PHOTOSYNTHETIC LUMINESCENCE ASSAY FOR DETERMINATION OF TRIAZINE RESISTANCE OF RAPE PLANTS

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Introduction

Breeding rape varieties resistant to triazine would facilitate weed killing and simplify crop rotation. At present, in many countries research, work on transfer of triazine resistance found in wild turnip rape to new bred strains is carried on (Souza Machado and Bandeen, 1982).

In breeding rape plants resistant to triazine herbicides a simple biotest, which could differentiate resistant plants from sensitive ones quickly and easily is required. For the last few years the method of fluorescence tests has been applied to solve this problem. Evaluating changes in fluorescence intensity of leaf discs floating on triazine solution, it is possible to distinguish resistant plants from sensitive during a few hours (Ahrens et al., 1981, Ali and Souza Machado, 1981). Another possibility of triazine resistance monitoring arises from the influence of triazine herbicides on photosynthetic luminescence. Herbicides - inhibitors of photosynthesis result in significant changes in intensity and kinetics of photosynthetic luminescence decay caused by disturbance of primary photosynthesis reactions (Assche et al., 1982, Jachetta and Radosevich, 1981).

The comparative studies of fluorescent test and a test using photosynthetic luminescence detection are conducted in our laboratory (Devlin et al., 1980, Devlin et al., 1983). The purpose of this paper is presentation of application possibility of photosynthetic luminescence to investigate of triazine resistance of different rapeseed varieties.

Material and methods

Studies were conducted on plants of four varieties:

A - Brassica napus cv Górczański

B - Brassica campestris cv Perko

C - Brassica napus cv Akela x Brassica campestris ssp. pekinensis cv Granaat

D - Brassica campestris ssp. pekinensis cv Granaat x Brassica oleracea ssp. acephala cv Normal

Plants were grown in containers with sand and Hoagland nutrient diluted with water /1:1/ under white fluorescent light /400 W mercury lamp LRFR 400; light intensity 200 $\mu E \cdot cm^{-2} \cdot s^{-1}$, PAR/. Photoperiod was 12 hours. The temperature was 15°C during the light period and 10°C during the dark period. Measurements were taken on 14-day first leaves. Discs of 13 mm diameter were out from the first leaf and placed on the surface of water /control samples/ or atrazine solution /10^{-6} M, 10^{-5} M/. Measurements were done in 5 repetitions and standard deviation for each series was calculated on level 0.95.

I. Fluorescence measurement

Fluorescence of leaf discs was measured with the set shown on Fig. 1. The source of exciting light was mercury lamp /1/ type HQE 40 L being the part of spectrofluorometer SPECOL /2/. made by Zeiss. The examined sample /3/ was exposed to mercury spectral line /λ = 436 nm/. Fluorescence of an investigated sample /λ_{max} = 685 nm/ after transition through the red filter /4/ and mirror reflection /5/ was measured by photomultiplier /6/ EMI 9558B type. The photomultiplier working in the single electron pulse counting system is supplied with high stabilized voltage from ZWN-21 /8/ made by POLON. Voltage pulses from the photomultiplier are being passed through pre-amplifier to the scaler P-21 /9/ made also

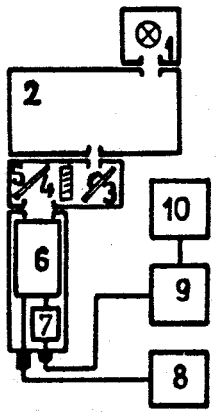

Fig. 1. Block diagram of set up to fluorescence measurement

by POLON. The sum of counted pulses is recorded by printing recorder /10/ during 100 s.

II. Measurement of photosynthetic luminescence /PSL/

PSL is recorded within a time range from several miliseconds to several minutes from the moment of switching exciting light off and its spectrum is similar to fluorescence spectrum /λ_{max} = 685 nm/. PSL of leaves was measured by means of the set shown on Fig. 2. The source of exciting light was projector lamp 150 W /2/. PSL was measured at the temperature 22°C within a range of time from 1 s to 6 s after turning exciting light off.

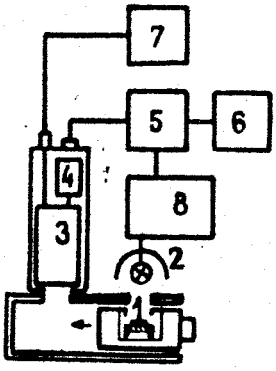

Fig. 2. Block diagram of set up to photosynthetic luminescence measurement

An examined disc of leaf /1/ was placed in a pulled out drawer of the camera under a projector lamp /2/. The programmed system /8/ switches a lamp on for 6 seconds to expose the leaf tissue. After switching exciting light off, the drawer containing the sample is moved to the other terminal position so as to introduce the exposed disc under the photomultiplier /3/. The photomultiplier connected to high voltage power supply /7/ should be sensitive in far red as for example EMI 9558B, S12FC51 or similar. Since PSL is very weak, the photomultiplier works in the single electron pulse counting system. The voltage pulses from the photomultiplier after amplifying then 200 times by pre-amplifier /4/ are transferred to the scaler input /5/. One second after turning light off, the programmed system /8/ switches on the pulse scaler for 1 s. The number of pulses N_1 is recorded them by the printing recorder /6/. After 4-second break the pulse scaler is switched on again and the printing recorder records the next number of pulses N_2 as shown on Fig. 3.

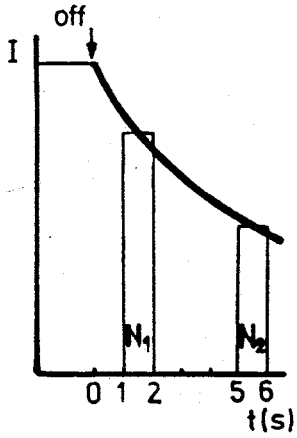

Fig. 3. Curve of photosynthetic luminescence decay

Results and discussion

After the 2 hour treatment with atrazine solutions at the concentration 10^{-6} M and 10^{-5} M /at 25°C; 200 $\mu E \cdot m^{-2} \cdot s^{-1}$, PAR/ of the investigated discs I_{Fl}, N_1 and N_2 were measured. Obtained results are presented in the table 1.

In all cases the relative values of N_1 /intensity of the first component of PSL/ and the relative values of N_1/N_2 ratio /characterizing PSL decay rate/ are higher then relative changes of I_{Fl}. The same regularity was observed earlier in investigations of the influence of weed killers - inhibitors of photosynthesis on fluorescence and PSL of chlorella /Devlin et al., 1983/. The fluorescence of photosynthetic apparatus is connected with the emigration of excited states across the photosynthetic unity toward the reaction center of photosystem II /Bertsch et al., 1971/. PSL is connected with primary photosynthesis processes and its intensity and decay kinetics provides information about efficiency of electron transport chain and co-operation of both photosystems /Govindjee and Jursinic, 1979; Lavorel and Dennery, 1982/. Various physico-chemical factors affecting plant physiological functions bring about changes also in fluorescence and PSL emission /Murkowski, 1973, 1974; Gwizdek et al., 1985/.

The effect of atrazine on luminescence of four varieties Brassica

Varieties	Fluorescence intensity I_{F1} /% of control/	Photosynthetic luminescence intensity N_1 /% of control/	Photosynthetic luminescence decay N_1/N_2 /% of control/
A			
10^{-6} M	163 ± 28	297 ± 38	383 ± 52
10^{-5} M	218 ± 15	418 ± 50	776 ± 99
B			
10^{-6} M	144 ± 27	489 ± 93	521 ± 99
10^{-5} M	214 ± 15	675 ± 91	1190 ± 114
C			
10^{-6} M	121 ± 15	271 ± 67	329 ± 47
10^{-5} M	207 ± 29	500 ± 52	762 ± 56
D			
10^{-6} M	125 ± 20	310 ± 69	375 ± 58
10^{-5} M	217 ± 19	565 ± 75	822 ± 110

A – Górczański
B – Perko
C – Akela x Granaat
D – Granaat x Normal

Conclusions

1. The most sensitive indicator of the herbicide reaction on chloroplasts is N_1/N_2 ratio.It is convenient also because its values are dimensionless and there is no need to calculate mass of the sample or to determine its area.
2. The most sensitive to atrazine proved to be turnip of Perko variety.
3. The use of photodetectors working in the single electron pulse counting system enables to apply professional electronic devices /e.g. radiometric equipment/ and to record the obtained results by means of computers.

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