Fluorescence techniques as suitable methods to discriminate wheat genotypes under drought and high temperature condition

Maria Balota[a], Malgorzata Sowinska[b], Claus Buschmann[c], Hartmut K. Lichtenthaler[c], Francine Heisel[b] and Fardbardha Babani[d]

[a]Cereals and Industrial Crops Institute, 8264 Fundulea, Romania

[b]Groupe d'Optique Appliquée/CNRS, F-67037 Strasbourg Cedex 2, France

[c]Botanisches Institut II, Universität Karlsruhe, 76128 Karlsruhe, Germany

[d]Institute of Biological Research, Academy of Sciences, Tirana, Albania

ABSTRACT

The chlorophyll fluorescence parameters Fv/Fo and Fd/Fs (=Rfd690), related to the quantum conversion capacity at dark-adapted and light-adapted state of the photosynthetic apparatus respectively, have been evaluated as possible indicators of drought and heat tolerance in winter wheat. The measurements were carried out on primary leaves of 8-day old seedlings.
Rfd values decreased in 8 days by 20% ($p \leq 0.01$) only under severe water limitation and for the drought susceptible genotype. The photosynthetic apparatus was more sensitive to high temperature with both ratios, Fv/Fo and Rfd690, showing mean decrease ($p \leq 0.001$) of 27% and 43%, respectively, in 5 days at 35°C. The susceptible cultivars decreased of up to 42% and 65% and the drought and heat tolerant genotypes only 7% and 12% for Fv/Fo and Rfd690, respectively.
The Fv/Fo ratio correlated well ($p \leq 0.05$ and $p \leq 0.01$) with seedling responses to oxidative and osmotic stresses. The Rfd690-values correlated better with all physiological parameters considered and with the deviations from linear regression of drought susceptibility index DSI ($r = -0.84$, $p \leq 0.01$) on yield potential showing the highest potential to predict drought and heat tolerance.
In addition the blue, green, red and far-red fluorescence have been determined using a laser-induced-fluorescence imaging system in entire seedlings of wheat and triticale grown under optimal laboratory conditions. The ratios F690/F740 and F440/F520 correlated well ($p \leq 0.05$) with the total chlorophyll content (detected by the SPAD-chlorophyll-meter) and the specific leaf dry weight (SLDW) showing the potential of the both fluorescence ratios to discriminate genetic differences between cultivars for these leaf structural sources of water use efficiency (WUE) improvement.

Key-words: wheat, drought and high temperature stress, chlorophyll fluorescence, LIF imaging system.

Acronyms: stress adaptation index (Ap), chlorophyll (Chl), drought susceptibility index (DSI); Chl fluorescence decrease from Fm to Fs (Fd); maximum Chl fluorescence (Fm); steady state Chl fluorescence (Fs); variable Chl fluorescence (Fv); ground Chl fluorescence (Fo); ratio of the chlorophyll fluorescence at 690 and 735 nm (F690/F735); PS II photochemical efficiency in the light-adapted state ($\Delta F/Fm'$); PS II photochemical efficiency in the dark-adapted state (Fv/Fm); laser-induced fluorescence (LIF); laser induced two-wavelengths chlorophyll fluorometer (LITWAF); fluorescence emission band in the blue region between 430 and 450nm (F 440); green fluorescence band between 520 and 530 nm (F520); red chlorophyll fluorescence maximum near 690 nm (F690); far-red chlorophyll fluorescence emission maximum near 740 nm (F740); fluorescence ratio blue/red (F440/F690); fluorescence ratio blue/far-red (F440/F740); Chl fluorescence ratio red/far-red (F690/F740); fluorescence ratio blue/green (F440/F520); variable Chl fluorescence ratios measured at 690 and 735 nm, respectively (Rfd690, Rfd735); specific leaf dry weight (SLDW); estimation of total chlorophyll content on a leaf area basis using the SPAD-chlorophyll meter (SPAD); water use efficiency (WUE).

M.B. (correspondence) Email: abalota@pcnet.pcnet.ro ; Phone: 40 (1) 311 0722; Fax: 40 (1) 311 0722
M.S. ,F.H. Email: sowinska@goa.c-strasbourg.fr ; Phone: 33 (0)3 88 10 65 59; Fax: 33 (0)3 88 10 65 48
H.K.L., C.B. Email: Hartmut.Lichtenthaler@Bio-Geo.Uni-Karlsruhe.de ; Phone: 49 721 608 3833; Fax: 49 721 608 4874
F.B. Email: babani@altavista.net ; Phone: 355 42 226 38; Fax: 355 42 226 38

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1. INTRODUCTION

Bread wheat is cultivated on 2.5 million ha in Romania, from which about 1.5 million ha are subjected to drought and heat. Recent data based on 50 field trials have shown that in Romania over 2/3 of the yield variation was due to weather fluctuations from one year to another and that total rainfall during wheat growth explained more than 40% of the yield variation [1]. Different types of drought have been identified in the South Plain of Romania in the wheat fields [2] and most of the drought time came together with heat (as in 1998 when the mean temperature of July exceeded by 3.1°C the multi annual mean of this month). For this reason it is well accepted that more effort should be directed towards advancing basic knowledge, discovering new genetic diversity and finding efficient screening methods in breeding for resistance to drought and heat [1]. Among physiological methods proposed to discriminate genotypes for their response to water and high temperature stresses various types of fluorescence signatures have been considered [3, 4]. Chlorophyll fluorescence has been extensively used to investigate the photosynthetic light processes and quantum conversion [5, 6]. Different types of chlorophyll signatures have been proposed as indicators to detect damage of the photosynthetic apparatus including various ratios of the variable chlorophyll fluorescence (Fv/Fm, Fv/Fo, Fd/Fs) determined from the Kautsky effect of pre-darkened leaves as well as quenching coefficients [3-7] and the ratio red/far-red (F690/F740) which is an indicator of the in vivo chlorophyll content [7-9]. Blue-green fluorescence of the green leaves has been also found to change consistently under stress events and mineral deficiencies [10-12]. Recently, a high resolution, ultraviolet (UV-A) laser-induced fluorescence (LIF) imaging system was developed, which images all four fluorescence bands: blue, green, red and far-red [10, 12-14]. LIF imaging system detects efficiently very small fluorescence gradients, so permitting early stress detection. Besides, all these techniques are non-invasive methods and less time consuming than other conventional techniques.

Here we have evaluated the potential of different fluorescence systems and parameters to detect wheat and triticale winter genotype differences in photosynthetic capacity under water shortage and high temperature stresses. We have also used the new LIF imaging system to evaluate some leaf structural indicators of photosynthetic capacity of the cultivars, highly contributing to the increase of water use efficiency (WUE).

2. MATERIALS AND METHODS

2.1. Wheat seedlings

Eight-day old seedlings were grown in plastic pots on buffered mineral peat (TKS II) at 18 to 25 °C, a photo period of 14 h per day with mean irradiance of 100 μmol m^{-2} s^{-1}. Three water levels were imposed in drought experiment: control, well watered (80 ml of water per pot, daily), moderate drought (30 ml of water per pot, daily), and severe drought (30 ml of water per pot till emergence and no supplement of water for the next 8 days). Two winter wheat genotypes with differences in their field reaction have been compared.

A second set of winter wheat and triticale have been used in the high temperature experiment where 8-day old seedlings were exposed permanently to 35 °C in a growth chamber for 0 h, 4 h, 24 h, and 5 days.

The same set of genotypes have been used for the measurements of the blue, green, red and far-red fluorescence using the LIF imaging system in entire seedlings under optimal growth conditions.

2.2. Genotypes

Dropia, Flamura 85 and Fundulea 4 are recent released Romanian cultivars recommended for the South-East part of the country. They have shown a similar reaction to environment being at present the most drought or heat resistant bread wheat cultivars in the country [1, 15]. Fundulea 29, Lovrin 34, Lovrin 41 and Alex cultivars, and H 70022, an advanced line are known to be less stable in its response to environment and more drought sensitive [1]. Bezostaya is an old Russian cultivar of winter wheat grown in Romania in the 70's on over 80% of the wheat area due to its high and stabile grain yield at that time. Atlet (resistant) and Atol (sensitive) are two triticale (X *Triticosecale* Witt) new advanced lines having opposite response to drought and heat in the field.

2.3. Measurements

The Chl fluorescence induction kinetics, the parameters of the variable chlorophyll fluorescence, and the net photosynthesis have been measured at the Botanisches Institut II, Universität Karlsruhe. The blue, green, red and far-red fluorescence of entire seedlings have been determined at the Groupe d'Optique Appliquée/CNRS, Strasbourg.

SLDW, SPAD and other physiological tests on seedlings and the deviations from linear regression of drought susceptibility index (DSI) on yield under irrigation were measured at Fundulea, Romania.

2.3.1. Chlorophyll fluorescence induction kinetics

The Chl fluorescence induction kinetics (Kautsky effect) of pre-darkened leaves (15 min) were measured simultaneously at 690 nm and 735 nm using the two-wavelength fluorometer LITWAF with a He/Ne laser excitation beam (Spectra Physics 5 mW; λ= 632.8 nm, light intensity ca. 500 μmol m^{-2} s^{-1} at the sample level). Measurements were carried out with leaf segments of primary leaves from the adaxial leaf side 20 mm below the leaf tip. The chlorophyll fluorescence ratios F690/F735 at maximum (Fm) and at steady-state conditions (Fs) of fluorescence were calculated from the fluorescence induction kinetics at 690 nm and 735 nm, respectively. The Rfd-values as vitality index, i.e. the ratio of fluorescence decrease Fd (= Fm - Fs) to the steady-state fluorescence Fs (Rfd=Fd/Fs) were determined at 690 nm and 735 nm (Rfd690 and Rfd735) (see Lichtenthaler and Rinderle, 1988 [6]). Stress adaptation index Ap=1-[(1+Rfd735)/(1+Rfd690)] was calculated according to Strasser et al. [16].

2.3.2. PAM fluorometer

The parameters of the variable chlorophyll fluorescence: ground fluorescence Fo, variable fluorescence Fv (=Fm-Fo), maximum fluorescence Fm (in the dark), ΔF(=Fm'-Fs) and Fm' (in the light) were measured using the pulse amplitude modulation chlorophyll fluorometer PAM (Walz, Effeltrich, Germany) as already described [6, 17-21]. Due to the wavelengths of the excitation light and the filter combinations, the PAM fluorometer senses the chlorophyll fluorescence only in the 720 nm region [6], which shows a lower amplitude and sensitivity than the 690 nm fluorescence. The chlorophyll fluorescence was induced by the weak modulated measuring red light (1.6 kHz of 0.01 μmol m^{-2} s^{-1}), which did not induce photosynthetic activity. Maximum fluorescence Fm was obtained by a pulse of saturating white light (2500 μmol m^{-2} s^{-1}) of 1 s duration directly after dark adaptation. A second pulse (1 s duration) was applied at a steady-state fluorescence (5 min) to obtain the value of maximal fluorescence Fm' at fully activated photosynthetic conditions. The light intensity of the red actinic light at the sample was 200 μmol m^{-2} s^{-1} PAR and the measuring beam during the illumination with actinic light was modulated with 100 kHz. The value of Fo' was obtained by switching off the actinic light, and giving a pulse (5 s duration) of weak far-red light to obtain the fully oxidized state of PS II. The chlorophyll fluorescence ratios Fv/Fm, Fv/Fo and ΔF/Fm' were determined according to Genty et al. [21] from the PAM kinetics measured on the leaf segments of the primary leaf from the adaxial leaf side 20 mm below the leaf tip.

2.3.3. Photosynthetic CO_2 fixation

The light induced CO_2 fixation (P_N) was measured using a CO_2/H_2O porometer (Walz, D 91090 Effeltrich). The irradiance on the leaves level was of 700 μmol m^{-2} s^{-1} PAR saturating with respect to P_N. The calculation of the photosynthetic rates was carried out according to van Caemmere and Farquhar [22].

2.3.4. Laser induced fluorescence imaging

The experimental set-up for laser-induced fluorescence imaging, already described [10, 12-14] is presented in Fig. 1.
The excitation source was a pulsed laser (Spectra Physics, pulse width of 7 ns, repetition rate 10 kHz) emitting in the UV-A at 355 nm and/or in the green at 532 nm, which enlarged by a beam expander which enables to obtain a 20cm spot diameter for the leaves (plants) excitation. A gated intensified and digitized CCD camera (Photonetics GmbH), via lenses and interferential filters (10 nm bandwidth) allows to record the fluorescence images of the leaves at four characteristic fluorescence bands (440, 520, 690 and 740 nm). Laser synchronisation, recording and storage of images as well as image treatment was carried out by a computer equipped with the appropriate interface cards. All the images were recorded in the presence of ambient light (light adapted steady state of the chlorophyll fluorescence induction kinetic (Kautsky effect)) with an optimized signal to noise ratio and they were corrected for: (i) the non uniformity of the laser excitation, (ii) spectral sensitivity of the camera and (iii) attenuation factor of the interference filters and focusing lens. As the fluorescence intensity for all measured leaves was rather uniform we could calculate the mean fluorescence intensity for each leaf (and for each fluorescence band, blue=F440, green=F520, red=F690 and far-red=F740) by averaging the pixel intensities over the leaf surface. The ratio images were formed by a pixel to pixel division procedure (R(440/520), R(440/690), R(440/740) and R(690/740)) and a fluorescence intensities ratio as a simple ratio of the mean fluorescence intensity values at different wavelength (F440/F520, F440/F690, F440/F740 and F690/F740).

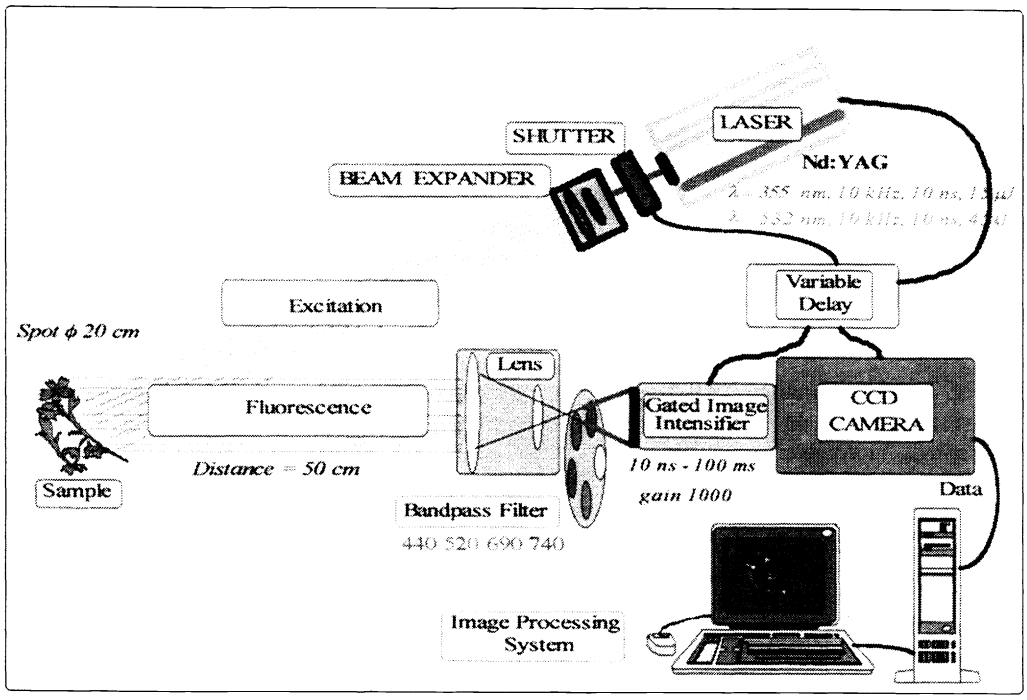


Fig. 1. Fluorescence imaging system.

A statistical analysis by variance analysis and comparison of means by a Newman-Keuls test was then applied in order to determine the significance of the differences for the measured populations.

2.3.5. Chlorophyll content and specific leaf weight

Total chlorophyll content was measured in intact leaves using a portable chlorophyll meter (SPAD-502, Soil-Plant Analysis Development (SPAD) Section, Minolta Camera Co., Osaka, Japan). The leaf blades used in the SPAD measurement were subsequently sampled and used to determine leaf area (L*l*0.7) and dry matter.

3. RESULTS AND DISCUSSION

3.1. Water stress

The effect of water stress on the chlorophyll fluorescence induction kinetics and the net photosynthesis is presented in Fig. 2. The quantum conversion capacity at the light-adapted state of the photosynthetic apparatus is presented as the fluorescence decrease ratios Fd/Fs which is referred to as the Rfd values [6]. Two *T.eastivum* genotypes have been tested: Flamura 85, resistant, and H 70022, sensitive, under field condition. About 40% reduction of soil water content had no influence on Rfd ratios and the CO_2 assimilation (Fig. 2A, B). Daily supplement of water to keep constant soil water level (30 ml of water per pot) allowed an adjustment of plant's biomass, from 38 mg to 15 mg/plant (data not shown) to its water availability without any major effect on the leaf photosynthetic processes. When soil water decreased below 30 ml per pot imposing a more severe drought stress both Rfd ratios, at 690 nm and at 735 nm, and the CO_2 fixation, significantly ($p \leq 0.01$, according to Student's t-test) decreased by 20% and 13%, respectively only in the sensitive genotype (Fig. 2C, D). The observed drought tolerance of PS II is in accordance with evidence on other wheat and herbaceus species [4, 23]. Very similar to our findings, in durum wheat Flagella et al.[4] obtained a significant decrease of the photosynthetic electron transport efficiency only under severe stress conditions and for the drought susceptible cultivar. In addition, we have obtained evidence that the discrimination for the capacity of quantum conversion among genotypes is detectable at very early growth stages, and this is very desirable as screening procedures in breeding programs.

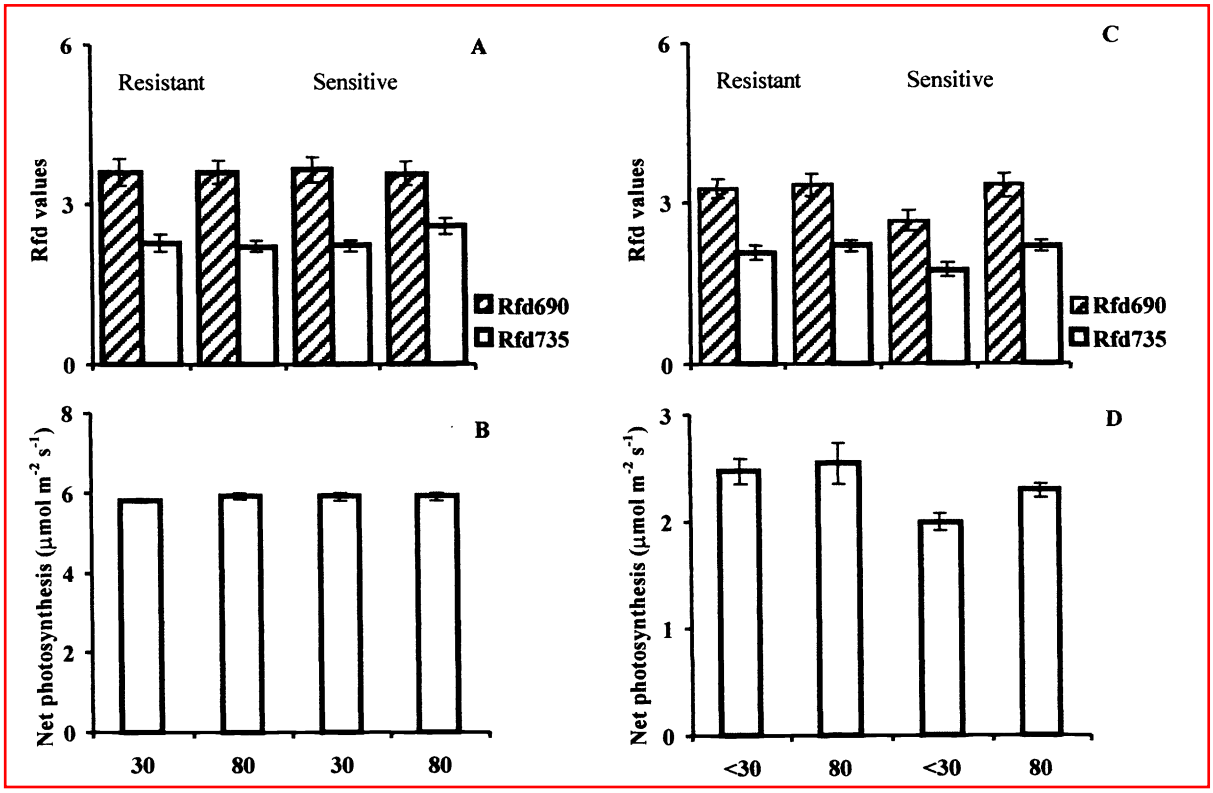


Fig. 2. Effect of moderate (A, B) and severe (C, D) water limitation on the variable fluorescence ratios Rfd 690 and Rfd 735 (A, C) and the photosynthetic net CO_2 fixation rates (B, D) measured in primary leaf of winter wheat. Mean of 4 replications (±SD).

In our experimental conditions, the CO_2 fixation rates showed a similar pattern as the photosynthetic quantum conversion with no significant changes under moderate water stress but significant ($p \leq 0.05$) decrease in the sensitive genotype under a more severe water limitation (Fig. 2B, D). The decrease of the net photosynthesis was partially due to the stomata closure that significantly ($p \leq 0.01$) occurred only under severe stress and in the sensitive genotype (data not shown).
To have a measure of the relationship between the two photosynthetic parameters we have correlated the Rfd at 690 nm and the CO_2 assimilation at the 3 water levels and in the 2 genotypes ($n = 6$). We have found a linear relationship ($y = ax+b$) and a coefficient of determination (R^2) of 0.86 significant at $p \leq 0.01$. This shows a high dependence of the photosynthetic rate on the light reactions of the PS II under drought and not only on the CO_2 limitation due to stomata closure, at least in our water stress conditions. This, also, shows that the Rfd ratio may be considered as good indicator of drought tolerance in winter wheat.

3.2. High temperature stress

The effect of high temperature on photosynthetic apparatus has been investigated on a set of 10 *T aestivum* cultivars and 2 triticale lines. These cultivars were compared with regard to heat tolerance on basis of Fv/Fo and ΔF/Fm' using a PAM fluorometer and the fluorescence decrease ratios, Rfd values, the ratio F690/F735 and Ap, using a LITWAF. An 11% mean decrease ($p \leq 0.01$) in Fv/Fo was observed after 4 h of high temperature exposure but no changes in ΔF/Fm' (Fig. 3). After 24 h at 35 °C similar values were recorded for both ratios, Fv/Fo and ΔF/Fm', in comparison with control plants indicating a trend for adjustment of the photosynthetic function. After 5 days, the high temperature induced a 27% decrease ($p \leq 0.001$) in the ratio Fv/Fo and of 7% in the ΔF/Fm' ratio. The decrease in Fv/Fo was due to a decrease in the Fv, from a mean value of 80 to 68 after 5 days, in all cultivars (Fig. 3), whereas the ground fluorescence Fo was increased significantly ($p \leq 0.001$) from 22 to 29, only in Fundulea 29 and Bezostaya known to be drought and heat susceptible under field condition.

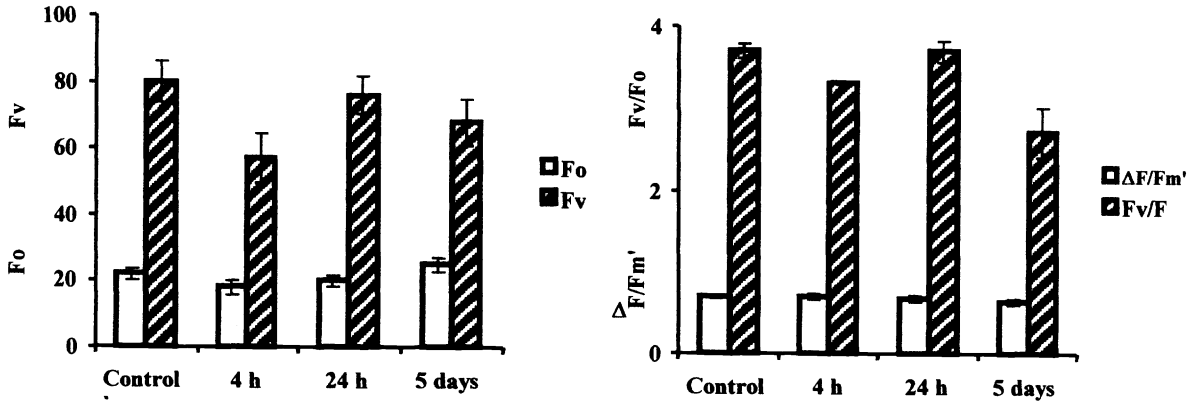


Fig. 3. Effect of high temperature (35 °C) on the variable fluorescence Fv, ground fluorescence Fo, and the variable fluorescence ratios of the dark-adapted (Fv/Fo) and light-adapted (ΔF/Fm') state of the photosynthetic apparatus in primary wheat leaves. Mean of 12 genotypes and 4 replications per genotype (±SD).

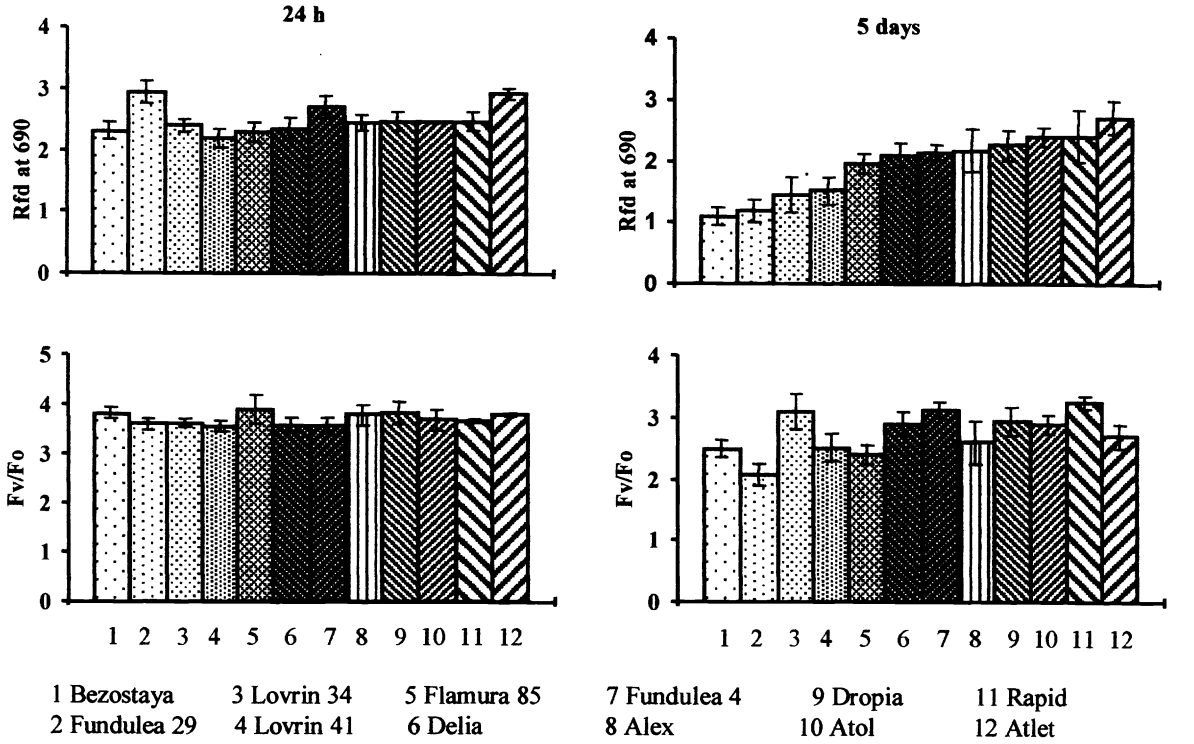


Fig. 4. Genotypic variability for the variable chlorophyll fluorescence ratios of the dark-adapted (Fv/Fo) and light-adapted (Rfd 690) state of the photosynthetic apparatus under 24 h and 5 days of exposure to 35 °C in primary wheat leaves. Mean of 4 replications (±SD).

The genotypic variability after 5 days of stress was much higher for the Fv/Fo ratio (minimum value = 2.06, maximum value 3.25) (Fig. 4) than for ΔF/Fm' (minimum value = 0.59, maximum value = 0.67) (data not shown). In control plants (0 h) and stressed plants after 24 h there were no or very little differences between the 12 cultivars, the Fv/Fo ratio showing a mean value of 3.70 (minimum value = 3.56, maximum value = 3.84) (Fig. 4). The variable fluorescence ratio Fv/Fo have been proposed as a much better indicator of changes in the rates of photosynthetic quantum conversion than the ratio Fv/Fm [7, 24]. In our experiment as well, the mean variation of Fv/Fm from 0.79 in controls to 0.72 after 5 days at 35 °C (data not shown), as the variation of the ΔF/Fm', were much smaller than the variation of the Fv/Fo.

In the fluorescence ratio Fv/Fo the changes of both components, the variable fluorescence Fv and the ground fluorescence Fo, were observed at any time, and thus this ratio responded very sensitively to any changes in Fv and /or Fo. In contrast, the ratio Fv/Fm was slower in response to small changes in Fv or Fo, since Fm is the sum of Fv + Fo. This also applied to the ratio ΔF/Fm', and both, Fv/Fm and ΔF/Fm', are considered as a pair of related fluorescence ratios of the dark-adapted (state 1) and the light-adapted (state 2) state of the photosynthetic apparatus [7, 22]. A similar pair of related variable fluorescence ratios describing the dark-adapted and light-adapted state of photosynthetic apparatus are Fv/Fo and the fluorescence decrease ratio Fd/Fs, which is referred to as Rfd-values. The Fv/Fo and the Rfd ratios are considered a better and fairly closer measure of the potential photosynthetic capacity and the net CO_2 fixation of leaves [6, 7]. Our present results are in full agreement with the above mentioned observation. Not only Fv/Fo showed the highest variation among heat duration and genotypes but also the Rfd ratios which by mean decreased ($p \leq 0.001$) from 3.5 and 2.4 in controls to 2.0 and 1.4 after 5 days at 35 °C (Rfd-values at 690 nm and at 735 nm, respectively) (Fig. 5). The genotypic variability was also high (minimum for Rfd690 = 1.09, maximum = 2.72), the most susceptible genotypes in field showed Rfd-values of 2 or less and the most drought and heat resistant higher than 2 (Fig. 4). In control plants (0 h) and stressed plants after 24 h there were no or very little differences between the 12 cultivars for the Rfd-values (Fig. 4). There were not significant changes in the ratio F690/F735, nor between treatments neither between genotypes, and Ap decreased significantly ($p \leq 0.01$) below 0.15 in the most susceptible cultivars: Fundulea 29, Bezostaya, Delia, Lovrin 34 and Lovrin 41 (data not shown).

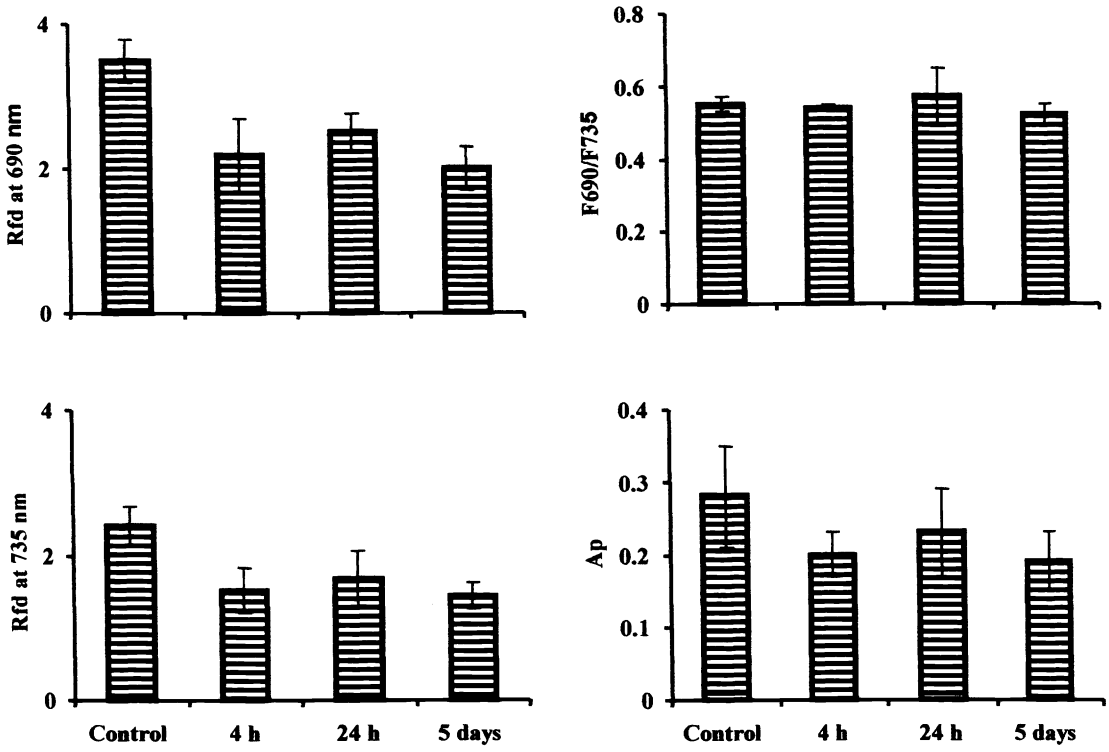


Fig. 5. Effect of high temperature (35 °C) on the chlorophyll fluorescence ratios Rfd690, Rfd735 , as vitality indices, F690/F735, and Ap, as stress adaptation index in primary wheat leaves. Mean of 12 genotypes and 4 replications per genotype (±SD).

In order to assess the predictive value for drought and heat tolerance of the fluorescence parameters Fv/Fo and Rfd, we correlated their values after 5 days at 35 °C with the deviations from linear regression of drought susceptibility index (DSI) on yield under irrigation and with some other physiological parameters of tolerance (Table 1). The physiological parameters considered were: tolerance to oxidative-induced stress (water loss), plasma membrane stability under heat shock and osmotic-induced stress (relative injury), and biomass accumulation in seedlings in water-deficient soil (dry matter). DSI was calculated according to Fisher & Maurer [25] from data in irrigated and dryland yield trials in five locations from South Romania. The Fv/Fo ratio correlated well ($p \leq 0.05$ and $p \leq 0.01$) with seedling response to oxidative and osmotic stresses. The Rfd690 correlated better ($p \leq 0.05$ and $p \leq 0.01$) with all physiological parameters considered (Table 1) and with the

deviations from linear regression of DSI ($r = -0.84$, $p \leq 0.01$) showing the highest potential to predict drought and heat tolerance. This is in agreement with our previous findings [3].

Table 1. Correlation coefficients between the ratio Fv/Fo and Rfd at 690 nm and other physiological and agronomical traits in 10 winter wheat (*T. aestivum*) cultivars grown in Romania.

	Tolerance to oxidative stress	Thermal membrane stability	Membrane stability to osmotic stress	Growth of seedlings under water stress	Deviations from linear regression on yield under irrigation
Fv/Fo (at 5 days at 35 °C)		- 0.31	- 0.84**		0.36
[Fv/Fo (difference from control)]	[- 0.87**]		[0.75*]	[0.50]	
Rfd690 (at 5 days at 35 °C)	0.91***	- 0.61*	- 0.87**	- 0.61*	- 0.84**

*,**,***, Significant at $p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$, respectively.

3.3. Fluorescence imaging

The same set of 12 genotypes as above has been used for the determination of the blue, green, red, and far-red fluorescence using the LIF imaging system. Entire seedlings grown under optimal conditions in laboratory have been used and measurements were performed on 40 replications for each genotype. Examples of the fluorescence images of primary leaves of two triticale genotypes with opposite response to the drought and heat (Atlet as resistant and Atol as sensitive) are shown in the Fig. 6. The fluorescence intensities in the blue and green region are very similar in both genotypes, while in the red and far-red bands (chlorophyll fluorescence) the sensitive variety show much higher fluorescence intensity than the resistant one.

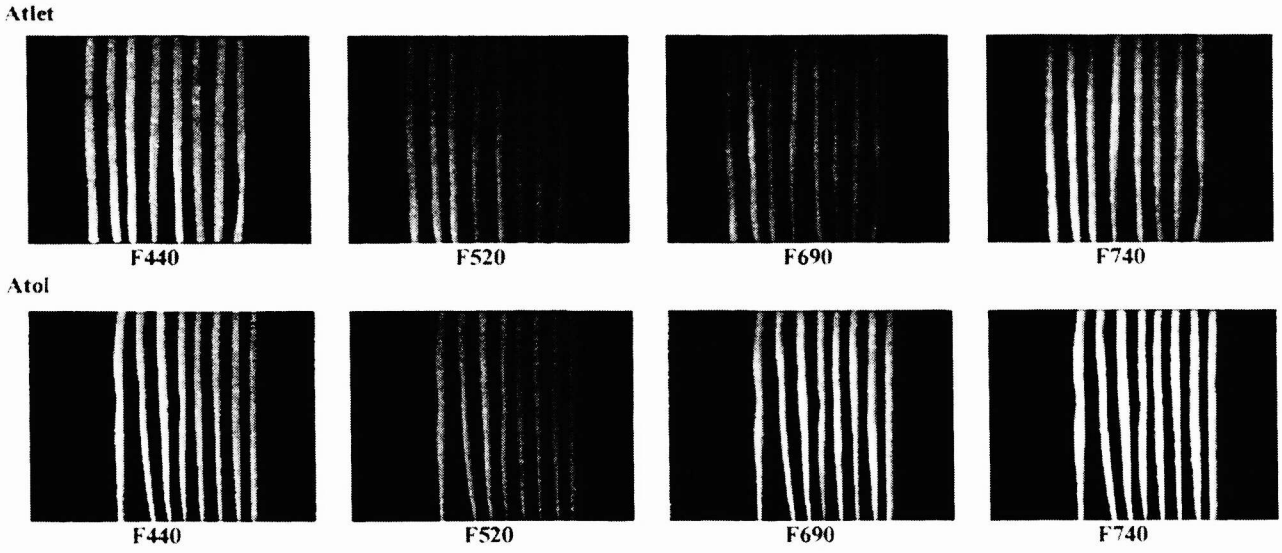
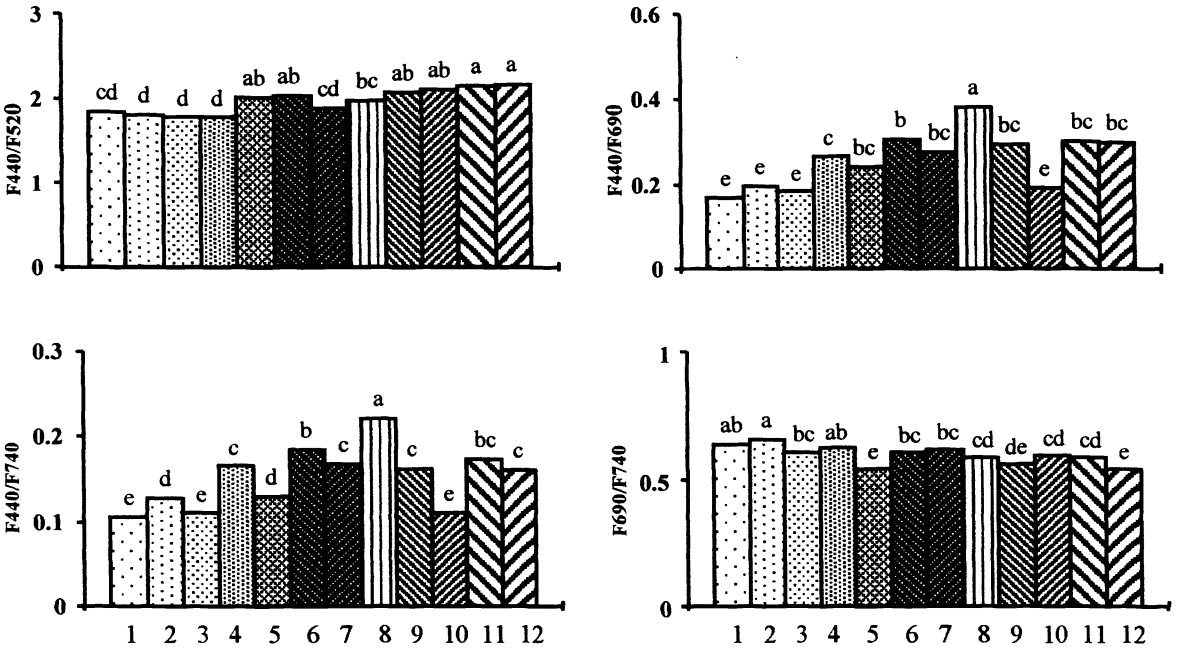


Fig. 6. Fluorescence images of primary leaves of two wheat genotypes with opposite response to drought and heat – Atlet (resistant) and Atol (sensitive) recorded at 440, 520, 690 and 740 nm. The gradual black to white scale color coding corresponds to increasing intensity (same scalar for both genotypes).

The ratios F440/F520, F440/F690, F440/F740 and F690/F740 (mean values of 40 replications) are presented in Fig. 7. In contrast to the previously presented measurements where no differences in controls (optimal grown condition) for any of fluorescence parameters have been observed, using the new imaging system a genotypic variability was identified for all fluorescence ratios. These differences are significant according to Newman-Keuls ($\alpha = 0.05$) for the mean values with

different letters (Fig. 7). These differences may be due to genetic differences between cultivars for some leaf structural indicators of photosynthetic capacity, such as the specific leaf dry weight (SLDW) and the chlorophyll content (SPAD) per unit leaf area. SLDW and SPAD have been identified as important leaf structural sources to improve water use efficiency under irrigation but also under rainfed conditions [26-28]. In our set of cultivars SLDW showed marked differences ($p \leq 0.001$) between cultivars from 57 g m^{-2} per sample (five primary leaves of 10-day old seedlings) to 73 g m^{-2} and the chlorophyll content (SPAD) from 29 to 39 per unit area of leaf (data not shown). We have found a good correlation ($p \leq 0.05$) between SPAD and the ratio F690/F740 and between SLDW and the ratio F440/F520 (Fig. 8).

Fig. 7. Genotypic variability for the fluorescence images ratios blue/green (F440/F520), blue/red (F440/F690), blue/far-red (F440/F740), and red/far-red (F690/F740). Means (of 40 replication) with the same letter are not significantly different by Newman-Keuls ($\alpha = 0.05$).



The chlorophyll fluorescence ratio red/far-red (F690/F740) has been demonstrated as a good, non-destructive indicator of the *in situ* chlorophyll content. This ratio is inversely correlated to the chlorophyll content [6, 8, 9]. In our experiment as well, the ratio F690/F740 correlated negatively (r = - 0.75) with the chlorophyll content of the genotypes (Fig. 8). It has also been shown that grasses and crop plants exhibit a high blue-green fluorescence, which can be greater than the chlorophyll fluorescence [11, 29, 30]. The blue-green fluorescence signal emanates predominantly from the chlorophyll-free epidermal cells and the leaf veins, whereas the cell walls of green mesophyll cells contribute very little to this fluorescence [29]. This is because in green mesophyll cells the blue-green fluorescence is reabsorbed by photosynthetic pigments, the lowest signal being found in fully green leaves with high chlorophyll content [30]. However, in our experiment no correlation was found between the chlorophyll content (SPAD) and F440 (r = 0.13) or F520 (r = -0.18) or the ratio F440/F520 (r = 0.69). In turn, the ratio F440/F520 was correlated (r = 0.75, $p \leq 0.05$) with the specific leaf dry weight SLDW (Fig. 8), which depends on leaf thickness, size and arrangement of mesophyll cells [26, 27].

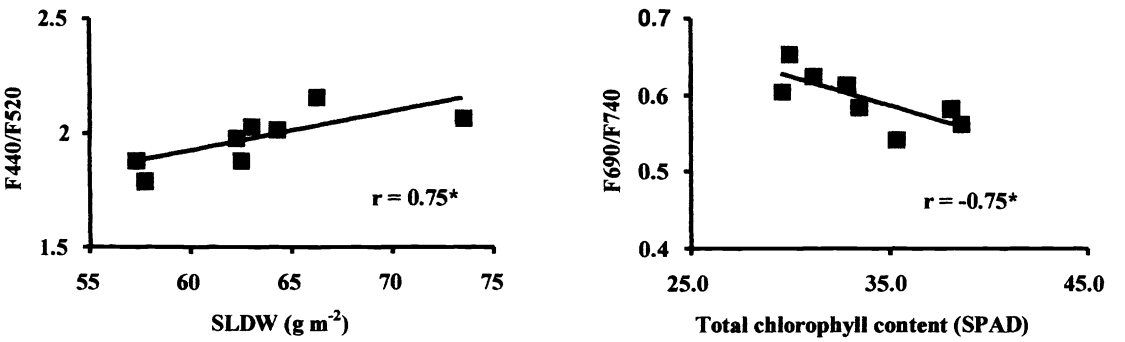


Fig. 8. Linear correlation between the specific leaf dry weight (SLDW) and the blue/green fluorescence (F440/F520), and between the chlorophyll content (SPAD) per unit of leaf areas and the red/far-red (F690/F740) fluorescence ratios in 2-leaf seedlings of winter wheat.

The improvement of WUE under rainfed condition may be attained by increasing of the internal photosynthetic activity, without any concomitant decrease in stomatal conductance, therefore the development of wheat cultivars with thicker or more dense leaves presenting higher photosynthetic rates per unit area of the leaf have been proposed [26, 27, 31]. In this respect, the possibility of recording the fluorescence images to screen genotypes for variation in intrinsic photosynthetic capacity is very desirable for breeding. In addition, the new LIF system allows analysis of entire plants and larger number of replications (40) in contrast to point-data measurements with other fluorometers (4 replications).

In summary, the chlorophyll fluorescence ratios Fv/Fo and Rfd may be considered suitable tests for drought and heat tolerance in winter wheat. The new LIF imaging system opens new possibilities to investigate genotype differences for WUE in the absence of stress at very early developmental stages. Fluorescence techniques are very suitable for screening drought and heat tolerance in wheat breeding programs.

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