Chlorophyll fluorescence in two strawberry (*Fragaria* x *ananassa* Duch.) cultivars

Fluorescencja chlorofilu u dwóch odmian truskawki (*Fragaria* x *ananassa* Duch.)

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Abstract

The study of chlorophyll fluorescence (CF) parameters i.e.: Fo, Fm, Fv/Fm, Y, qP, qN, F'o, F'm was conducted in two strawberry cultivars 'Honeoye' and 'Teresa'. Measurements of their values were done twice: in an early autumn and late spring. Fluorescence of five dark-adapted mature leaves derived from each cultivar was measured in the laboratory at room temperature with the use of a portable pulse amplitude modulation (PAM) fluorometer in 15. replicates for each parameter. Statistical analysis of the obtained results showed significant differences between values of CF parameters in both cultivars. Mean values of CF, excluding F'o and F'm were significantly higher in cv.'Teresa' when compared with cv.'Honeoye'. In both cultivars, the values of Fo, F'o, Fm, F'm were significantly higher in the spring measurements. In cv.'Teresa' the CF parameters i.e. Fv/Fm, Y, qP and qN achieved the higher values in the autumnal measurement, but in cv.'Honeoye' values of these parameters except Y, were significantly higher in the spring. In the conclusion, it could be emphasized, that cv.'Teresa' distinguished a more efficient photosynthetic apparatus than cv.'Honeoye' and the significant differences observed between the autumnal and spring CF values in the analyzed cultivars revealed the different, dependent on the genotype, response in the functioning of this apparatus to the various environmental conditions characterizing both seasons of the year.

Keywords: fluorometer, genotype, measurements, photochemistry, photosynthetic efficiency

Streszczenie

Przeprowadzono badania fluorescencji chlorofilu (FC) u dwóch odmian truskawki 'Honeoye' i 'Teresa'. Pomiary wartości parametrów fluorescencji takich jak: Fo, Fm, Fv/Fm, Y, qP, qN, F'o, F'm wykonano dwukrotnie: wczesną jesienią i późną wiosną. Fluorescencja pięciu zaadaptowanych w ciemności dojrzałych liści pobranych z obu odmian truskawki była mierzona w temperaturze pokojowej za pomocą fluorometru w 15. powtórzeniach dla każdego parametru. Statystyczna analiza uzyskanych wyników wykazała istotne zróżnicowanie pomiędzy wartościami parametrów

fluorescencji u badanych odmian. Średnie wartości parametrów FC, z wyjątkiem F'o i F'm były istotnie wyższe u odmiany 'Teresa' w porównaniu z odmianą 'Honeoye'. U obu odmian wartości Fo, F'o, Fm, F'm były istotnie wyższe w pomiarach wiosennych. U odmiany 'Teresa' takie parametry jak: Fv/Fm, Y, qP i qN osiągnęły wartości wyższe w pomiarach jesiennych, natomiast u odmiany 'Honeoye' wartości tych parametów z wyjątkiem Y, były istotnie wyższe wiosną. W podsumowaniu należy podkreślić, że odmiana 'Teresa' charakteryzowała się sprawniejszym aparatem fotosyntetycznym niż odmiana 'Honeoye', a istotne różnice zaobserwowane pomiędzy jesiennymi i wiosennymi wartościami FC u badanych odmian ujawniły odmienną, zależną od genotypu reakcję w funkcjonowaniu ich aparatu fotosyntetycznego w obu porach roku.

Słowa kluczowe: fluorometr, genotyp, pomiary, fotochemia, wydajność fotosyntetyczna

Introduction

Photosynthesis belongs to a fundamental and very complex physiological process of plant, which is determined by internal and external factors. Each limitation of its intensity causes a decrease in height and quality of yielding. Chlorophyll fluorescence (CF) replaces partly conventional measurements of a photosynthetic efficiency and allows as a rapid, non-destructive technique to examine photosynthetic process *in vivo*. As was stated by Krause and Weis (1991) the measurement of chlorophyll fluorescence as an indicator of the primary photochemistry of photosynthesis is now well established. The measurements of CF parameters are particularly useful to assess the plant response to different environmental stresses (Bolhar-Nordenkampf et al.,1989; Guidi et al.,1997; Jimenez et al.,1997; Maciorowski et al.,1996 Smillie et al.,1987; Havaux and Lannoye,1985; Krause and Somersalo, 1989; Murkowski and Skórska,1988; Skórska and Murkowski, 1988). Fluorescence can give insights into the ability of a plant to tolerate environmental stresses and into the extent to which those stresses have damaged the photosynthetic apparatus. By measuring the intensity and nature of this fluorescence, plant ecophysiology can be investigated, also (Lichtenthaler et al., 1986).
It has long been known that chlorophyll fluorescence emission kinetics from plants provide an indicator of plant photosynthetic performance . More recently, fluorescence parameters have been shown to relate directly to the photosynthetic CO_2 assimilation rate of leaves (Genty et al., 1989; Cornic and Ghashghaie, 1991; Harbinson et al., 1990; Krall and Edwards, 1990, 1991; Krall et al., 1991; Edwards and Baker, 1993; Siebke et al., 1997) and have been widely used to study leaf photosynthetic performance (Maxwell and Johnson, 2000). Another application where fluorescence may be useful is in examining the acclimation of plants to different microenvironments. By measuring the light dependency of Φ_{PSII} it is possible to make simple and rapid estimates of the light saturation behavior of different plants under field conditions. In recent years no investigations into photosynthetic performance of plants under field conditions seems complete without some fluorescence data. The technique of chlorophyll fluorescence has become ubiquitous in plant ecophysiology studies. A number of excellent reviews exist that discuss the theoretical background of both measurement and analysis of chlorophyll fluorescence, however, these are typically written from a biophysicist's or a molecular

plant physiologist's point of view (Horton and Bowyer, 1990; Krause and Weis, 1991; Govindjee, 1995).

Phenotypic values of CF parameters are mostly depended on the genotypic response to environmental conditions. The objective of this study was to analyze the variability of CF parameters in different strawberry genotypes. In order to evaluate this differentiation, the values of CF parameters were measured in an early autumn and late spring in leaves of two strawberry cvs. 'Honeoye' and 'Teresa' grown in the same field conditions. Both cultivars are commercially cultivated in Poland and belong to early and mid-early genotypes (respectively).

Material and methods

Eight parameters of chlorophyll fluorescence (CF) were assessed in leaves of two strawberry cvs. 'Honeoye' and 'Teresa'. All measurements were done using plants growing outdoors at the Experimental Station belonging to the University of Life Sciences in Lublin (51.240° N, 22.570° E). Chlorophyll fluorescence was evaluated twice for each cultivar-after yielding in the early autumn (September 2009) and in the late spring next year (June 2010). In order to measure the values of chlorophyll fluorescence parameters five mature leaves were taken from plant of each cultivar in both terms of evaluation. The leaves of 'Teresa' and 'Honeoye' were placed into the clip, darkened for 20 min and then illuminated with red light emitting diodes (peak at 650 nm, maximum photosynthetic photon flux density-PPFD at leaf surface was 600 $\mu mol \times m^{-2} \times s^{-1}$). Fluorescence of dark-adapted leaf samples was measured in the laboratory at room temperature, with the use of a portable pulse amplitude modulation (PAM) fluorometer (PAM 2000, Heinz Walz GmbH, Effeltrich, Germany) in fifteen replicates for each parameter (three replicates per leaf). The following parameters of chlorophyll fluorescence were measured:

1. F_o – minimal fluorescence of dark-adapted leaves
2. F_m – maximal fluorescence of dark-adapted leaves
3. F_v/F_m – ratio of variable fluorescence (Fv=Fm-Fo) to maximal fluorescence (Fm); an indicator of maximum quantum photochemical efficiency of PSII (maximum quantum yield), an indicator of photoinhibition
4. Y – Yield of PSII, a light adapted test normally taken at steady state photosynthesis levels, to estimate of the effective portion of absorbed quanta used in PSII reaction centers
5. qP –photochemical quenching
6. qN – non-photochemical quenching
7. F'o – minimal fluorescence in the light-adapted leaves
8. F'm – maximal fluorescence in the light-adapted leaves.

The obtained results were statistically evaluated using the two factorial analysis of variance. The significance of differences between means was evaluated by Duncan's multiple range test at P=0.05.

Results and discussion

Statistical analysis of the obtained results showed significant differences between values of chlorophyll fluorescence (CF) parameters measured during the experiment in the strawberry leaves and gave the following estimations:

Table 1. Mean values of Fo

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.1233 a•	0.2031 c	0.1632 b
'Honeoye'	0.1189 a	0.1533 b	0.1361 a

• - means followed by the same letter are not significantly different within and between columns at P=0.05

Table 2. Mean values of Fm

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.5835 a	0.7019 b	0.6427 b
'Honeoye'	0.5839 a	0.6041 a	0.5940 a

Table 3. Mean values of Fv/Fm

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.7532 d	0.7141 c	0.7336 b
'Honeoye'	0.6539 a	0.6784 b	0.6662 a

Table 4. Mean values of Y

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.6851 c	0.6542 b	0.6697 b
'Honeoye'	0.6013 a	0.5916 a	0.5965 a

Table 5. Mean values of qP

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.7055 c	0.5823 b	0.6439 b
'Honeoye'	0.5418 a	0.6896 c	0.6157 a

Table 6. Mean values of qN

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.4522 c	0.2019 a	0.3270 b
'Honeoye'	0.1937 a	0.3361 b	0.2649 a

Table 7. Mean values of F'o

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.0639 a	0.0993 c	0.0816 a
'Honeoye'	0.0645 a	0.0905 b	0.0775 a

Table 8. Mean values of F'm

Cultivar	Date of evaluation		Total mean
	30.09.2009	1.06.2010	
'Teresa'	0.2251 a	0.3139 c	0.2695 a
'Honeoye'	0.2707 b	0.3093 c	0.2900 b

In this study, mean values of CF parameters, excluding F'o and F'm were significantly higher in cv.'Teresa' when compared with cv.'Honeoye' (Tab.1, 2, 3, 4, 5, 6). In the case of F'o, measured in cv.'Teresa', its mean value was insignificantly higher than that observed in cv.'Honeoye' (Tab.7). Values of F'o measured in the spring 2010 were significantly higher in both cultivars when compared with values observed in the autumn 2009 (Tab.7).
The similar differences in the values of F'm measured in the autumn and spring were observed in both cultivars, where significantly higher values had been notified in the spring measurement (Tab.8). In general, the mean value of this parameter in cv.'Teresa' was significantly lower in comparison with cv.'Honeoye'.
Besides, the spring values of Fo and Fm were significantly higher in both cultivars in comparison with the autumnal measurements. The autumnal values of Fv/Fm, Y, qP and qN were significantly higher in cv.'Teresa' when compared to the spring values, but in cv.'Honeoye' was observed the different tendency (Tab.3,4,5,6). Only in the case of Y (Tab.4) its value was insignificantly lower in the spring in this cultivar.
CF parameters in the strawberry were evaluated by many scientists. In the study conducted by Borkowska (2001) the ratio Fv/Fm values were similar (on average over 0.8) in leaves of 'Senga Sengana' plantlets rooted by both methods-*in vitro* and *ex vitro* which indicated a well-developed and functioning photosynthetic apparatus. In the present study the ratio Fv/Fm reached comparatively average values between 0.65 and 0.75. Borkowska (2006) used the chlorophyll A fluorescence method as a physiological marker of plant response to light stress and endo-mycorrhiza in the strawberry plants cv. 'Elsanta ' originated by micropropagation. The photosynthetic status of *in vitro* strawberry shoots was assessed by Zenkteler and Borkowska (2002). In this study values of Fv/Fm were higher in leaves from sucrose containing medium than in leaves from glucose medium. Murkowski et al. (1998) used CF for quality evaluation of *in vitro* regenerated strawberry plants. Strawberry plants cv. 'Ananasowa' showed significantly higher values of luminescence parameters indicating better condition of plants of this variety in comparison with the variety 'Senga Sengana'. After temperature lowering these values were more reduced than for plants of cv.'Senga Sengana' which was interpreted as higher susceptibility of this variety to chilling. In the studies conducted by Hdider and Desjardins (1994) the apparent quantum yield and the ratio of variable fluorescence to maximum fluorescence were reduced in strawberry plantlets cultured with 3 or 5 % sucrose as compared to those with 0 or 1%. Razavi et al. (2008) used the chlorophyll fluorescence as a tool for evaluation of drought stress in strawberry. Also Wu et al. (2010) examined the effects of drought stress and rehydration on chlorophyll fluorescence characteristics in strawberry cv. Toyonoka. Khanizadeh et al. (1999, 2000, 2002) also used the chlorophyll fluorescence as a technique to screen for tolerance of strawberry flowers to spring frost. Archbold et al. (2002) identified heat tolerant *Fragaria* accessions using CF. Kadir et al. (2006) measured Fv and Fv/Fm parameters as the indicies of thermotolerance of photosynthesis in 'Chandler' and 'Sweet Charlie' exposed to three temperature regimes. Xu et al. (2005) observed that reduction rate of Fm, Fv/Fm decreased in strong light and increased during

subsequent dark recovery. Maximum quantum efficiency of PSII Fv/Fm was measured by Valkama et al. (2003) to assess the combined effects of enhanced UV-B radiation and selenium in strawberry treated in the field. Xu et al. (2006, 2007) measured the Fo, Fm, Fv/Fm parameters to assess the effects of elevated CO_2 on photoinhibition of strawberry leaves and photosynthetic acclimation to elevated CO_2 under different nitrogen level. Keutgen et al. (1997) examined the responses of strawberry leaf chlorophyll fluorescence to elevated CO_2 , where an elevated CO_2 caused a distinct decrease of optimal quantum yield and an increase of non-photochemical energy dissipation in old leaves.

In the present study, plants of cv.'Teresa' showed, in general, the better functioning photosynthetic apparatus in comparison with cv.'Honeoye', because in the first cultivar the values of CF parameters were significantly higher when compared to the values observed in cv.'Honeoye'. Besides, significant differences in the autumn and spring values of CF parameters in both cultivars were likely affected by various environmental conditions (temperature and/or light) characterizing these seasons of year and by their impact on the functioning of the photosynthetic apparatus.

In the conclusion, it can be stated, that considerably differentiated phenotypic values of CF parameters observed in the analyzed plant material probably resulted from the specific response of photosynthetic apparatus, dependent on genotype to environmental conditions.

No doubt, chlorophyll fluorescence analysis has become one of the most powerful and widely used techniques available to plant physiologists and ecophysiologists. No investigation into the photosynthetic performance of plants under field conditions seems complete without some fluorescence data. In spite of the simplicity of the measurements, however, the underlying theory and the interpretation of data still remains complex and in places, controversial.

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