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Acta Universitatis Carolinae. Mathematica et Physica, Vol. 20 (1979), No. 2, 3--9

Persistent URL: http://dml.cz/dmlcz/142436

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Fluorescence, Absorption and Photosynthetic Rate Measurements of Potato Leaves (Solanum tuberosum L.) During Their Vegetation

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Received 27 October 1978

A certain correlation between ontogenetic dependences of spectral properties (absorption, fluorescence and polarized fluorescence) and the photosynthetic rate of in vivo potato leaves has been found. The spectral properties reflect the state of the leaf pigment system which can be slightly different for different potato varieties.

Была обнаружена взаимосвязь между онтогенетической зависимостью спектральных свойств (поглощение, флуоресценция и поляризация флуоресценции) и скоростью фотосинтеза листьев картофеля. В спектральных свойствах отражается состояние пигментной системы листьев, которое может отличаться для разных разновидностей картофельного растения.

Byla zjištěna určitá korelace mezi ontogenetickou závislostí spektrálních vlastností (absorpce, fluorescence a polarizace fluorescence) a rychlostí fotosyntézy listů brambor in vivo. Ve spektrálních vlastnostech se odráží stav pigmentové soustavy listů, který může být poněkud odlišný u různých odrůd.

1. Introduction

Relatively many papers have been devoted to the absorption and luminescence measurements of plant pigments in the material in vivo (e.g. [1], [2], [3], [4]), but only few of them have studied the change of the mentioned properties during the ontogenesis (e.g. recently [5], [6]). Although it is difficult to gain quantitative conclusions from such measurements, mainly due to a number of disturbing effects (scattering, non-homogenous pigment distribution in the tissue in vivo), these measurements can provide some qualitative information concerning, for example, relative amounts of different pigments, the changes of average pigment concentration or its aggregation during the ontogenesis of the leaf etc. The information about the

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correlation between physical and photosynthetic properties of the leaves and the final products of photosynthesis (i.e. tubers) can contribute to the application aspects of these experiments.

2. Material and Methods

Four potato varieties Blaník, Cira, Nora and Radka were chosen for optical studies during a vegetation period of 4 weeks in the greenhouse. All spectroscopic measurements were carried out on the terminal leaflet of the fourth leaf (counted from the top of the plant) at room temperature. Fluorescence was excited and detected at the upper side of the leaf. The experiments were three times repeated during the years 1975-7.

The relative absorption was measured by modified spectrophotometer Specord UV-VIS (neutral filters), the fluorescence was measured by means of laboratory spectrofluorometer (excitation prism monochromator SPM 1 and measuring grating monochromator SPM 2) with a cooled photomultiplier M12FD35 connected to registration potentiometer. Monochromators and photomultiplier are products of Carl Zeiss, Jena. The experimental arrangement is shown in Fig. 1. The absorption and fluorescence spectra are not corrected for the scattering and reabsorption.



Fig. 1. Design of the laboratory-made spectrofluorometer. 1 — Excitation high pressure mercury lamp HBO 500; 2 — monochromator SPM1 (Carl Zeiss, Jena); 3 — interference filter; 4 — sample holder; 5 — Schott filter RG 2; 6 — monochromator SPM 2 (Carl Zeiss, Jena); 7 — photomultiplier M 12 FD 35; 8 — recorder EZ 4 (Laboratorní přístroje, Praha)

The rate of photosynthesis was measured by the method of Bartoš et al. [7] adapted for potato plants by Zrust [8].

Two series of experiments were performed. In the first series all varieties were planted simultaneously, in the second one the plants were grown in such a way that

the beginning of the bud growth was approximately the same for all varieties. The results of the first and second series are shown in Fig. 4-5 and in Fig. 6, respectively. The basic character of dependences and all conclusions are the same for both series of experiments. The first measurement was performed at the beginning of bud formation.

3. Results

Fig. 2 shows a typical spectrum of the relative absorption of green potato leaf at room temperature. The main absorption maxima are numbered from the shortto the longwavelength side of the spectrum. The spectrum reflects the presence of main photosynthetic pigments – chlorophylls and carotenoids. Both the optical density ratio of the 2nd and the 7th bands (D_2/D_7) and also the position of the 7th maximum λ_7 decreased during the studied period of ontogenesis. There was found no unambiguous difference among the varieties in these dependences.



Fig. 2. Relative absorption spectrum of the terminal leaflet of the fourth potato leaf from the top (variety Blaník, two weeks after commencement of bud formation) at room temperature

Fig. 3 presents the fluorescence spectra of leaf at room tempetature excited by the light of different wavelengths. The fluorescence band intensity ratio I_1/I_2 decreases with the change of the excitation wavelength from 435, 465 nm to 578 nm. For $\lambda_{ex} = 578$ nm the first fluorescence maximum is shifted to longer wavelengths in comparison with that excited by $\lambda_{ex} = 435$ and 465 nm. These changes were proved for all measurements.

For example the I_1/I_2 ontogenetic dependence for $\lambda_{ex} = 435$ nm and for four potato varieties is shown in Fig. 4. The I_1/I_2 ratio for all three excitation wavelengths slightly increases in the followed time interval. In mutual comparison of different varieties Blaník and Radka show an extreme behaviour. For $\lambda_{ex} = 435$ and 465 nm



Fig. 3. Fluorescence spectrum of the terminal leaflet of the fourth potato leaf from the top (variety Blanik, three weeks after the full plant emergence) at room temperature for three different excitation wavelengths

the band ratio I_1/I_2 is, in the average, the greatest one and the smallest one for the variety Blaník and Radka, respectively (see Fig. 4). For $\lambda_{ex} = 578$ nm the opposite situation was found.

Fig. 5 illustrated the ontogenetic dependence of fluorescence polarization degree P of the first fluorescence band (684 nm) excited by $\lambda_{ex} = 435$ nm at room temperature. The decrease is not monotonous but "sinusoidally modulated". The average absolute value of P is the greatest one and the smallest one for the variety Blaník and Radka, respectively.

In the Fig. 6 the corresponding ontogenetics dependence of photosynthetic rate is shown. In the course of ontogenesis the photosynthetic rate decreases. Again the varieties Blaník and Radka behave extremely (maximum mean rate for Blaník and minimum for Radka).

A greet natural dispersity of plant material and hence a great statistical error is demonstrated in all measured values. Furthermore, the influence of a systematic error (the influence of weather, illumination etc.) cannot also be excluded. Due to



Fig. 4. The change of the fluorescence intensity ratio of the shortwavelength (684 nm) to the longwavelength (735 nm) band I_1/I_2 during the studied ontogenesis interval. Fourth leaf from the top, room temperature. Curves: 1 — variety Blanik, 2 — variety Cira, 3 — variety Nora, 4 — variety Radka



Fig. 5. The change of the fluorescence polarization degree P during the ontogenesis of potato plant. Fourth leaf from the top, room temperature. Curves: 1 — variety Blaník, 2 — variety Cira, 3 — variety Nora, 4 — variety Radka

the above reasons, the accuracy of measurements is lowered, and only qualitative conclusions can be drawn from our data.



Fig. 6. The change of the photosynthetic rate of the potato leaf during the studied ontogenesis interval. Curves: 1 — variety Blanik, 2 — variety Cira, 3 — variety Nora, 4 — variety Radka

4. Discussion

The relative increase of the second fluorescence band intensity and simultaneous shift of the first band to the longer wavelengths with increasing excitation wavelength (Fig. 3) were observed also in a model system (chlorophyll a in polymer matrix [9]). These facts belong to the general properties of higly concentrated pigment systems, and they reflect a series of phenomena due to low distance of pigment molecules (different pigment forms, aggregated states, electron excitation energy transfer, reabsorption and, in leaves, also number of chloroplasts in a cell).

During the studied part of potato plant ontogenesis the local concentration of chlorophyll *a* (fluorescent species) decreases and hence the ratio I_1/I_2 and the absolute value of polarization degree *P* increase. Simultaneously the relative amount of other pigments in comparison with chlorophyll *a* decreases (decrease of D_2/D_7). The auxiliary pigments (chlorophyll *b* and carotenoids) as well as chlorophyll *a* contribute to the second absorption band, the 7th band represents mainly the absorption of chlorophyll *a*.

A systematic study of spectral parameters and photosynthetic rate also revealed the distinct behaviour of the potato varieties Blaník and Radka. In our opinion the variety Blaník shows some features of a more effective photosynthetic system (a greater photosynthetic rate, lower local chlorophyll *a* concentration) than other varieties, the variety Radka represents the opposite case.

The found correlation of the spectral and photosynthetic parameters of the leaf indicates the possibility of the use of nondestructive optical and luminescence methods for the determination of the photosynthetic leaf activity in different plants.

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