

Chlorophyll fluorescence characteristics of different winter wheat varieties (*Triticum aestivum* L.)

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Abstract. The objective of the study was to estimate the fluorescence level of newly developed winter wheat varieties. It was detected that the minimum (actual) fluorescence yield (F_i) and the maximum fluorescence yield (F_m), measured with every saturation pulse, as well as the quantum yield (Y) and rate of electron transport (ETR) in the illuminated leaves of the winter wheat varieties *Ada* and *Alma* were higher than in the control variety *Širvinta*. The fluorescence parameters of the variety *Seda* were weaker. Nevertheless, the grain yield of this variety was higher compared with the other varieties. The variety *Seda* is late ripening and more resistant to leaf diseases. Therefore, at the time of the experiment, the total area available for photosynthesis of the variety *Seda* was higher compared with the other varieties. The maximum electron transport rate in rapid light curve in the leaves of the wheat varieties *Ada* and *Alma* was measured to be $850 \mu\text{mol m}^{-2}\text{s}^{-1}$ and for the variety *Seda* $800 \mu\text{mol m}^{-2}\text{s}^{-1}$ of photosynthetically active radiation. In order to select plant breeding material using the chlorophyll fluorescence method more precisely, it is necessary to group the varieties or breeding lines according to the dates of maturity.

Key words: winter wheat, varieties, fluorescence characteristics

INTRODUCTION

Grain yield of winter wheat is one of the desirable traits for intensive cultivation as well as for ecological farming. Winter wheat productivity is limited by a host of factors, the most important of which is the ability of the plants to absorb photosynthetically active radiation (PAR). The factors depend on plant structure and genetic characteristics of the winter wheat variety. The upper limit of dry matter (DM) production for wheat crops ranges between 3 to 4 g DM MJ⁻¹ PAR. Further efforts to improve wheat yield need to be focused on extending the duration of efficient photosynthesis and improving the conversion to grain (Loomis & Amthor, 1996).

Photosynthesis may be considered the most fundamental biological process. Photons are absorbed by molecules of antenna pigments, the excitation energy is transferred to reaction centres of the photosystems. The energy drives primary photochemical reactions that initiate the photosynthetic energy conversion in photochemical and biochemical pathway. Other competitive pathways represent the thermal dissipation and the chlorophyll fluorescence. Fluorescence is the energy predominantly emitted (3–5% absorbed energy) from chlorophyll complexes of PSII (Govindjee, 1995).

The quantitative relationship between chlorophyll fluorescence and the efficiency of photosynthetic energy conversion opens new ways of application in plant breeding, developing of new varieties, evaluation of plant productivity (Schreiber & Bilger, 1995; Schreiber, 1997; Koaček & Bortak, 1999; Baker et al., 2001). There is a fundamental relationship between the quantum yield of fluorescence and photochemical energy conversion.

Experiments with a host of plants and different photosynthetic metabolism processes, which can be induced by varieties of plants and many biotic and abiotic factors, can directly or indirectly produce modification to fluorescence induction kinetics (Percival & Baker, 1991; Crudace, 2000).

The essence saturation pulse method of the fluorescence lies in the application of a very strong pulse of light, by which photochemical conversion is saturated in the photosynthetic system (PS II). In this way the quantum yield of fluorescence and non-radiative energy dissipation are transiently increased to maximum values. Quantum yield (Y) represents fluorescence yield at a given moment and maximum fluorescence, which can be induced by a saturation light at the same moment.

Measuring of chlorophyll fluorescence provides information on qualitative and quantitative changes in photosynthesis. There is also a possibility to analyse intact live leaves.

Fluorometer working on the principle of pulse amplitude modulation (PAM) is used in many fields of plant investigation. The saturation pulse method assumes photochemical and non-photochemical pathways of photosynthesis. The method of pulse amplitude modulation was chosen for measuring some chlorophyll fluorescence parameters for the assessment of individual winter wheat varieties.

Chlorophyll fluorescence can provide useful information about leaf photosynthetic performance, nevertheless, thousands of papers written on chlorophyll fluorescence of agricultural and horticultural plants showed a complicated situation for the estimation of relations between the productivity and fluorescence of wheat. It is not the intention of this investigation to provide a review of chlorophyll fluorescence and applications. Our aim was the adaptation of same parameters of light-adapted leaves of winter wheat for the assessment of varieties.

MATERIALS AND METHODS

Field experiments were set up in the replicated trials at the Lithuanian Institute of Agriculture during 2002–2003. The plots were 16.5 m², soil type loam, clay content 24–27%, pH 6.8; the percentage of organic matter 2.5–2.7; P₂O₅ 190–240; K₂O 185–264 mg kg⁻¹ soil. N₉₀P₆₀K₆₀ was applied annually. The tests involved the new varieties *Ada*, *Alma* and *Seda* developed at the Lithuanian Institute of Agriculture. *Alma* is a very early ripening variety, characterised by high grain protein content and moderate yield, *Ada* is a high yielding, early ripening variety with good bread-making qualities, *Seda* is a high yielding, late ripening variety suitable for confectionery or alcohol production. *Širvinta* was used as a control variety. The stem of this variety is tall, and its grain yield is moderate.

The chlorophyll fluorescence was measured by a chlorophyll fluorometer (chlorophyll fluorometer PAM-210, Walz GmbH, 1997). The minimum (actual) fluorescence yield of the illuminated sample (F₀) and the maximum fluorescence yield

(F_m) of the illuminated light-adapted sample were measured with every saturation pulse. F_t corresponds to the momentary value of fluorescence yield at a given measuring light intensity ($320 \mu\text{mol m}^{-2}\text{s}^{-1}$). F_m is defined as the maximum fluorescence yield of an illuminated sample induced by a saturation pulse. Saturation pulse reached $3,500 \mu\text{mol}$ quanta per square metre per second ($\mu\text{mol m}^{-2}\text{s}^{-1}$), being higher than the outdoor light intensity. The maximum light-acclimated photochemical quantum yield of PSII (Y) was estimated according to the relationship $Y = (F_m - F_t) : F_m = \Delta F : F_m$ and the electron transport rate $\text{ETR} = c \times 0.5 \times \text{PAR} \times Y$ (Schreiber, 1997). Using this equation, it is assumed that 84% of the incident quanta are absorbed and 50% of the absorbed quanta are distributed to PS II. Rapid light curve runs (RLCR) standardised automatic recording of a light response curve which involves 12 Y -measurements at 20 sec intervals (total run-time of 220 sec). Intensity of light runs from 10 to $1250 \mu\text{mol m}^{-2}\text{s}^{-1}$ PAR energy.

All measurements were carried out at the heading stage of winter wheat (late ripening varieties at the stage DC 58, early ripening ones at DC 59); light measurements were used for each variety of wheat in 8 replications. The results received from the experiments were processed by using Microsoft Excel 97 programme as well as the programmes Microsoft Graph Chart and ANOVA (Tarakanovas, 1999).

Field measurements were performed in a cloudy period or at low daylight. Daylight was measured by a quantum-photo-radiometer HD 9021. The daylight intensity of photosynthetic active radiation was usually lower than the maximum saturation pulse of PAM-210 ($3,500 \mu\text{mol m}^{-2}\text{s}^{-1}$). For measuring rapid light curve runs, the wheat leaves were placed in an opaque leaf holder.

RESULTS AND DISCUSSION

Selection of winter wheat lines at earlier generations is one of the possibilities to improve the effectiveness of plant breeding. To achieve this, it is necessary to develop indirect assessment methods. On the other hand, it is important to know the physiological background of the newly developed varieties and the correlation between physiological and agronomic characteristics of winter wheat plants.

Light energy drives primary photochemical reactions that initiate the photosynthetic energy conversion. Photochemical pathway involving a charge separation and electron transport is, nevertheless, not the only way in which the excitation energy is consumed. Other two competitive pathways represent the thermal dissipation and chlorophyll fluorescence. Consequently, the objective of our experiment was to measure chlorophyll effect in different varieties using the fluorescence method for comparison.

Chlorophyll fluorescence is measured as a reflection of electron transport in the photosynthetic systems of plants.

Fluorometric parameters of different winter wheat varieties were identified (Figs 1–4). The maximum (F_m) and minimum (F_t) fluorescence, the quantum yield and rate of electron transport (ETR) in the photosynthetic system (PS II) of leaves of the varieties *Ada* and *Alma* were higher than those of the variety *Širvinta*. This relationship showed a negative correlation between the plant height and fluorescence parameters (Tables 1, 2). The strongest negative relationship was established in the variety *Alma*,

whose plants were shorter, but the fluorescence parameters were similar to or higher than those of the other varieties. An exception is the variety *Seda*, whose fluorescence parameters were less intensive.

Despite its weaker fluorescence parameters, the grain yield of the variety *Seda* was higher compared with the other wheat varieties investigated (Tables 1, 2). *Seda* is a late ripening variety and more resistant to foliar diseases. During the experimental period, the plants of the variety *Seda* had 5 full functional erect leaves. This suggests that weaker fluorescence was compensated by a larger area available for photosynthesis.

Semi-erect leaves and high tillering capacity combined with a good fluorescence ability resulted in a satisfactory grain yield of the variety *Ada*. The variety *Alma* is very early ripening, therefore, its leaf senescence occurs more rapidly. The high rate of electron transport resulted in a relatively high grain yield and protein content.

Table 1. Grain yield and morphometric parameters of winter wheat.

Varieties	Grain yield		Plant height, cm	Number of productive tillers, m ²	Number of producing leaves	Characteristics of leaves
	kg / 16.5 m ²	%				
<i>Širvinta</i>	9.365	100.0	115	458	3	Prostrate
<i>Ada</i>	11.483	122.6	95	543	4	Semi-erect
<i>Alma</i>	9.6375	102.9	89	427	3	Semi-erect
<i>Seda</i>	12.555	134.1	93	491	5	Erect
LSD ₀₅	1.39		11	61		
LSD ₀₁	1.59					

Table 2. Fluorometric parameters of winter wheat leaves.

Variety	Fluorescence parameters			
	F _t	F _m	Y	ETR
<i>Širvinta</i>	0.249	0.407	0.383	49.9
<i>Ada</i>	0.299	0.495	0.395	51.5
<i>Alma</i>	0.269	0.480	0.439	57.1
<i>Seda</i>	0.259	0.380	0.317	41.3
LSD ₀₅				0.129

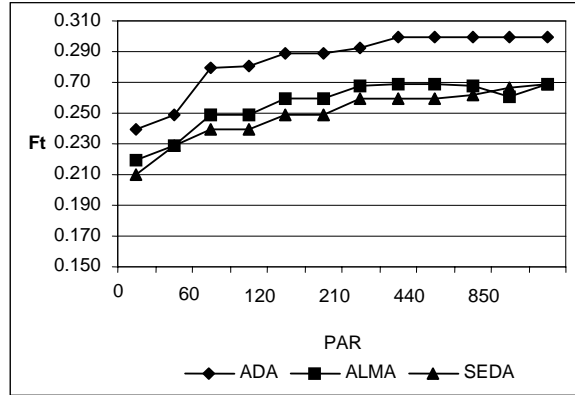


Fig. 1. Minimum fluorescence (F_t) in leaves of winter wheat varieties in variable PAR flux 10–1250 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

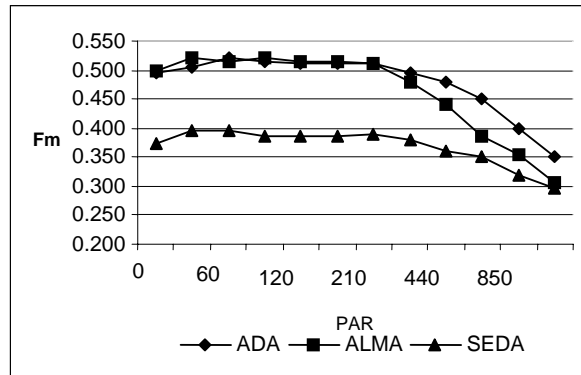


Fig. 2. Maximum fluorescence (F_m) in leaves of winter wheat varieties in variable PAR flux 10–1250 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

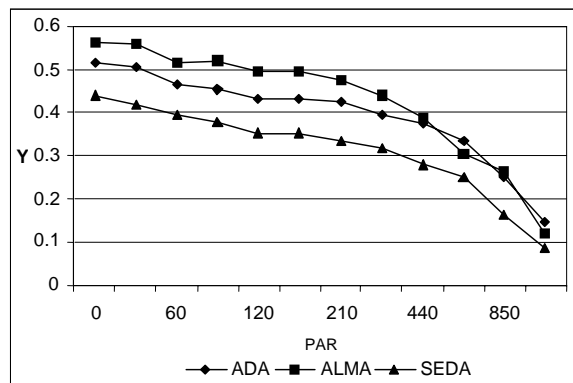


Fig. 3. Quantum yield of electron transport (Y) in leaves of winter wheat varieties in variable PAR flux 10–1250 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

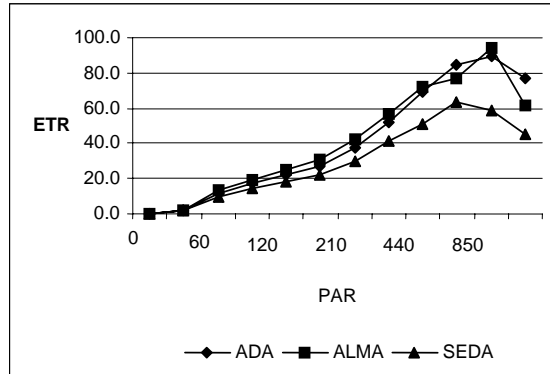


Fig. 4. Electron transport rate (ETR) in leaves of winter wheat varieties in variable PAR flux 10–1250 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

A similar relationship between grain yield and plant height was established at fluorescence parameters' rapid light curve run recording a light response at 20 sec intervals. The peak of electron transport ratio (ETR) was identified in the leaves of the wheat varieties *Ada* and *Alma* at 850 $\mu\text{mol m}^{-2}\text{s}^{-1}$ energy of PAR light. The saturation light of electron transport rate for the variety *Seda* was established at 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ level of PAR. The other fluorescence parameters (F_t , F_m , ETR) in leaves of the variety *Seda* in variable PAR flux ranging from 10 to 1250 $\mu\text{mol m}^{-2}\text{s}^{-1}$ were less intensive.

CONCLUSIONS

The maximum (F_m) and minimum (F_t) fluorescence, the quantum yield (Y) and rate of electron transport (ETR) in the photosynthetic system of the winter wheat varieties *Ada* and *Alma* were higher than those for the control variety *Širvinta*.

The chlorophyll fluorescence parameters of the variety *Seda* were weaker compared with the control variety *Širvinta*. However, grain yield of this variety was higher compared with that of the other varieties due to the larger total leaf area available for photosynthesis. The variety *Seda* is late ripening and more resistant to leaf diseases. Therefore, at the time of the experiment, the total area available for photosynthesis of the variety *Seda* was higher compared with the other varieties.

The peak of electron transport rate in active radiation flux in the leaves of the wheat varieties *Ada* and *Alma* was measured 850 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and for the variety *Seda* 800 $\mu\text{mol m}^{-2}\text{s}^{-1}$.

In order to select plant breeding material using the chlorophyll fluorescence method more precisely, it is necessary to group the varieties or breeding lines according to the dates of maturity.

Fluorescence parameters of the photosynthetic system II correlated with plant characteristics and crop productivity.

REFERENCES

- Baker, N.R., Oxborough, K., Lawson, T., & Morison J.I.L. 2001. High resolution imaging of photosynthetic activities of tissues, cells and chloroplasts in leaves. *J. of Exp. Botany* **52**, 615–621.
- Bartak, M., Nijs, I. & Impens I. 1998. The susceptibility of PS II of *Lolium perenne* to a sudden fall in air temperature – response of plants grown in elevated CO₂ and/or increased air temperature. *Environ.exp.Bot.* **39**, 85–95.
- Buffoni, M., Testi, M.G., Pesaresi, P., Garlaschi, F.M. & Jennings, R.C. 1998. A study of the relation between CP29 phosphorylation, zeaxanthin content and fluorescence quenching parameters in *Zea mays* leaves. *Physiol.Plant* **102**, 318–324.
- Burbulis, N., Jakienė, E. & Šlapakauskas, V. 2001. Physiological examination of synthetic plant growth regulators-stilts. *Horticulture and vegetable growing*. Babtai, **20**(4)-2, 48–54. (in Lithuanian).
- Crudacea, A.J. 2000. The investigation of the *in vivo* behavior of maize herbicide-Isoxaflutole. PhD thesis. University of Essex, Colchester, UK.
- Govindjee, R. 1995. Sixty-three years since Kautsky: Chlorophyll a fluorescence. *Aust. J. Plant Physiol.* **22**, 131–160.
- Kornyejev, D.J. 2002. *Informatic possibilities of induction fluorescence of chlorophyll*. Alterpress, Kyiv (in Russian).
- Krause, G.H. & Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: the basics.- *Annu.Rev. Plant Physiol. Plant mol.Biol.* **42**, 313–349.
- Loomis, R.S. & Amthor, J.S. 1996. Limits to yield revisited. *Increasing yield potential in wheat: breaking the barriers*. Mexico, D.F.: CIMMYT, 76–89.
- Percival, M.P. & Baker, N.R. 1991. Herbicides and photosynthesis. In Baker, N.R. & Percival, M.P.(eds): *Herbicides*. Elsevier, Amsterdam, 1–26.
- Rascher, U., Liebig, M. & Lüttge, U. 2000. Evaluation of instant light-response curves of chlorophyll fluorescence parameters obtained with a portable chlorophyll fluorometer on site in the field. Technical report. *Plant, Cell and Environment* **23**, 1397–1405.
- Rohaček, K. & Bartak, M. 1999. Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. *Photosynthetica* **39**(3), 339–363.
- Schreiber, U., Bilger, W. & Neubauer, C. 1995. Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of *in vivo* photosynthesis. In Schulze, E.D. & Caldwell, M.M. (eds): *Ecophysiology of Photosynthesis*. Springer-Verlag, Berlin, Heidelberg, 49–70.
- Šlapakauskas, V. & Varkulevičienė, J. 2004. Fluorescentic evaluation of growth and ornamentality of primrose (*Primula Malacoides* Franch.). *Horticulture and vegetable growing*. Babtai, **23**(2), 241–247 (in Lithuanian).
- Tarakanovas, P. 1999. Package for statistical data processing “Selekcija”. Dotnuva (in Lithuanian).
- Van Kooten, O. & Snel, I.F.H. 1990. The use of chlorophyll fluorescence nomenclature in plant stress physiology. *Photosynth. Res.* **25**(3), 147–150.