Fluorescence excitation spectra of drought resistant and sensitive genotypes of triticale and maize

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Abstract

An influence of soil drought (7 or 14 d) and 7 d recovery on changes of leaf fluorescence excitation spectra at wavelengths of 450, 520, 690, and 740 nm (F_{450}, F_{520}, F_{690}, F_{740}) for drought resistant and sensitive genotypes of triticale and maize was compared. In non-stressed plants the differences between maize and triticale were observed for F_{450} and F_{520}, but not for F_{690} and F_{740}. Drought caused the increase in F_{450}, F_{520}, and F_{690} and this increase was more distinct for drought sensitive genotypes. After re-hydration, chlorophyll fluorescence mostly recovered to values of control plants. Drought caused significant increase in F_{690}/F_{740} but not in F_{450}/F_{690} and F_{450}/F_{520}. For triticale, highest increase in F_{690}/F_{740} was observed in the 4th and 7th leaves of resistant genotype and contrarily in maize for the sensitive one. After recovery, the F_{450}/F_{520}, F_{450}/F_{690}, and F_{690}/F_{740} ratios mostly returned to values of control plants.

Additional key words: *Triticale*; *Zea*.

Introduction

Measurements of the blue, green, red, and far-red fluorescence can be used to detect direct and indirect plant responses to stress. The blue and green fluorescence originates from plant phenolics, primarily from ferulic acid covalently bound to sugars of epidermal cell walls (Morales *et al*. 1994, 2005, Lichtenthaler 1996, 1998, Schweiger *et al*. 1996, Cerovic *et al*. 2002, Meyer *et al*. 2003). Phenolic compounds may function as UV-filter for the mesophyll tissues and can change radiation falling on leaf through absorption of UV-radiation and its transformation into the blue and/or green fluorescence. The plants phenolics in epidermal layer of leaves absorb about 90 % of UV-radiation and in this way can protect deep situated photosynthetic apparatus. The source of the red fluorescence is the protein-bound chlorophyll (Chl) *a* of the mesophyll cells. Measurement of the red fluorescence is a sensitive and rapid method for recognition of stress effects on plants before visible damages occur. The study of the light-induced red fluorescence of the plant leaves provides basic information on function of the photosynthetic apparatus and performance of photosynthesis. Disturbances of photosynthesis performance are connected with injuries of photosystems (PS2 and PS1) and light-harvesting complexes (LHC) (Lang *et al.* 1994, 1996, Lang 1995, Schweiger *et al*. 1996, Buschmann and Lichtenthaler 1998, Buschmann *et al*. 2000). When drought limits carbon metabolism and utilisation of light-phase products, the great amount of harvested radiant energy, which is harmful to PS2, cannot be converted to the chemical energy (Cornic and Massacci 1996). The increase in red fluorescence at the cost of photosynthetic radiant energy conversion is often connected with damages to PS2, PS1, and LHC under stress conditions (Schweiger *et al*. 1996, Buschmann and Lichtenthaler 1998, Buschmann *et al*. 2000). A consequence of the drought induced limitation of photosynthesis is the exposure of plants to excess energy, which may be harmful to PS2 (Demmig-Adams and Adams 1992, Cornic and

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Abbreviations: Chl – chlorophyll; FWC – field water capacity; PAR – photosynthetically active radiation; PS – photosystem.
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Massacci 1996). PS2 is more susceptible to water stress as compared to PS1 (van Rensburg and Krüger 1993).

In our earlier papers we described the differences between drought resistant and drought sensitive genotypes of triticale and maize in gas exchange, leaf water potential, leaf injury, and Chl content (Grzesiak 2004, Grzesiak *et al.* 2006, 2007). In this experiment we were focused mainly on the underlying mechanism of Chl fluorescence of the different aging leaves response to drought stress and compared the different responses to drought between C_3 and C_4 plants. We applied spectrofluorescence methods for determination of differences in response of triticale and maize plants to drought stress to provide information on plant ability to avoid and repair damages of photosynthetic apparatus.

Materials and methods

Plants and growing conditions: The experiment was carried out on two spring triticale (×*Triticosecale* Wittmack) breeding strains and two maize (*Zea mays* L.) single cross hybrids. The triticale grain was obtained from Polish Breeding Station, Choryn and maize grain from SEMPOL Holding, Trnava, Slovakia. For chosen genotypes the Drought-Susceptibility Index (DSI) was determined by the method by Fischer and Maurer (1978). According to Grzesiak (2004), triticale CHD-247 and maize Tina belong to drought resistant genotypes (DSI 0.368 and 0.381, respectively) and triticale CHD-12 and maize Ankora belong to the group of drought sensitive genotypes (DSI 0.544 and 0.650, respectively).

The plants were grown in air-conditioned growth cabinets: day/night temperature of 23/18 °C (±2.5 °C), relative humidity (RH) 70/60 % (±5 %), 16-h photoperiod with artificial irradiation from high pressure sodium lamps (*Philips SON-T AGRO,* 400 W) yielding photosynthetically active radiation (PAR) about 500 μmol m^{-2} s^{-1}. Plants were grown in Mitscherlich pots filled with mixture of soil, peat, and sand (1 : 1 : 3, v/v/v) and till the 28th d after sowing they were maintained well-watered (65 % of field water capacity, FWC). Subsequently, drought treatment (30 % FWC) was applied for 7 (D7) or 14 (D14) d. After this period, for the next 7 d well watering was re-established (R7). The pots were weighted every day, and the amount of water loss by transpiration was refilled to keep the constant mass of pots in each treatment.

Measurements: Fluorescence emission spectra of red fluorescence were recorded using a *Perkin-Elmer LS 50B* spectrofluorimeter. The fluorescence yield was measured at 450, 520, 690, and 740 nm with the excitation at 450 nm. The spectral band width was adjusted at 5 nm (excitation) and 10 nm (emission). Fluorescence emission spectra were recorded between 650 and 800 nm. The fluorescence excitation spectra of blue and green fluorescence were recorded with an excitation wavelength varied from 250 to 400 nm and an emission wavelength set at 450 nm. The slit widths for excitation and emission monochromators were adjusted to 10 nm. The cut-off filter (390 nm) was applied to study both blue and green fluorescence. Both excitation and emission spectra were recorded at room temperature.

Measurement of excitation spectra (F_{450}, F_{520}, F_{690}, and F_{740}) for the 4th and 7th leaf and for each treatment and each day of measurements were done in 10 replications. Data were statistically analysed using a Duncan's multiple range tests. Angular transformations (arc sin$\sqrt{x}$) were performed when variable involved was expressed in percent.

Results

In non-drought stressed plants differences between triticale and maize were observed in F_{450} and F_{520}, which were about six and four times higher in maize than in triticale. Between the 35th and 49th d of plant growth a tendency to increase fluorescence emission was observed, caused most likely by leaf ageing. Differences in F_{450} and F_{520} between sensitive and resistant genotypes of triticale and maize were in most cases significant, while differences in F_{690} and F_{740} were not significant. In non-stressed plants, the differences between the older (4th) and younger (7th) leaf were statistically significant for F_{450} and F_{520}, but not for F_{690} and F_{740} (Table 1).

Both short (D7) and prolonged (D14) drought caused an increase of emission and excitation of leaves in range of F_{450}, F_{520}, and F_{690} fluorescence. However, no impact of drought on F_{740} was observed. This increase in fluorescence within blue, green, and red range was more distinct in measurements taken for maize genotypes. Both in maize and triticale the increase in values of F_{450}, F_{520}, and F_{690} was more significant for measurements of sensitive forms (CHD12, Ankora) than of drought resistant ones (CHD247, Tina) and, moreover, more distinct in measurements on the 4th leaf. After 7-d-long recovery for plants of treatments D7 and D14, Chl fluorescence parameters in blue, green, and red range did not reach the values obtained for non-drought stressed plants (Fig. 1).

Relations between fluorescence ratio in different spectral ranges (F_{450}/F_{520}, F_{450}/F_{520}, and F_{690}/F_{740}) are shown in Table 2 and Fig. 2. In non-drought stressed plant the differences between drought-sensitive and drought-resistant genotypes of triticale and maize seedlings were in most cases significant. For plants subjected to drought (D7, D14) comparing to non-drought stressed plants mainly resulted in relatively small and

Table 1. Fluorescence emission of 4th and 7th leaves (detection at the wavelengths 450, 520, 690, and 740 nm) in non-drought stressed seedlings of triticale and maize genotypes. Means within columns followed by the same letter for particular leaf and days of vegetation do not differ significantly according to Duncan`s multiple range test (α = 0.05).

Plant	Leaf number	Day after sowing	Genotype	Excitation wavelength [nm] F_{450}	F_{520}	F_{690}	F_{740}
Triticale	4	35	CHD12	0.11 a	0.04 a	0.08 a	0.03 a
			CHD247	0.13 b	0.06 b	0.07 a	0.05 b
		42	CHD12	0.11 a	0.05 ab	0.08 a	0.04 ab
			CHD247	0.14 b	0.09 c	0.08 a	0.05 b
		49	CHD12	0.17 c	0.09 c	0.11 b	0.08 c
			CHD247	0.14 b	0.10 c	0.10 b	0.07 c
	7	35	CHD12	0.12 a	0.07 b	0.07 ab	0.07 b
			CHD247	0.11 a	0.05 a	0.06 a	0.05 a
		42	CHD12	0.12 a	0.07 b	0.07 ab	0.06 ab
			CHD247	0.16 c	0.08 bc	0.08 b	0.07 b
		49	CHD12	0.16 c	0.09 cd	0.10 c	0.06 ab
			CHD247	0.14 b	0.10 d	0.10 c	0.09 c
Maize	4	35	Ankora	0.69 a	0.35 b	0.09 a	0.05 ab
			Tina	0.71 b	0.30 a	0.09 a	0.04 a
		42	Ankora	0.70 a	0.33 b	0.10 a	0.04 a
			Tina	0.72 b	0.30 a	0.10 a	0.05 ab
		49	Ankora	0.88 c	0.45 d	0.12 b	0.08 d
			Tina	0.94 d	0.40 c	0.14 b	0.06 b
	7	35	Ankora	0.76 d	0.29 b	0.11 b	0.07 a
			Tina	0.74 c	0.25 a	0.09 a	0.06 a
		42	Ankora	0.72 b	0.31 bc	0.11 b	0.06 a
			Tina	0.70 a	0.32 c	0.11 b	0.07 a
		49	Ankora	0.78 e	0.35 d	0.13 c	0.10 b
			Tina	0.80 f	0.30 b	0.13 c	0.09 b

often statistically insignificant changes in fluorescence ratios of F_{450}/F_{690} and F_{450}/F_{520}. However, drought significantly increased the F_{690}/F_{740} ratio compared to non-drought stressed plants. For both maize and triticale genotypes increase of ratio F_{690}/F_{740} was higher for D14 treatments. Higher increase of F_{690}/F_{740} was observed in drought-sensitive maize genotype (Ankora), especially for the 4th leaf. For triticale a higher F_{690}/F_{740} was observed in the 4th and 7th leaves of drought-resistant genotype (CHD 247), however, for maize the differences were of reverse order. After a 7-d-long recovery (D7R7, D14R7) in most cases a complete return of F_{690}/F_{740} to the values obtained for non-drought stressed treatment was observed (Fig. 2).

Discussion

Drought stress causing functional and structural disturbances in leaves may shorten (weaken) or lengthen (amplify) the path of acting radiation (Lang *et al*. 1994, Lang and Lichtenthaler 1995, Lichtenthaler 1996, 1998, Lichtenthaler *et al*. 1996, Stober and Lichtentaler 1998). Leaf growth is highly responsive to the environment and the most characteristic effects of drought on plant growth are decrease in leaf number and leaf expansion. During drought the older leaves have lower photosynthetic rate and Chl content compared to the young leaves, indicating that old leaves senesce more profoundly (He *et al.* 2002, Chaves *et al*. 2003). In plants subjected to drought, leaves occur after a delay compared to control plants and the process of ageing is accelerated (Šesták and Šiffel 1997). In our experiment we confirmed the differences between maize and triticale leaf fluorescence in F_{450}, F_{520}, and F_{690}. Water deficit in leaves lead to the increase in intensity of F_{450} and F_{520}, which was most likely caused by leaf aging between 35th and 49th d of seedling age. Hence drought mainly adversely affected the activity of PS2 in older leaves, and the youngest leaves had higher plasticity for the response of Chl fluorescence to drought. Our earlier studies confirmed leaf age modified impact of drought on gas exchange, leaf water potential, and Chl content (Grzesiak *et al*. 2006, 2007).

Soil drought predisposes plants to injury of the photosynthetic apparatus through its co-acting with UV or visible radiation and the defence mechanism against such radiation may depend on the accumulation of plant phenolics in the leaf tissue (Bilger *et al*. 2001, Schmitz-Hoerner and Weissenbock 2003). In this case UV radiation is transformed into blue-green fluorescence, which

may be used in photosynthesis or partially reemitted as Chl fluorescence (Schweiger *et al*. 1996, Buschmann and Lichtenthaler 1998, Lichtenthaler and Schweiger 1998). In tobacco plants drought caused limited increase in

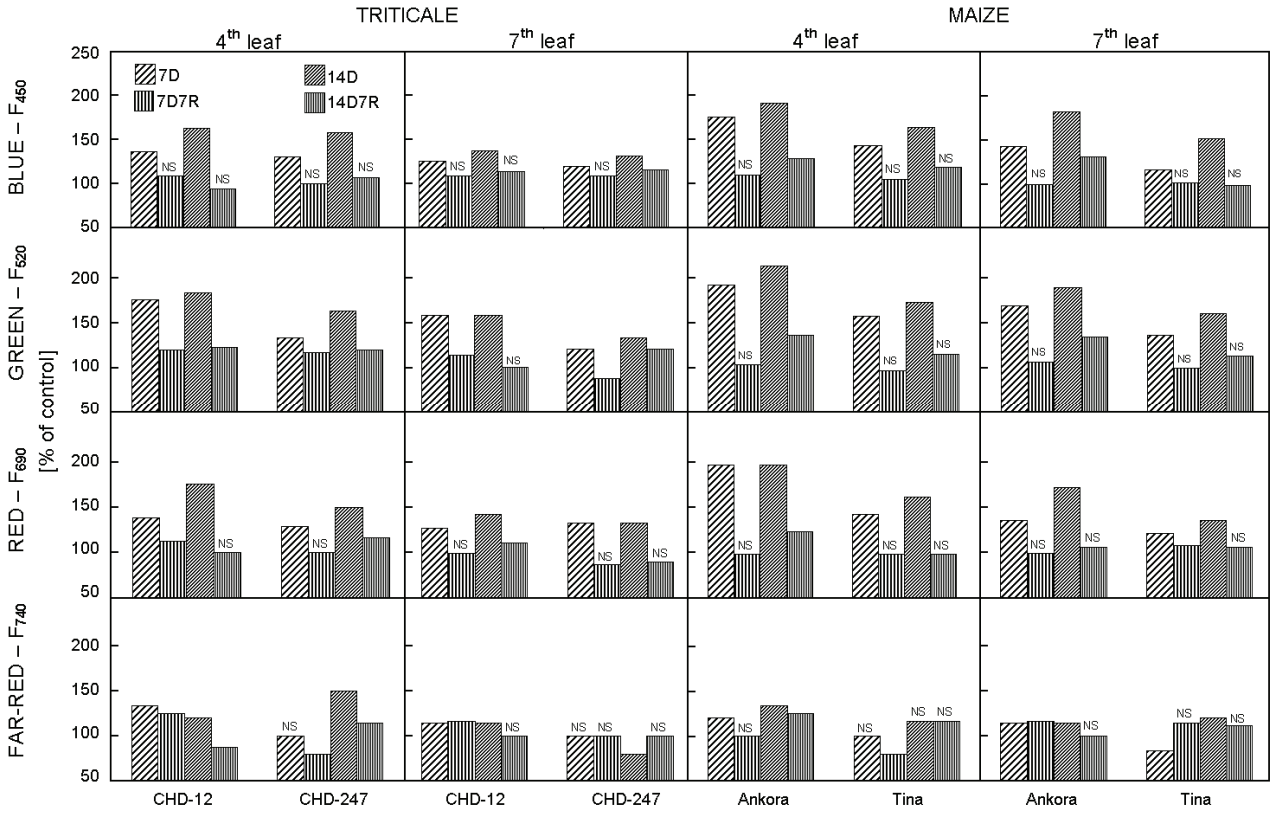

Fig. 1. The effect of 7- (7D) and 14-d-long (14D) soil drought and 7-d-long-recovery (7R) on parameters of fluorescence range emission at the wavelengths of 450, 520, 690, and 740 nm for 4th and 7th leaves of triticale and maize genotypes. Results presented as a percent of non-drought stressed plant. NS – non significant difference compared to non-drought stressed plant.

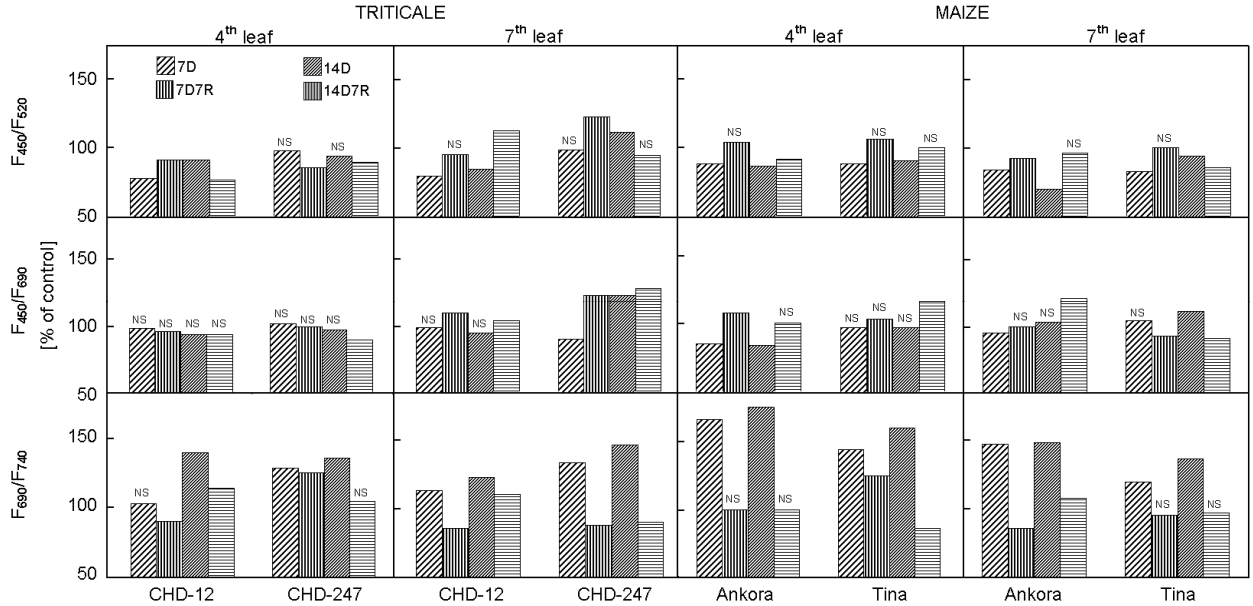

Fig. 2. The effect of 7- (7D) and 14-d-long (14D) soil drought and 7-d-long-recovery (7R) on fluorescence ratios (F_{450}/F_{690}, F_{450}/F_{520}, and F_{690}/F_{740}) for 4th and 7th leaves of triticale and maize genotypes. Results presented as a percent of non-drought stressed plant. NS – non significant difference compared to non-drought stressed plant.

Table 2. Fluorescence ratios in excitation spectra of 4th and 7th leaves (F_{450}/F_{690}, F_{450}/F_{520}, and F_{690}/F_{740}) in non-drought stressed seedlings of triticale and maize genotypes. Means within columns followed by the same letter for particular leaf and days of vegetation do not differ significantly according to Duncan`s multiple range test ($\alpha = 0.05$).

Plant	Leaf number	Day after sowing	Genotype	Fluorescence ratios F_{450}/F_{690}	F_{450}/F_{520}	F_{690}/F_{740}
Triticale	4	35	CHD12	1.38 a	2.75 e	2.67 b
			CHD247	1.86 d	2.17 c	1.40 a
		42	CHD12	1.38 a	2.20 cd	2.00 c
			CHD247	1.75 c	2.33 d	1.60 b
		49	CHD12	1.55 b	1.89 b	1.38 a
			CHD247	1.40 a	1.40 a	1.43 a
	7	35	CHD12	1.71 c	1.71 c	1.00 a
			CHD247	1.83 d	2.20 d	1.20 c
		42	CHD12	1.71 c	1.71 c	1.17 bc
			CHD247	1.63 b	1.63 b	1.14 b
		49	CHD12	1.60 b	1.78 c	1.67 d
			CHD247	1.40 a	1.40 a	1.11 b
Maize	4	35	Ankora	7.67 d	1.97 a	1.80 b
			Tina	7.89 e	2.37 c	2.25 cd
		42	Ankora	7.00 b	2.12 b	2.50 e
			Tina	7.20 c	2.40 c	2.00 b
		49	Ankora	7.33 c	1.96 a	1.50 a
			Tina	6.71 a	2.35 c	2.33 de
	7	35	Ankora	8.22 f	2.96 d	1.50 c
			Tina	6.91 e	2.62 c	1.57 d
		42	Ankora	6.55 d	2.32 b	1.83 e
			Tina	6.36 c	2.19 a	1.57 d
		49	Ankora	6.00 a	2.23 a	1.30 a
			Tina	6.15 b	2.67 c	1.44 b

intensity of F_{450} and F_{520} by partial re-absorption of radiation by assimilation pigments. However, in rhododendron plants a parallel impact of drought, high temperature, and irradiation on fluorescence intensity in F_{450} and F_{520} was caused by increase in the contents of various phenolic compounds in leaf epidermis (Lang *et al*. 1996).

The studies of Schweiger *et al*. (1996), Buschmann and Lichtenthaler (1998), and Hideg *et al*. (2002) demonstrate that fluorescence emission spectra of leaves can successfully be applied for stress detection in plants. The differing fluorescence yield in dependence of the excitation wavelength can be quantified by the fluorescence ratios F_{450}/F_{690} and F_{450}/F_{740}, as well as by F_{690}/F_{740} and F_{450}/F_{520}. The fluorescence ratios F_{450}/F_{690} and F_{450}/F_{740} provide the most sensitive and best suited stress indication (Schweiger *et al.* 1996). The fluorescence ratio F_{450}/F_{690} was recommended by Buschmann and Lichtenthaler (1998) as the best and very early stress indicator. These authors also showed that various abiotic and biotic stress factors cause increase, decrease, or lack of changes in F_{450}/F_{690}, F_{690}/F_{740}, and F_{450}/F_{520}. Water deficit and nitrogen deficiency cause increase in F_{450}/F_{690}, however, heat stress and UV-A irradiation cause its decrease. Deficiencies of water and nitrogen do not influence the value of blue/green fluorescence, but UV-A irradiation, heat, and temperature cause its decrease. Drought, heat, and UV-A irradiation do not have impact on the F_{690}/F_{740} fluorescence ratio, however, nitrogen deficiency causes an increase.

We observed an increase in F_{690}/F_{740} in leaves of triticale and maize seedlings and this discrepancy with the results of Buschmann and Lichtenthaler (1998) requires a further study. Our results also provide valuable information on the most suitable wavelength for fluorescence excitation which is different for the F_{450} and F_{520} fluorescence and F_{690} and F_{740} fluorescence.

Measurements of Chl fluorescence parameters, as a non-destructive method, may be useful for plant physiologists and breeders as stress tolerance tests. Our research demonstrates that parameters of fluorescence emission spectra of leaves can be successfully applied for detection of water stress in plants. Application of these methods to evaluation of drought tolerance between different genotypes of crop plants requires further study. Foremost in concern is the specification of testing conditions, especially selection of appropriate time and level of exposure to drought and selection of a leaf or leaves on which the measurements should be taken. Trials are undertaken to apply fluorescence to evaluate interspecies variability in plant tolerance to water stress but this requires further studies focused on unifying the indexes and testing conditions. Progress in application of fluorescence

measurements in physiological research seems to be possible and moreover it is undoubtedly stimulated by development of devices enabling wider spectrum of measurements of various Chl fluorescence parameters (Lichtenthaler 1998, Lichtenthaler *et al.* 2005).

References

Bilger, W., Johnsen, T., Schreiber, U.: UV-excited chlorophyll fluorescence as a tool for the assessment of UV-protection by the epidermis of plants. – J. exp. Bot. **52**: 2007-2014, 2001.

Buschmann, C., Langsdorf, G., Lichtenthaler, H.K.: Imaging of the blue, green, and red fluorescence emission of plants: An overview. – Photosynthetica **38**: 483-491, 2000.

Buschmann, C., Lichtenthaler, H.K.: Principles and characteristics of multi-colour fluorescence imaging of plants. – J. Plant Physiol. **152**: 297-314, 1998.

Cerovic, Z.G., Ounis, A., Cartelat, A., Latouche, G., Goulas, Y., Meyer, S., Moya, I.: The use of chlorophyll fluorescence excitation spectra for the non-destructive in situ assessment of UV-absorbing compounds in leaves. – Plant Cell Environ. **25**: 1663-1676, 2002.

Chaves, M.M., Maroco, J.P., Pereira, J.S.: Understanding plant responses to drought – from genes to whole plant. – Funct. Plant Biol. **30**: 239-264, 2003.

Cornic, G., Massacci, A.: Leaf photosynthesis under drought stress. – In: Baker, N.R. (ed.): Photosynthesis and the Environment. Pp. 347-366. Kluwer Academic Publ., Dordrecht – Boston – London 1996.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Fischer, R.A., Maurer, R.: Drought resistance in spring wheat cultivars. I. Grain yield responses. – Aust. J. agr. Res. **29**: 897-912, 1978.

Grzesiak, M.T.: [Effect of Drought Stress on Photosynthetic Apparatus and Productivity of Triticale and Maize Genotypes Differing in Drought Tolerance.] – Doctor Thesis. Cracow Agricultural University, Cracow 2004. [In Polish.]

Grzesiak, M.T., Grzesiak, S., Skoczowski, A.: Changes of leaf water potential and gas exchange during and after drought in triticale and maize genotypes differing in drought tolerance. – Photosynthetica **44**: 561-568, 2006.

Grzesiak, M.T., Rzepka, A., Hura, T., Hura, K., Skoczowski, A.: Changes in response to drought stress of triticale and maize genotypes differing in drought tolerance. – Photosynthetica **45**: 280-287, 2007.

He, P., Osaki, M., Takebe, M., Shinano, T.: Changes of photosynthetic characteristics in relation to leaf senescence in two maize hybrids with different senescent appearance. – Photosynthetica **40**: 547-552, 2002.

Hideg, E., Juhasz, M., Bornman, J.F., Asada, K.: The distribution and possible origin of blue-green fluorescence in control and stressed barley leaves. – Photochem. photobiol. Sci. **1**: 934-941, 2002.

Lang, M.: Studies on the blue-green and chlorophyll fluorescence of plants and their applications for fluorescence imaging of leaves. – Plant Physiol. **29**: 1-110, 1995.

Lang, M., Lichtenthaler, H.K.: Changes in the blue-green and red fluorescence-emission spectra of beech leaves during the autumnal chlorophyll breakdown. – J. Plant Physiol. **138**: 550-553, 1995.

Lang, M., Lichtenthaler, H.K., Sowinska, M., Heisel, F., Miehé, J.A.: Fluorescence imaging of water and temperature stress in plant leaves. – J. Plant Physiol. **148**: 613-621, 1996.

Lang, M., Lichtenthaler, H.K., Sowinska, M., Summ, P., Heisel, F.: Blue, green and red fluorescence signatures and images of tabacco leaves. – Bot. Acta **107**: 230-236, 1994.

Lichtenthaler, H.K.: Vegetation stress: an introduction to the stress concept in plants. – J. Plant Physiol. **148**: 4-14, 1996.

Lichtenthaler, H.K.: *In vivo* chlorophyll fluorescence as a tool for stress detection in plants. – In: Lichtenthaler, H.K. (ed.): Applications of Chlorophyll Fluorescence. Pp. 129-142. Kluwer Academic Publ., Dordrecht 1998.

Lichtenthaler, H.K., Buschmann, C., Knapp, M.: How to correctly determine the different chlorophyll fluorescence parameters and the chlorophyll decrease ratio R_{Fd} of leaves with the PAM fluorometer. – Photosynthetica **43**: 379-393, 2005.

Lichtenthaler, H.K., Lang, M., Sowinska, M., Heisel, F., Miehé, J.A.: Detection of vegetation stress via a new high resolution fluorescence imaging system. – J. Plant Physiol. **148**: 599-612, 1996.

Lichtenthaler, H.K., Schweiger, J.: Cell wall bound ferulic acid, the major substance of the blue-green fluorescence emission of plants. – J. Plant Physiol. **152**: 272-282, 1998.

Meyer, S., Cartelat, A., Moya, I., Cerovic, Z.G.: UV-induced blue-green and far-red fluorescence of long wheat leaves: a potential signature of leaf ageing. – J. exp. Bot. **54**: 757-769, 2003.

Morales, F., Cartelat, A., Álvarez-Fernández, A., Moya, I., Cerovic, Z.G.: Time-resolved spectral studies of blue-green fluorescence of artichoke (*Cynara cardunculus* L. var. *scolymus*) leaves: identification of chlorogenic acid as one of the major fluorophores and age-mediated changes. – J. agr. Food Chem. **53**: 9668-9678, 2005.

Morales, F., Cerovic, Z.G., Moya, I.: Characterization of blue-green fluorescence in the mesophyll of sugar beet (*Beta vulgaris* L.) leaves affected by iron deficiency. – Plant Physiol. **106**: 127-133, 1994.

Schmitz-Hoerner, R., Weissenbock, G.: Contribution of phenolic compounds to the UV-B screening capacity of developing barley primary leaves in relation to DNA damage and repair under elevated UV-B levels. – Phytochemistry **64**: 243-55, 2003.

Schweiger, J., Lang, M., Lichtenthaler, H.K.: Differences in fluorescence excitation spectra of leaves between stressed and non-stressed plants. – J. Plant Physiol. **148**: 536-547, 1996.

Šesták, Z., Šiffel, P.: Leaf-age related differences in chlorophyll fluorescence. – Photosynthetica **33**: 347-369, 1997.

Stober, F., Lichtenthaler, H.K.: Changes of the laser-induced blue, green and red fluorescence signatures during greening of etiolated leaves of wheat. – J. Plant Physiol. **140**: 673-680, 1998.

van Rensburg, L., Krüger, G.H.J.: Differential inhibition of photosynthesis (*in vivo* and *in vitro*), and changes in chlorophyll *a* fluorescence induction kinetics of four tobacco cultivars under drought stress. – J. Plant Physiol. **141**: 357-365, 1993.