Luminescence variations in cucumber (*Cucumis sativus* L.) leaves derived from different regeneration systems

Murkowski, A.[1], Wróblewski, T.[2], Burza, W.[2] and Skórska, E.[1]

[1] *Department of Physics, Agricultural University of Szczecin, ul. Pawła VI 3, 71-454 Szczecin*
[2] *Department of Plants Genetics, Breeding and Biotechnology of SGGW-AR, ul. Nowoursynowska 166, 02-766 Warszawa*

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Summary: Plants obtained from *in vitro* culture can show increased susceptibility to environmental stress conditions. In the process of their adaptation to natural conditions it requires monitoring of their physiological state. The methods used to check this phenomenon should estimate quickly and exactly the tolerance to suboptimal environmental factors. Such requirements are satisfied by the methods of measuring chlorophyll luminescence *in vivo*, e.g. fluorescence induction and delayed luminescence. The objects of our studies were cucumber plants regenerated from cultures of callus and embryogenic cell suspension, as well as the plants obtained from seeds. The plants derived from *in vitro* cultures displayed a poor physiological condition at the early phase of adaptation characterised by higher susceptibility both to stress caused by increased density of the light flux (ca. 700 $\mu E \sum m^{-2} \sum s^{-1}$) and low temperature (4 °C) in comparison with the plants obtained from seeds.

Introduction

Plants obtained from *in vitro* culture show usually increased susceptibility to suboptimal environmental conditions. The process of their adaptation to natural condition is complex and requires time. A control of environmental conditions is necessary as well as an estimation of the plant's physiological state, particularly under conditions of stress. Most of the stress factors disturb the functions and induce destruction of biological membranes (also in chloroplasts) thus impair the correct course of the process of photosynthesis. It affects, directly, both characters of photoinduced chlorophyll luminescence emission, particularly fluorescence induction, and delayed luminescence (*Briantais et al.* 1986; *Havaux* and *Lannoye* 1985; *Veselovsky* and *Veselova* 1992). Using adequate methods of monitoring we can seize and estimate, exactly, the tolerance of the plant to unfavourable environmental factors and their actual physiological state as well as potential productivity (*Krause* and *Weis* 1984, *Krause* and *Somersalo* 1989, *Gwizdek et al.* 1985, *Murkowski* and *Skórska* 1988; *Skórska* and *Murkowski* 1988, *Schapendonk et al.* 1992).

In a previous work (*Burza et al.* 1994), we described how the plants were raised from callus on media with different growth regulators, and that they show differences in various parameters of chlorophyll fluorescence induction and delayed luminescence decay kinetics. The purpose of the present work is the comparison of the same parameters in the plants regenerated from two different systems: callus and cell suspension.

Material and methods

The objects of our studies were cucumber plants (*Cucumis sativus* L. cv. Borszczagowski), regenerated from two kinds of *in vitro* cultures – callus and embryogenic cell suspension. First of them arose from leaf explants according to *Malepszy* (1988) and the second from the shoot meristem initiated in an embryogenic cell suspension (*Wróblewski et al.* 1995). The control plants arose from sterile seeds and grew in the same conditions as the former plant of *in vitro* origin according to *Burza et al.* (1994). Growth conditions: 22/18 °C – day/night, light intensity 100 $\mu E \sum m^{-2} \sum s^{-1}$; photoperiod 12 h. The plants with 1–2 leaves were submitted to stress of 2-hours: S2 – increased density of light flux (ca. 700 $\mu E \sum m^{-2} \sum s^{-1}$) at a temperature of 22 °C and S3 – increased density of light flux (ca.700 $\mu E \sum m^{-2} \sum s^{-1}$) and low temperature 4 °C. The control plants were at the same time under conditions S1, i.e. 22 °C, 100 $\mu E \sum m^{-2} \sum s^{-1}$.

We used luminescence tests, similar as in the publication of *Burza et al.* (1994): two parameters of fluorescence induction Fv/Fm (index of photoinhibition destructions in PSII), Rfd (vitality rate, informs about interaction of primary

photosynthetic reactions with dark enzymatic reactions, leading to CO_2 assimilation) (Lichtenthaler *et al.* 1986) and coefficient of Ld delayed luminescence decay kinetics, defining efficiency of electron transport between primary acceptors QA and QB in PS II. Measurements of chlorophyll fluorescence induction were done on the universal fluorimeter steered by microcomputer, and delayed luminescence – on high-sensivity luminometer working in the system of one electron pulse counting.

Measurements were conducted on disks of 13 mm diameter, cut from the first leaves of plants. Results presented in a table are means from 5–6 repetitions and LSD values were statistically calculated by means of test t on the 0.05 significance level. Statistically significant differences are marked by stars.

Results and discussion

Results show that in conditions S1 plants from *in vitro* culture don't show photoinhibition destruction, but their Rfd vitality index and L_d defining photosynthetic electron transport in PSII are lower than analogous parameters in plants from seeds. Particularly low values of both parameters show the cucumber plants from callus culture *(Table 1)*.

Increased light intensity level at optimal temperature (S2) causes lowering of the three parameters studied in plants raised both from seeds as well as from in *vitro* culture. Also in this case plants from callus culture reacted most strongly. Low temperature at increased light intensity (S3) caused further reduction of all parameters.

We can observe similar changes of the L_d parameter, which is more reduced in plants from callus culture compared with control plants. Submitted to stress conditions of S2 and S3 a reduction of 2- to 6-times ensued, respectively. Photosynthetic apparatus of leaves of the plants obtained from embryonic cell suspension appeared more resistant to studied stresses. In conditions of increased light intensity, the Rfd index was somewhat lower in leaves of plants cultivated from seeds, however, at low temperature it was reduced, but not significantly in relation to the other two cultures.

In stress conditions of S3 the efficiency of electron transport between both photosystems in plant leaves of *in vitro* cultures studied was worse in comparison with plants raised from seeds. It shows a higher sensitivity of the L_d parameter than the vitality index Rf_d. The parameter F_v/F_m was most efficient in proving the differences between the three cultures under stress conditions of S3.

Both regeneration systems in question produced plants on the same way i.e. somatic embryogenesis (Malepszy 1988; Wróblewski *et al.* 1995). However, the differences between the systems clearly exist in susceptibility expressed by luminescence. The poor physiological state of callus derived plants is expressed in their low yield, as was shown previously in the same cucumber variety (Malepszy *et al.* 1990). This supports the potential utility of our method for a rapid evaluation of the physiological state of *in vitro* regenerated plants.

Conclusions

1. Cucumber plants raised from in vitro cultures and studied in the pre-adaptation phase displayed a poor physiological state and higher susceptibility to light stress and low temperature in comparison with plants raised from seeds.
2. Plants obtained from callus culture have worse parameters than plants regenerated from embryogenic cells in all studied stress conditions.
3. Luminescence parameters are good indexes of the plant's physiological state during its onthogenesis. The method presented facilitates a fast and sensitive analysis of the photosynthetic processes under optimal as well as suboptimal stress conditions.

Table 1 **Chlorophyll luminescence parameters of cucumber leaves of plants raised from seeds and regenerated from *in vitro* systems**

Cucumber plants	S1			S2			S3		
obtained from:	Fv/Fm	Rfd	Ld	Fv/Fm	Rfd	Ld	Fv/Fm	Rfd	Ld
seeds	0.811	3.26	469	0.698	2.57	245	0.567	0.65	92.9
suspension	0.809	2.62	353	0.549	1.49	213	0.492	0.70	36.3
callus	0.762	2.03	268	0.530	1.00	139	0.362	0.50	42.3
LSD	0.018*	0.31*	41*	0.045*	0.24*	56*	0.069*	0.23	8.7

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