Assessment of the photosynthesis-related traits and high temperature resistance in tetraploid wheat (*Triticum* L.) genotypes

Hodnotenie fotosyntetických charakteristík a rezistencie na vysokú teplotu pri tetraploidných genotypoch pšenice (*Triticum* L.)

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Abstract

The collection of 10 parental lines of tetraploid wheat genotypes with various origin was cultivated in growth chamber. Leaf and growth traits such as assimilation pigments content, dry mass and leaf area of plants were measured. The genotype differences were recognized by chlorophyll a fluorescence fast kinetics method applied on penultimate young wheat leaves. Consequently, heat stress susceptibility based on the exposure of whole plants in pots to air temperature of 42^{o}C for 6 hours was measured, too. Rapid chlorophyll a fluorescence kinetics method gave us better knowledge about differences among tetraploid wheat genotypes collection comparing to classic assimilation pigments analyse. Genotypes with higher content of pigments did not always exhibit good resistance against heat stress. Using the chlorophyll fluorescence parameters, the rating of genotypes based on photosynthetic performance as well as photosystem II (PSII) thermostability was done. We identified the genotypes TRG7 (*Triticum turgidum* subsp. *turgidum*, PI 384230, Ethiopia) and RONCAL (*Triticum turgidum* subsp. *dicoccon*, TRI 17700, Spain) as the perspective donors of genes for better thermostability of photosynthetic apparatus in changed climate conditions and material into wheat efficiency breeding programs.

Keywords: chlorophyll *a* fluorescence, heat stress, JIP-test, photosynthetic performance, photosynthetic pigments, photosynthetic traits, tetraploid wheat genotypes

Abstract in Slovak language

Kolekcia 10 rodičovských línií tetraploidných pšeníc rôzneho pôvodu bola pestovaná v rastovej komore. Merané boli listové a rastové charakteristiky, obsah asimilačných pigmentov, suchá hmota a listová plocha rastlín. Zisťované boli aj genotypové

rozdiely na základe rýchlej kinetiky fluorescencie chlorofylu a aplikovanej na predposledný plne vyvinutý list pšeníc. Následne bola skúmaná citlivosť na teplotný stres vystavením celých rastlín v kvetináčoch teplote vzduchu 42^{o}C po dobu 6 hodín. Metóda rýchlej kinetiky chlorofylu *a* nám poskytla lepšie poznatky o rozdieloch v kolekcii tetraploidných pšeníc než klasická metóda analýzy asimilačných pigmentov. Genotypy s vyšším obsahom pigmentov nepreukázali vždy dobrú rezistenciu voči teplotnému stresu. Na základe meraní fluorescencie chlorofylu boli genotypy zatriedené do poradia tak podľa fotosyntetickej výkonnosti ako aj podľa teplotnej stability fotosystému II (PSII). Na základe našich výsledkov môžeme preto odporučiť pre účely šľachtenia pšenice genotypy TRG7 (*Triticum turgidum* subsp. *turgidum*, PI 384230, Etiópia) a RONCAL (*Triticum turgidum* subsp. *dicoccon*, TRI 17700, Španielsko) ako perspektívnych donorov génov lepšej termostability fotosyntetického aparátu v meniacich sa klimatických podmienkach.

Keywords: asimilačné pigmenty, fluorescencia chlorofylu *a*, fotosyntetická výkonnosť, fotosyntetické charakteristiky, JIP-test, teplotný stres, tetraploidné genotypy pšenice

Detailed abstract in Slovak language

Do experimentu s tetraploidnými pšenicami rôzneho pôvodu bolo vybraných 10 genotypov: TRI 6158 (*Triticum turgidum* subsp. *dicoccon*, Irán); RONCAL (*Triticum turgidum* subsp. *dicoccon*, TRI 17700, Španielsko) – donorom týchto dvoch genotypov bol IPK Gatersleben; ARM 7 (*Triticum timopheevii* subsp. *armeniacum*, PI 352265, Azerbajdžan); CAR 1 (*Triticum turgidum* subsp. *carthlicum*, PI 61102, Gruzínsko); DCS 9 (*Triticum turgidum* subsp. *dicoccoides*, PI 487262, Sýria); ISP 6 (*Triticum ispahanicum*, PI 330548, Irán); PLN 8 (*Triticum turgidum* subsp. *polonicum*, PI 254215, Irak); TIM 1 (*Triticum timopheevii* subsp. *timopheevii*, PI 119442, Turecko); TRG 7 (*Triticum turgidum* subsp. *turgidum*, PI 384230, Etiópia); TRN 8 (*Triticum turgidum* subsp. *turanicum*, PI 624892, Irán) – donorom týchto 8 genotypov bol GRIN, Beltsville, USA (USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network - GRIN). Semená všetkých genotypov boli jarovizované v chlade pri teplote 0-2^{o}C 6 týždňov. Následne boli klíčence vysadené do kvetináčov, umiestnené do rastovej komory a pestované pri fotoperióde 16/8 h (deň/noc), teplote 21/18^{o}C a intenzite svetla 250 µmol.m^{-2}.s^{-1} na úrovni listov. Po 6 týždňoch bol zisťovaný obsah asimilačných pigmentov, suchá hmota, listová plocha rastlín a merané vybrané parametre fotosyntézy aplikáciou fluorescenčnej techniky na nestresované rastliny. Následne boli rastliny podrobené teplote 42^{o}C v rastovej komore na svetle počas 6 hodín, závlahou a prechodným zvýšením vlhkosti vzduchu bolo zabránené dehydratácii listov. Následne boli mladé, plne vyvinuté predposledné listy (po predchádzajúcej 30 minútovej adaptácii na tmu pomocou klíps zadržiavajúcich svetlo) merané metódou rýchlej kinetiky fluorescencie chlorofylu *a* na. Indukčné krivky fluorescencie chlorofylu *a* boli získané pomocou prenosného fluorimetra Handy PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd., Kings Lynn, UK). Takto získané rýchle fluorescenčné prechody (tranzity) boli analyzované tzv. JIP testom. Merané a počítané parametre JIP-testu sú zobrazené v tab. 1.

Obsah chlorofylu je zvyčajne v korelácii s fotosyntetickou výkonnosťou a úrodovým potenciálom. Produkcia (tvorba) vysokej biomasy závisí od veľkosti listovej plochy a jej štruktúry, zahŕňajúc obsah chlorofylov, karotenoidov a ich funkčných prejavov. Podľa nameraných hodnôt koncentrácie pigmentov, suchej hmoty a listovej plochy rastlín v tab. 2 môžeme povedať, že genotypy ISP6 a TRG7 dosiahli v týchto parametroch predbežne lepšie výsledky (t.j. vyššie hodnoty) než ostatné genotypy. Predbežne horšie výsledky (t.j. nižšie hodnoty) dosiahli CAR1, TIM1 a ARM7. Vysoké hodnoty suchej hmoty a listovej plochy nemusia vždy korelovať s vysokým obsahom pigmentov – dokonca môžu byť v ostrom rozpore pre určitý genotyp, ako napr. v prípade DCS9. OJIP-krivka (obr. 1) určuje diverzitu fotochemických vlastností listov jednotlivých genotypov. Numerické analýzy JIP-testov (tab. 3 a 4) zobrazujú značné genotypové rozdiely. Hodnoty F_o, F_m, F_v a F_v/F_m v tab. 3 poukazujú na to, že genotypy mali normálny fotosyntetický aparát bez anomálií a bez evidentných stresových prejavov. Hodnoty F_v/F_0, V_J a V_i už vykazujú určitú diverzitu, ktorá je ďalej analyzovaná parametrami vyjadrujúcimi toky energie a celkovú výkonnosť fotochemických procesov v tab. 4. Radarový graf (obr. 2) vyjadruje relatívne hodnoty všetkých parametrov JIP-testu. Najmä parametre RE_o/CS_m a $PI(cs_m)$ ukazujú značné rozdiely medzi genotypmi. V podmienkach bez stresu dosiahol najlepšie hodnoty genotyp PLN8; genotypy TRI6, ARM7 a najmä TIM1 boli výrazne podpriemerné. Situácia sa však zmenila vystavením rastlín teplote 42^{o}C na 6 hodín v podmienkach s plnou závlahou. Obr. 3a) zobrazuje abnormálne krivky pri rastlinách ošetrených teplom oproti nestresovanej kontrole. Najcitlivejšími na teplotný stres boli genotypy TRI6, TIM1 and CAR1. Najnižšiu senzitivitu a teda najvyššiu rezistenciu voči teplotnému stresu sme zistili pri genotypoch TRG7, RONCAL a DCS9. Ostatné genotypy boli citlivé na vysokú teplotu. To potvrdzuje aj malý graf v ľavom hornom rohu obr. 3a), s parametrom F_o ako indikátorom nefunkčných reakčných centier. Najmä TRG7 a RONCAL odporúčame preto ako perspektívnych donorov génov lepšej termostability fotosyntetického aparátu pre účely šľachtenia pšenice do možných poľných experimentov. Obr. 3b) prezentuje zmenené krivky fluorescenčnej kinetiky po teplotnom strese, kde vysoký nárast v čase 0,3 ms poukazuje na poškodenie kyslík uvoľňujúceho komplexu (neboli zaradené najcitlivejšie genotypy TIM1 a CAR1 s extrémnym priebehom kriviek). Na obr. 4 bola rezistencia genotypov vyjadrená parametrom F_v/F_o, ktorý reprezentuje pomer fotochemických procesov (teda potenciálne využiteľnej energie) ku nefotochemickým procesom (teda energie neproduktívne stratenej). V prípade najcitlivejších genotypov na teplotný stres (CAR1, TIM1, TRI6) bol podiel zachytenej energie svetla využiteľný vo fotosyntéze zanedbateľný.
Aplikácia rýchlej kinetiky fluorescencie chlorofylu *a* môže napomôcť identifikovať línie odolné alebo citlivé na teplotný stres v početných skupinách genotypov pšenice. Tieto línie môžu následne slúžiť pre ďalší výskum ako zdroj tolerancie voči vysokej teplote pre potreby šľachtenia.

Keywords: asimilačné pigmenty, fluorescencia chlorofylu *a*, fotosyntetická výkonnosť, fotosyntetické charakteristiky, JIP-test, teplotný stres, tetraploidné genotypy pšenice

Introduction

Tetraploid wheat species are inseparable part of hexaploid wheat species evolution. Currently, we can see the increase of significance of these species; they are used for

scientific purposes, e.g. for study of domestication processes but their huge genetic diversity is used also practically. Tetraploid wheat species are included in different breeding programs for wheat resynthesis, as the donors of resistance, etc. (Švec et al., 2011). Globally, the greatness of wheat production as well as the yield stability are restricted by abiotic stresses - drought and high temperature, mainly. Plants responses to stress are non-specific and genetically conditioned. The decrease of photosynthesis rate and inhibition of many molecular mechanisms are the early responses of plants to unsuitable environment (Olšovská, Brestič, 2001). Diversity of physiological reactions can serve for screening tolerant wheats to water stress or heat stress (Švec et al., 2010).

The techniques based on chlorophyll fluorescence records represent an efficient tool for assessment of negative effects of water deficit, high temperatures and other abiotic stressors on photosynthetic apparatus (for review see e.g. Maxwell, Johnson, 2000; Brestic, Zivcak 2013). The fluorescence itself has the origin in photosynthetic pigments of green plants and it screens a large scale of photophysical processes, which perform in thylakoid membranes inside the chloroplasts during the transformation of sun radiation energy into the biochemically available form (Govindjee, 2004). Chlorophyll fluorescence measurement is rapid, nondestructive, quantitative and diagnostic method used now widely as technique for estimating of photosynthetic performance in plants (Janušauskaitė et al., 2012; Kalaji et al. 2012). It reflects reliably the status of photosystem II (PS II) because of its damage is often the first manifestation of a stress impact. PS II informs us about plant ability to tolerate stress (Rathod et al. 2010; Holá et al. 2010; Gogoláková, Štrba, 2011; etc.). Therefore we used this method for measuring selected photosynthetic traits in the tetraploid wheat species collection.

Materials and Methods

The genotypes of 10 tetraploid wheat species with different origin and identification were used in experiment, as follows: TRI 6158 (*Triticum turgidum* subsp. *dicoccon*, Iran); RONCAL (*Triticum turgidum* subsp. *dicoccon*, TRI 17700, Spain) – the donor of those two genotypes was IPK Gatersleben; ARM 7 (*Triticum timopheevii* subsp. *armeniacum*, PI 352265, Azerbaijan); CAR 1 (*Triticum turgidum* subsp. *carthlicum*, PI 61102, Georgia); DCS 9 (*Triticum turgidum* subsp. *dicoccoides*, PI 487262, Syria); ISP 6 (*Triticum ispahanicum*, PI 330548, Iran); PLN 8 (*Triticum turgidum* subsp. *polonicum*, PI 254215, Iraq); TIM 1 (*Triticum timopheevii* subsp. *timopheevii*, PI 119442, Turkey); TRG 7 (*Triticum turgidum* subsp. *turgidum*, PI 384230, Ethiopia); TRN 8 (*Triticum turgidum* subsp. *turanicum*, PI 624892, Iran) – the donor of those eight genotypes was GRIN, Beltsville, USA (USDA, ARS, National Genetic Resources Program. Germplasm Resources Information Network, Online Database). Seeds from all genotypes were vernalized in cooling box under 2^{o}C for 6 weeks. After this period plantlets were planted into small plastic pots, displaced into growth chamber (incubator-type) and grown under photoperiod 16/8 h (day/night), temperature 21/18^{o}C and light intensity 250 μmol.m^{-2}.s^{-1} at the leaf level. After 6 weeks (stem elongation growth stage) the assimilation pigments content, dry mass, leaf area and selected photosynthetic parameters (based on chlorophyll fluorescence technique) were assessed.

Table 1: Measured and calculated JIP-test parameters

Tabuľka 1: Parametre JIP-testu použité vo výpočtoch

Parameter	Name and basic physiological interpretation
F_t	Fluorescence level at time t
F_o	Basal fluorescence, fluorescence level at time 0.05 ms
$F_m = F_P$	Maximum fluorescence (the measured "peak" F_P value)
$V_t = (F_t - F_{50\ \mu s})/(F_m - F_{50\ \mu s})$	Relative variable fluorescence at time t
$V_J = (F_{2ms} - F_{50\ \mu s})/(F_m - F_{50\ \mu s})$	Relative variable fluorescence at time of J-step (2ms)
$V_I = (F_{30ms} - F_{50\ \mu s})/(F_m - F_{50\ \mu s})$	Relative variable fluorescence at time of I-step (30 ms)
$F_V/F_m = \varphi_{Po} = 1 - F_{50\ \mu s}/F_m$.	Maximum quantum yield of primary PSII photochemistry
$F_V/F_o = (F_m - F_{50\ \mu s})/F_o$	The ratio of maximum quantum yield of photochemistry (F_V/F_m) and competitive non-photochemical processes (F_o/F_m) of PSII in dark adapted state
$dV/dt_0 = 4\cdot(F_{300\mu s} - F_{50\ \mu s})/(F_m - F_{50\ \mu s})$	Initial slope of relative variable fluorescence
$RC/CS_m = [(\varphi_{Po}\cdot V_J)/(dV/dt_o)]\cdot F_m$	Number of active PSII RC per (excited) leaf cross section CS_m
$TR/CS_m = \varphi_{Po}\cdot F_m$	Maximal trapping rate of absorbed photons per CS_m
$ET_o/CS_m = \varphi_{Po}\cdot(1 - V_J)\cdot F_m$	Electron transport flux from reduced Q_A to Q_B per excited cross-section CS_m
$DI/CS_m = (1 - \varphi_{Po})\cdot F_m$	Effective dissipation energy in active RCs per CS_m
$REo/CS_m = \varphi_{Po}.(1 - V_I).F_m$	Electron transport flux from reduced Q_B to PSI end acceptors per excited cross-section CS_m
$PI_{CSm}=[RC/CS_m].[\varphi_{Po}/(1- \varphi_{Po})]\cdot[\psi_{ET2o}/(1-\psi_{ET2o})]$	Performance index (PI_{ABS}) for the photochemical activity

Abbreviations: PS I = photosystem I; PS II = photosystem II; RC = reactive centres; CSm – optical cross-section; QA – primary PSII quinone acceptor

Leaf area of whole plants was detected by computer scanning, dry mass by weighting after drying (80°C, in drier chamber). Assimilation pigments contents were measured in control leaves as follows: The segments of the youngest mature leaves of tetraploid wheat genotypes were homogenized with using sea sand, $MgCO_3$ and 100% acetone and then extracted with 80% acetone. Extracts were centrifuged 2 minutes at 2500 rpm. Absorbance (A) of the solution was measured by UV-VIS spectrophotometer (Jenway, UK), at 470 nm, 647 nm, and 663 nm, with correction for scattering at 750 nm; the measurements were done in three repetitions. The concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids (Car) in $mg*l^{-1}$ were determined by using the equations of Lichtenthaler (1987):

Chl *a* = $12.25*(A_{663} - A_{750})-2.79*(A_{647} - A_{750})*D$

Chl *b* = $21.50*(A_{647} - A_{750})-5.10*(A_{663} - A_{750})*D$

Chl *a*+*b* = $7.15*(A_{663} - A_{750})+18.71*(A_{647} - A_{750})*D$

Car = $[(1{,}000*(A_{470} - A_{750}) -1.82*(\text{Chl } a) - 85.02*(\text{Chl } b))/198]*D$

The concentrations of the pigments were calculated in mg dm^{-3}; A_n was the absorbance at given wavelengths (n) after correction for scattering at 750 nm; D was the optical thickness of cuvette; results were also recalculated in mg m^{-2} using the volume of solution and the area of leaf segments: [mg m^{-2}] = V/1000*1/A, when V is volume of 80% acetone and A is area of leaf segments. Statistical analyses were

performed by one way analysis of variance (results not presented here), the weighted means and standard errors were plotted in tables.

Subsequently plants were treated by 42^{o}C temperature in light for 6 hours in fully watered conditions, and then the measurements of rapid chlorophyll *a* fluorescence kinetics on fully developed young (penultimate) plant leaves were done. Intact leaves were adapted to darkness for 30 min using light-withholding clips and then measured using continuous illumination with intensity 3500 μmol.m^{-2}.s^{-1} for 1 second (saturation pulse). Chlorophyll *a* fluorescence induction curves were obtained by using portable Handy PEA fluorimeter (Plant Efficiency Analyser, Hansatech Instruments Ltd., Kings Lynn, UK). Fast fluorescence transients (the OJIP phase, where O stands for origin (minimal fluorescence); J and I are inflections, and P stands for the peak (maximum fluorescence), thus obtained, were analyzed by the "JIP test" (Strasser, Strasser 1995; Strasser et al., 2004; for review see Stirbet, Govindjee, 2011). The measured and calculated JIP test parameters are described in table 1.

Results and Discussion

Chlorophyll *a* and *b* participate on light energy conversion to biochemical energy together with carotenoids. Carotenoids also serve as a protection component against excessive solar radiance (Havaux, 1998; Govindjee, 2004). Chlorophyll content correlates with photosynthetic performance and crop yield potentials. Production of high biomass depends on leaf area greatness and its structure - including content of chlorophylls, carotenoids, and their functional demonstrations (Nelson, 1988). These functional demonstrations we measured by rapid chlorophyll *a* fluorescence kinetics method.

The table 2 shows that genotypes ISP6 and TRG7 achieved in evaluated parameters better results (i.e. higher values) in comparison with remaining genotypes. The genotypes with preliminary worse results (i.e. lower values) were CAR1, TIM1 and ARM7. High values of plant dry mass and plant leaf area do not correlate always with high values of pigments content – they can be in a sharp contradiction for specific genotype even, as in a case DCS9.

Table 2: Measured values of assimilation pigments, dry mass and leaf area

Tabuľka 2: Namerané hodnoty koncentrácie pigmentov, suchá hmota a listová plocha rastlín

Genotypes	Chlorophyll *a* (mg m^{-2})	Chlorophyll *b* (mg m^{-2})	Carotenoids (mg m^{-2})	Plant dry mass (g plant^{-1})	Plant leaf area (m^{2}.plant^{-1})
TRG7	209,5 ± 3.9	96 ± 3.4	57.3 ± 2.4	0.962 ± 0.12	0 .0294 ± 0.0037
ISP6	206.6 ± 10.4	118.1 ± 9.9	56.5 ± 1.3	0.488 ± 0. 028	0.0174 ± 0.001
ARM7	134.9 ± 15.6	47.7 ± 6.9	36.8 ± 6.9	0.74 ± 0.07	0.0192 ± 0.0018
RONC	165.2 ± 3.3	85.9 ± 6.6	42.6 ± 0.8	0.71 ± 0.05	0.0118 ± 0.0008
DCS9	152.3 ± 8.2	61.9 ± 0.9	40 ± 4.5	1.333 ± 0.064	0.0368 ± 0.0018
TRN8	168 ± 3.0	93.3 ± 2.8	39.3 ± 1.4	0.697 ± 0.082	0.0138 ± 0.0016
PLN8	165.2 ± 7.9	128.1 ± 1.6	38.2 ± 1.9	0.6 ± 0.05	0.019 ± 0.0016
TRI6	194.2 ± 8.7	113.9 ± 4	53.8 ± 3.5	0.548 ± 0.031	0.0155 ± 0.0009
CAR1	121.4 ± 5.9	116.8 ± 4.9	27.7 ± 2.3	1.31 ± 0.07 9	0.0277 ± 0.0017
TIM1	152.8 ± 6.5	117.3 ± 0.8	34.7 ± 1.6	0.354 ± 0.0 64	0.0237 ± 0.0017

* mean ± standard error

Illumination of dark adapted photosynthetic samples leads to emission of the chlorophyll *a* fluorescence. The chlorophyll fluorescence intensity plotted as a function of time is denoted as chlorophyll fluorescence induction. Such a curve measured under continuous light has a fast (less than one second) exponential phase and a slow decay phase (duration of few minutes). The nomenclature for "OJIP" curve includes O for origin or $F_o = F_0$ level measured at 50 µs (or less) after illumination, J and I represent intermediate states measured after 2 ms and 30 ms, and P is the peak or $F_p = F_m$ (maximal fluorescence). In heat treated samples another peak arises between F_o and F_J level at 300 µs application, which is usually called K-step (Guisse et al., 1995; Srivastava et al., 1997; Strasser et al., 2004). The OJIP transient can be used for the estimation of the photochemical quantum yield of PS II photochemistry and the electron transport properties. The numerical analysis of OJIP fluorescence curve called the JIP-test (Strasser et al., 1995) can be used to monitor the effect of various biotic and abiotic stresses and photosynthetic mutations affecting the structure and function of the photosynthetic apparatus (Strasser et al., 2000).

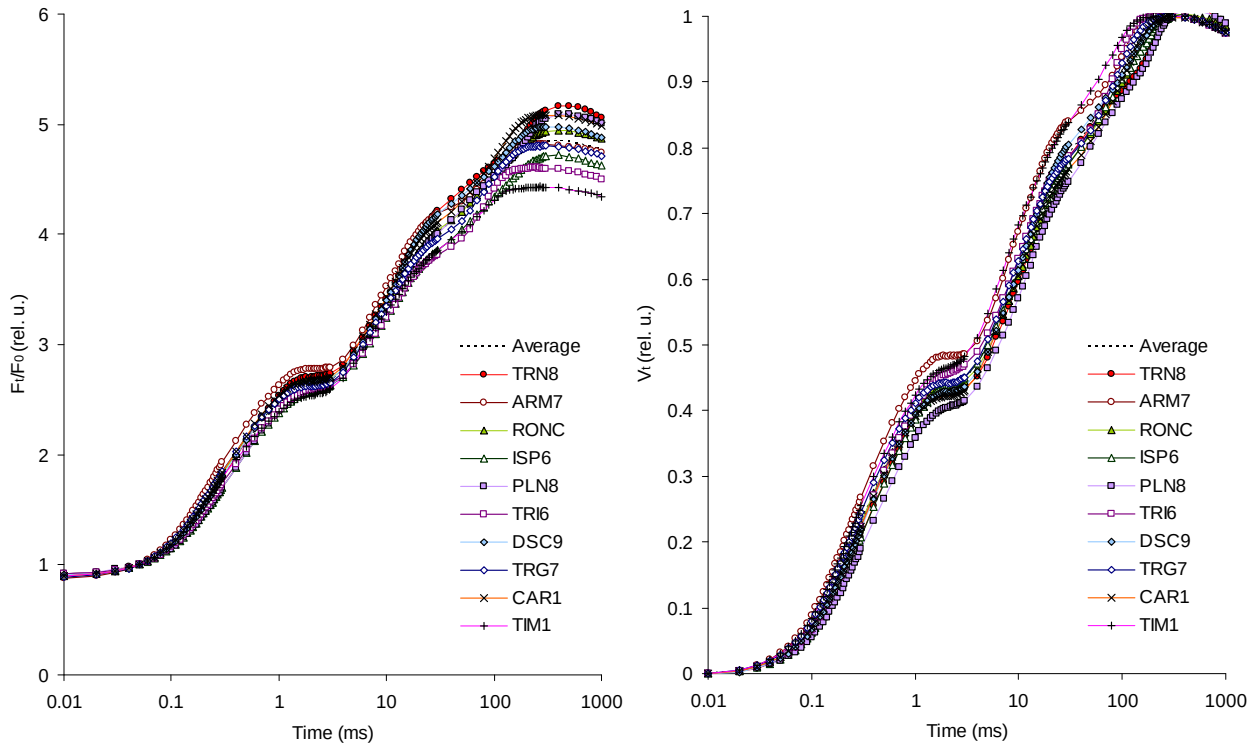

Figure 1: a) The rapid chlorophyll fluorescence kinetics in plants of tetraploid wheat genotypes. The curves are shown with normalised F_o. The average curve is shown as a short dashed line. b) The graph of relative variable fluorescence (V_t) observed in samples showing the different proportionality of JIP-transients in individual genotypes.

Obrázok 1: a) Kinetika rýchlej fluorescencie chlorofylu pri rastlinách tetraploidných genotypov pšenice. Krivky sú zobrazované s normalizovanou F_o. b) Graf relatívnej premennej fluorescencie (V_t) pozorovanej vo vzorkách ukazuje rozdielnu proporcionalitu JIP-prechodov v genotypoch.

In our case the OJIP-curve (fig. 1) indicates diversity of photochemical properties in leaves of individual genotypes. Numerical analyses of JIP-tests (table 3 and 4) depict serious differences among genotypes. The values of F_o, F_m, F_v and F_v/F_m in table 3 show us that genotypes had normal photosynthetic apparatus without anomalies and without evident stress symptoms. The F_v/F_o, V_J and V_i values determinate diversity which is further analysed by parameters which describe energy fluxes and total performance of photochemical processes in table 4. RC/CS_m parameter expresses relative number of reactive centres in chlorophylls per area unit. TR_o/CS_m represents efficiency of photon energy transmission by the assimilation pigments (i.e. the first step of photochemistry). ET_o/CS_m shows the first phase of electron transport in the photochemistry. DI_o/CS_m presents a part of energy dissipated in the photochemistry. RE_o/CS_m involves a part of energy transported beyond the PSI, i.e. to its electron acceptors. $PI(cs_m)$ is an integral parameter, which reflects global performance of the primary processes of photosynthesis. It was proved that $PI(cs_m)$ value is in a good correlation with markers of vitality and photosynthetic productivity (Živčák et al., 2008b). Therefore we regard it as a reliable indicator of plant photosynthetic performance.

Table 3: Values of basic JIP-test parameters

Tabuľka 3: Hodnoty základných parametrov JIP-testu

Genotype	F_o	F_m	F_v	F_v/F_m	F_v/F_o	V_J	V_i
TRN8	458 ± 2.9	2363 ± 17.3	1905 ± 15.7	0.81 ± 0.001	4.45 ± 0.035	0.41 ± 0.005	0.77 ± 0.008
ARM7	537 ± 4.5	2587 ± 13.1	2050 ± 12.8	0.79 ± 0.002	4.2 ± 0.047	0.47 ± 0.004	0.84 ± 0.005
RONC	518 ± 3.7	2521 ± 21.1	2004 ± 20.2	0.79 ± 0.002	4.17 ± 0.047	0.42 ± 0.004	0.77 ± 0.004
ISP6	508 ± 10	2395 ± 28.1	1888 ± 20.9	0.79 ± 0.003	3.97 ± 0.062	0.42 ± 0.007	0.77 ± 0.009
PLN8	490 ± 10.6	2483 ± 44.9	1993 ± 34.9	0.8 ± 0.001	4.28 ± 0.037	0.39 ± 0.002	0.74 ± 0.007
TRI6	490 ± 3.6	2256 ± 53.6	1766 ± 51.9	0.78 ± 0.005	3.84 ± 0.104	0.44 ± 0.013	0.78 ± 0.007
DCS9	508 ± 6.1	2530 ± 28.1	2022 ± 22.9	0.8 ± 0.001	4.27 ± 0.031	0.41 ± 0.005	0.8 ± 0.006
TRG7	551 ± 7.1	2661 ± 27.5	2110 ± 22.4	0.79 ± 0.002	4.16 ± 0.037	0.42 ± 0.002	0.78 ± 0.003
CAR1	487 ± 7.1	2477 ± 22.4	1990 ± 16.5	0.8 ± 0.002	4.4 ± 0.033	0.41 ± 0.008	0.76 ± 0.009
TIM1	519 ± 5.7	2298 ± 31.9	1779 ± 33.2	0.77 ± 0.004	3.72 ± 0.091	0.45 ± 0.008	0.83 ± 0.006

* mean ± standard error of estimate

From the comparison of evaluated parameters in table 4 (in conditions without stress) we found out that genotype PLN8 reached the best results, based on relative highest achieved values (with exception DI_o/CS_m parameter, where the lowest values are just required). Good results were achieved for genotypes TRN8, CAR1 and DCS9, too. The worse (i.g. lower) values (with the same exception for DI_o/CS_m parameter) were recorded for TRI6, ARM7 and TIM1, mainly. The radar plot (fig. 2) illustrates the relative values of all JIP-test parameters (mean value represents unity) measured in penultimate leaves of observed genotypes. It is a multiparametric description of the structure and function of each photosynthetic sample. This type of presentation provides a direct visualization of sample behaviour and thus facilitates the comparison of plant material as well as classification of the effect of different environmental stressors on it in terms of the modifications it undergoes to adapt to new conditions (Strasser et al., 2004). Parameters RE_o/CS_m a $PI(cs_m)$ show large genotypic differences. The best genotype in condition without stress was PLN8,

genotypes TRI6, ARM7 and TIM1 mainly were markedly below the average. However, situation was changed when genotypes were treated by high temperature (42°C) for 6 hours under fully watered conditions.

Table 4: Values of phenomenological energy fluxes (in relative units) derived from chlorophyll fluorescence measurements in leaves of observed genotypes

Tabuľka 4: Hodnoty fenomenologických tokov energie (v relatívnych jednotkách) odvodené z meraní fluorescencie chlorofylu v listoch skúmaných genotypov

Genotype	RC/CS_m	TR_o/CS_m	ET_o/CS_m	DI_o/CS_m	RE_o/CS_m	$PI(cs_m)$
TRN8	1036 ± 10.1	1905 ± 15.7	1124 ± 12.8	458 ± 2.9	538 ± 21.8	62118 ± 1418
ARM7	982 ± 12.5	2050 ± 12.8	1095 ± 11.4	537 ± 4.5	425 ± 13.5	43290 ± 1524
RONC	997 ± 12	2004 ± 20.2	1170 ± 10.6	518 ± 3.7	579 ± 10.5	54362 ± 1189
ISP6	1059 ± 15.2	1888 ± 20.9	1088 ± 20.6	508 ± 10	553 ± 28	54585 ± 2863
PLN8	1136 ± 25.4	1993 ± 34.9	1220 ± 22.2	490 ± 10.6	650 ± 18.2	72980 ± 1709
TRI6	951 ± 32.8	1766 ± 51.9	989 ± 49.7	490 ± 3.6	494 ± 25.6	45125 ± 4764
DCS9	1041 ± 13.6	2022 ± 22.9	1184 ± 13.1	508 ± 6.1	507 ± 14.7	58964 ± 1852
TRG7	1015 ± 15.4	2110 ± 22.4	1214 ± 14	551 ± 7.1	594 ± 8.3	52874 ± 1357
CAR1	1000 ± 21	1990 ± 16.5	1179 ± 12.5	487 ± 7.1	591 ± 18.2	60485 ± 3826
TIM1	861 ± 15	1779 ± 33.3	982 ± 32.5	519 ± 5.7	382 ± 19.2	36780 ± 2596

* mean ± standard error of estimate

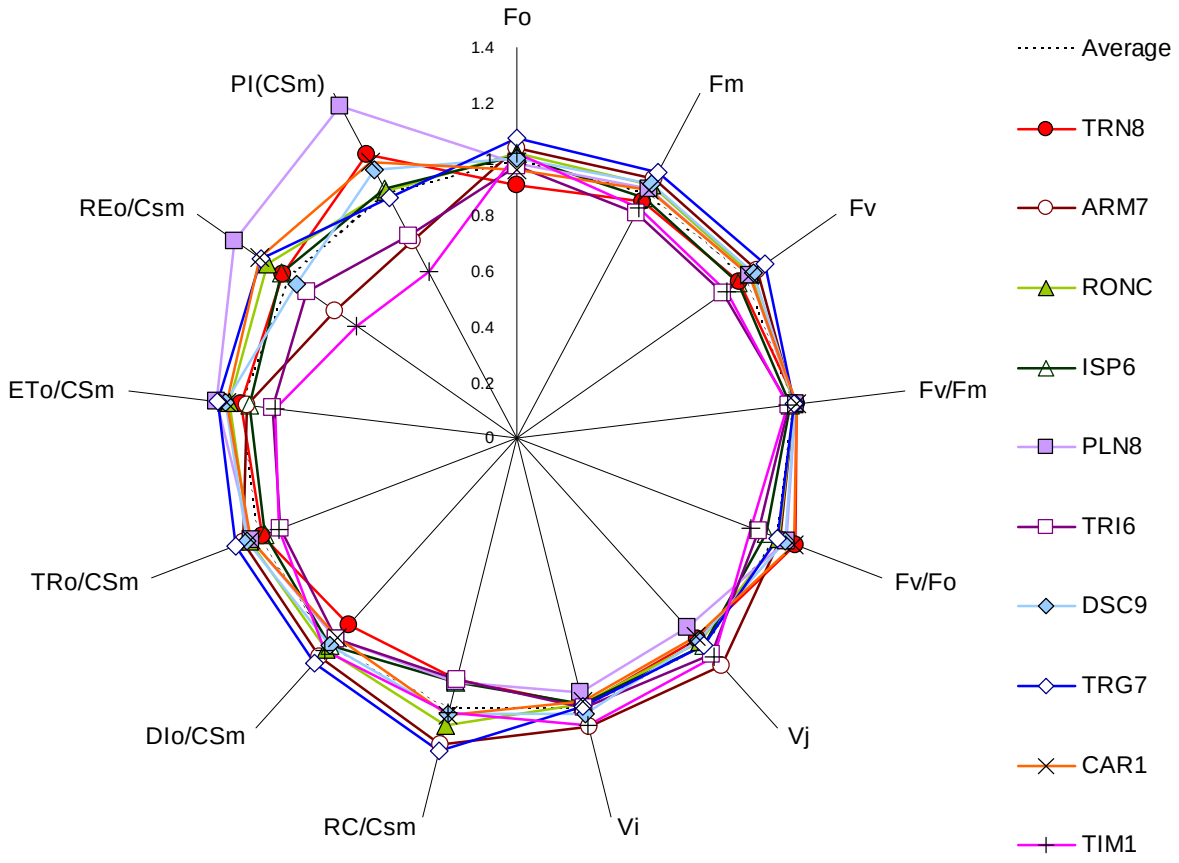

Figure 2: The radar plot showing the relative values of all JIP-test parameters (mean value represents unity) measured in leaves of observed genotypes.

Obrázok 2: Radarový graf zobrazujúci relatívne hodnoty všetkých parametrov JIP-testu (stredná hodnota sa rovná jednej) namerané v listoch skúmaných genotypov.

High temperature hits the photosynthesis by change of excitation energy distribution on thylakoid membrane level (Berry, Björkman, 1980); it changes activity of Calvin cycle and other metabolic processes. With increasing temperature the destruction of single parts of the photosystem occurs (at first, the light harvesting complexes avulsion) and thereafter protein denaturising. Fast kinetics of chlorophyll *a* fluorescence enables to determine regress of maximal quantum efficiency (yield) of PSII (F_v/F_m) as well as damage of oxygen evolving complex (OEC), which is manifested by a K-step appearance. Increase of a variable fluorescence in 0.3 ms represents parameter which is specific for high temperatures effect and is suitable for plant photosynthetic apparatus thermostability evaluation by a heat test according to Živčák et al., 2010. The temperature of 40°C permits to differentiate the genotypes according to their thermostability, in young plants especially (Živčák et al., 2008a); in adult plants grown in field conditions mainly the temperature of 42°C is recommended (Brestic et al., 2012).

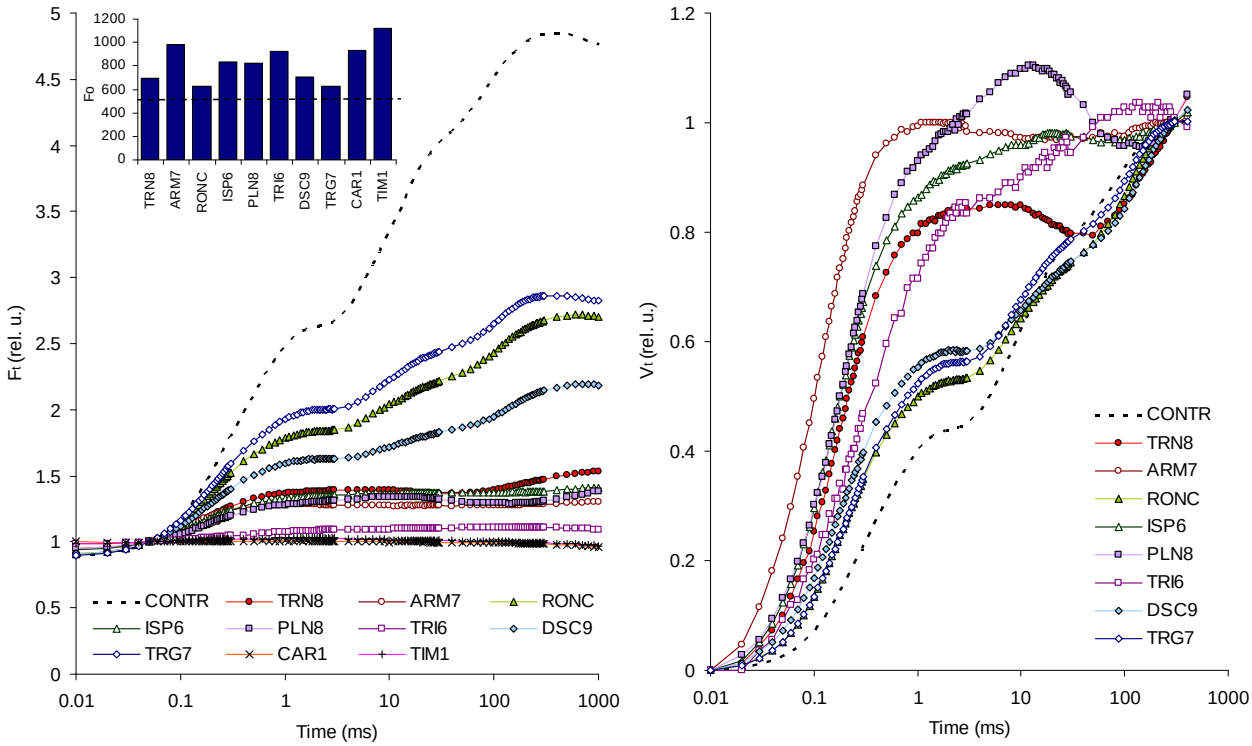

Figure 3: a) The rapid chlorophyll fluorescence kinetics in heat-stress exposed plants of wheat genotypes compared to average curve in non-stressed plants (CONTR; short-dashed line). The curves are shown with normalised F_o. The observed F_o values are shown in small graph above the curves (the short-dashed line shows average F_o value in non-stressed plants). b) The graph of relative variable fluorescence (V_t) observed in heat-stressed samples compared to average non-stressed curve (CONTR, short-dashed line).

Obrázok 3: a) Kinetika rýchlej fluorescencie chlorofylu v genotypoch pšenice vystavených teplotnému stresu v porovnaní s priemerom krivky nestresovanej

kontroly (CONTR; prerušovaná čiara). Krivky sú zobrazené s normalizovanou F_o. Pozorované F_o hodnoty sú zobrazené v malom grafe nad krivkami (prerušovaná čiara ukazuje priemer hodnôt F_o pri nestresovaných rastlinách). b) Graf relatívnej premennej fluorescencie (V_t) pozorovanej vo vzorkách stresovaných teplom v porovnaní s priemerom krivky pri nestresovaných kontrolných rastlinách (CONTR; prerušovaná čiara).

Results obtained from plants under heat stress in our experiment are visualised in figures 3 and 4. In figure 3a) we can see abnormal curves in heat treated plants in comparison with a non-treated control curve. Whatever regress from this control value is negative. The most sensitive to heat stress were genotypes TRI6, TIM1 and CAR1. The smallest sensitivity and so at once the best resistance toward heat stress displayed genotypes TRG7, RONCAL and DCS9. Other genotypes are more sensitive to heat stress as visualised on a small graph in the top left corner of figure 3a) with F_o parameter. F_o is an indicator of disrupted (non-functional) reactive centres. Figure 3b) presents a change in fluorescence kinetics, a change of a kinetics curves after heat stress, when an occurrence of peak at 0.3 ms indicates the damage of oxygen evolving complex (OEC). Keeping the kinetics near to the normal curve shape suggests the photosynthetic apparatus resistant to high temperature. Genotypes CAR1 and TIM1 were not included to the graph on figure 3b) as the mostly suppressed fluorescence growth made the mathematical normalisation inapplicable in those cases. Finally, in figure 4, the genotype resistance was numerically expressed by parameter F_v/F_o, which represents the ratio of photochemical processes (i.e. potential usable energy) to non-photochemical processes (i.e. energy which is vanished as unproductive). Whereas in the most resistant genotypes to heat stress approximatelly 50% decrease of sun (light) energy utilization happens thereafter, this decrease is substantially more accentuated in the remaining genotypes. In a case of the most sensitive genotypes to heat stress (CAR1, TIM1, TRI6) the portion of absorbed energy useful in the photosynthetic processes was negligible.

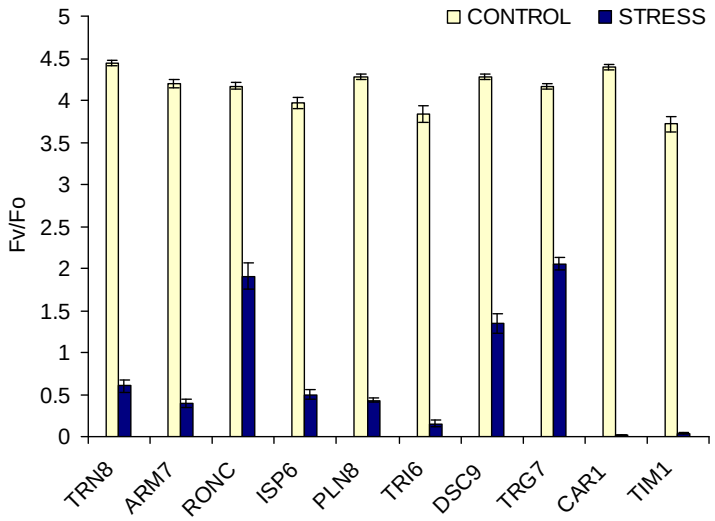

Figure 4: Values of F_v/F_o parameter recorded in non-stressed (yelow columns) and heat-stressed samples (blue columns). The error bars represent the standard error.

Obrázok 4: Hodnoty parametra F_v/F_o zaznamenaného v nestresovaných vzorkách (žlté stĺpce) a teplom stresovaných vzorkách (modré stĺpce). Chybové úsečky vyjadrujú štandardnú chybu. .

As it was shown above, application of fast chlorophyll *a* fluorescence kinetics enables to identify heat resistant or susceptible lines; this method is useful even in much larger collections of crop genotypes. Our results can serve for further research on high temperature tolerance or the proposed methods could be used practically, in different crop breeding programs (Brestic et al., 2012).

Conclusions

Combining the modern method of rapid chlorophyll *a* fluorescence kinetics with the assimilation pigments analyses enabled to compare both photosynthetic performance as well as the heat tolerance in a set of tetraploid wheat genotypes. According to the values of parameter performance index for the photochemical activity (PI_{CSm}) obtained in conditions without heat stress, the ranking of individual genotypes from the best to worst was arranged: PLN8 → TRN8 → CAR1 → DCS9 → ISP6 → RONCAL → TRG7 → TRI6 → ARM7 → TIM1. According to heat stress the resistance of genotypes indicated by values of F_v/F_o parameter, the ranking from the best to worst was as follows: TRG7 → RONCAL → DCS9 → TRN8 → ISP6 → PLN8 → ARM7 → TRI6→ TIM1→ CAR1. Based on our data, we suggest the genotypes TRG7 and RONCAL as perspective donors of better thermostability of photosynthetic apparatus useful within the wheat breeding programs aimed to increase of abiotic stress tolerance.

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