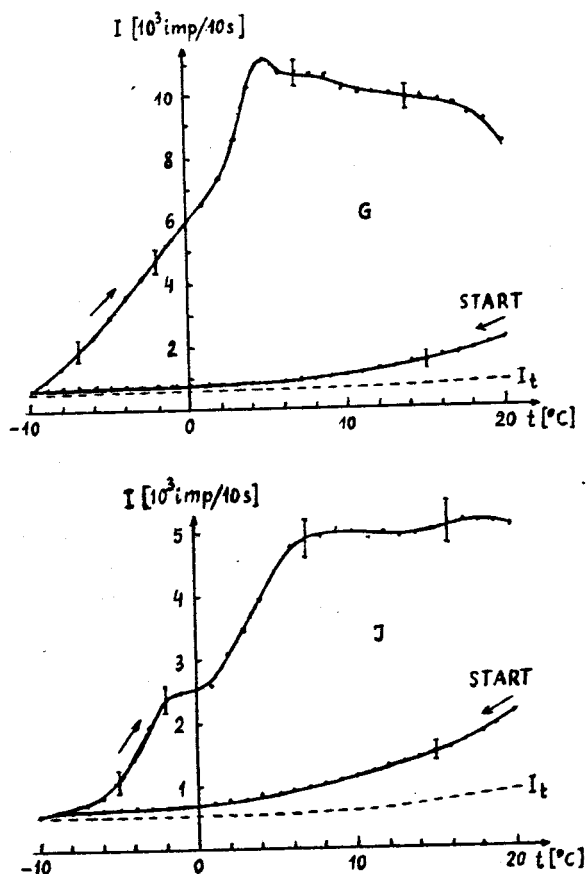


Fig. 2. Kinetic curves of the ultraweak biochemiluminescence intensity  $I$  of green tissues of leaves of two varieties within the range of temperature  $20^{\circ}\text{C} \rightarrow -10^{\circ}\text{C} \rightarrow 20^{\circ}\text{C}$ . G - Górczański, J - Jupiter,  $I_t$  - background.

ristic value of temperature at which a frost injury of their tissue occurs within the range from  $-5^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$ , whereas the hardened ones do so within the range from  $-10^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$ .

Acquired data suggest that the applied luminescence method can be helpful in the relative estimation of frost resistance of the rape plants at the early stages of their growth.

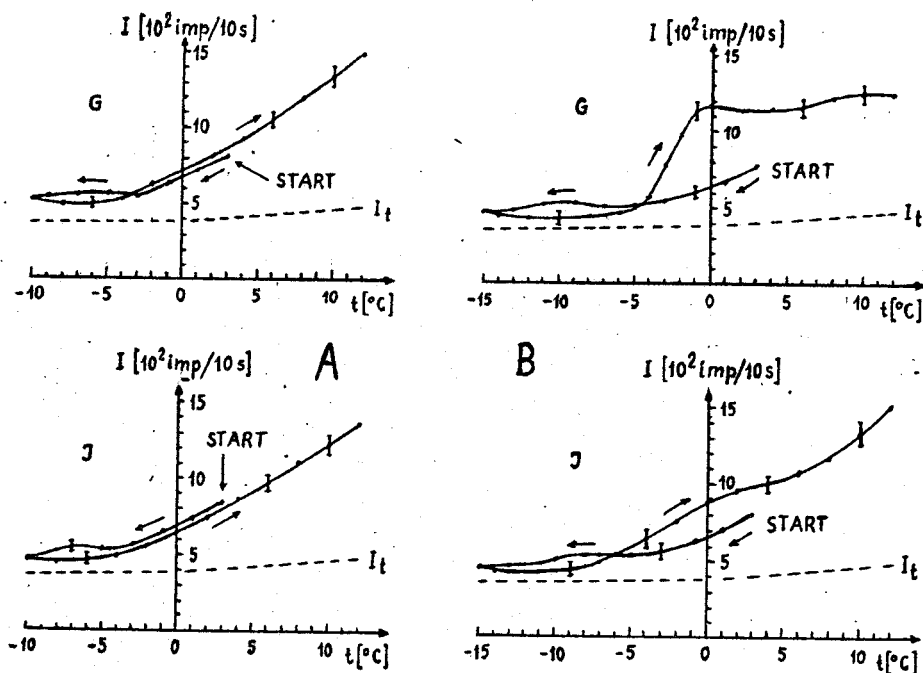


Fig. 3. Kinetic curves of the ultraweak biochemiluminescence intensity  $I$  of green tissues of leaves of two varieties within the range of temperature A:  $3^{\circ}\text{C} \rightarrow -10^{\circ}\text{C} \rightarrow 12^{\circ}\text{C}$ , B:  $3^{\circ}\text{C} \rightarrow -15^{\circ}\text{C} \rightarrow 12^{\circ}\text{C}$ . G - Górczański, J - Jupiter,  $I_t$  - background.

#### References

1. Bakradze N.G. et al., 1985. Osobiennosti niskotemperaturnovo plasmolisa kletok "zakalennykh" rastieni. Biofizika, 30, 3, 482-486.
2. Boveris A. et al., 1980. Spontaneous chemiluminescence of soybean seeds. FEBS Lett. 113, 29-32.
3. Grabikowski E., 1977. Badanie żywotności nasion roślin uprawnych przy pomocy ultrasłabej biochemiluminescencji. Praca doktorska, AR w Szczecinie.
4. Grabikowski E., Murkowski A., 1984. Zmodyfikowany test luminescencyjny do oceny mrozoodporności rzepaku. Biul. Inst. Hod. i Aklim. Roślin, 157, 21-23.

5. Grabikowski E., 1985. Influence of temperature on ultraweak bioluminescence of plants. 3<sup>rd</sup> International Conference Physical Properties of Agricultural Materials. Prague, Czechoslovakia, 273-277.
6. Kacperska-Palacz A., Długokęcka E., 1971. Metodyka przeprowadzania oceny odporności roślin na zamarzanie. Wiad. Botan., 15, 1, 79-90.
7. Mamiedov T.G., Popov G.A. and Koniev V.V., 1969. Svierchslaboje sviecenije razlicnych organizmov. Biofizika, 14, 6, 1047-1051.
8. Mamiedov T.G., Popov G.A. and Koniev V.V., 1969. Spektralnyj sostav svierchslaboj bioluminescencji rastieni. Dokl. AN SSSR, 187, 4, 928-930.
9. Salin M.L., Bridges M., 1981. Chemiluminescence in wounded root tissue. Evidence for peroxidase involvement. Plant Physiol., 67, 43-46.
10. Sławiński J., Majchrowicz I., Grabikowski E., 1981. Ultraweak luminescence from germinating resting spores of *Entomophthora virulenta* Hall et Dunn. Acta Mycologica, 17 /1-2/, 131-139.
11. Sławiński J. et al., 1986. Ultraweak luminescence and energy transfer to chlorophyll a in green plants. Inter. Sym. on Molec. Lumin. and Photoph., Toruń. Proceedings, 347-350.
12. Tarusov B.N., 1968. Svierchslaboje sviecenije biologičeskich sistem i perspektivy jevo ispolzovanija. Sielskochoz. biol., 3, 3, 336-344.
13. Vasiliev R.F., 1969. On the mechanism of the chemiluminescence in oxidation of organic substances and polymers. Die Makromolekular Chemie, 126, 3038, 231-238.
14. Veselovski V.A., 1982. O roli bioantioksidantov v ustojčivosti rastieni k neblagoprijatnym usloviam suscestvovanja. Bioantiokisliteli v regulacii metabolisma v normie i patologii. Trudy MOIP, Moskva, Nauka, 57, 150-152.