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Controlled Long-Term Water Deficiency and its Impact on the Fluorescence Emission of Tomato Leaves during Stress and Re-Watering

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Summary

We explored the potential of fluorescence ratios, based on multiple fluorescence excitation indices, as compared to chlorophyll fluorescence (ChlF) recorded with the pulse amplitude modulation (PAM) technique, for early and sensitive detection of water deficiency (WD) in tomato (*Solanum lycopersicum*) leaves. In this context, we hypothesized that the indices BFRR_UV (ratio of BF (blue fluorescence) to FRF (far-red fluorescence), both excited with UV-light), FLAV (logarithm of the ratio of red-excited FRF to the UV-excited FRF) and NBI (ratio of UV-excited FRF to the red-excited red fluorescence) of the multiple fluorescence excitation technique reveal the onset and intensity of WD in plants exposed to long-term WD, as well as during re-watering. For this purpose, three tomato genotypes were grown in the greenhouse under well-watered or long-term WD conditions. The decrease of the osmotic potential, relative water content and chlorophyll concentration as well as the increase in proline concentration confirmed the impact of WD leading to drought stress. ChlF recorded with the PAM technique showed significant differences between control and WD treatment in the relative apparent electron transport rate and the coefficient of photochemical quenching (qP). The indices BFRR_UV and FLAV significantly increased while the NBI significantly decreased during WD. As compared to the qP, the ratios BFRR_UV and FLAV provide information of equivalent quality concerning the genotype-specific responses to WD. Thus, the multiple fluorescence excitation technique establishes a valuable, fast and practical tool for the *in situ* monitoring of the physiological status of the plants without the need of dark adaption as required for the PAM recordings.

Key words. abiotic stress – drought stress – physiological responses – *Solanum lycopersicum* – water shortage

Introduction

The fluorescence emission of leaves has emerged as a fast and reliable approach to detect and evaluate the influence of stresses on plants, and might be used to amend or partially replace time consuming evaluations based on plant growth or lab analysis. Fluorescence datasets provide fast information about the plant's physiological status (Cerovic et al. 1999). In this context, the chlorophyll fluorescence (ChlF) has become a well-established tool in the last decades (Baker and Rosenqvist 2004). In particular, the chlorophyll (Chl) a fluorescence emission, recorded by pulse amplitude modulation (PAM) systems, provides extensive information about the photosystem II (PSII) (Bilger et al. 1995). On the other hand, the value of the blue fluorescence (BF) for a better understanding of plant physiological responses has received more attention (Morales et al. 1996).

Fluorescence can be excited by ultra-violet (UV)-radiation and by visible light from blue to orange-red. In order to generate fluorescence emissions carrying as much information as possible, the use of different excitation lights is indispensable. The UV-excitation (375 nm) mainly penetrates the upper cell layers of the tissue, being predominantly absorbed in the epidermis. Similar pattern has been observed with red light excitation (635 nm). In contrast, green light (510 nm) penetrates into deeper cell layers of the leaf (Buschmann et al. 2008). Thus, the combination of BF and ChlF, excited by more than one light source, seems to be a promising approach to get detailed information about genotype specific response in terms of water deficiency (WD) and during re-watering (RW).

Absolute fluorescence intensities contain essential evidences about the plant physiology. However, they are susceptible to morphological and external factors such as leaf geometry and measurement settings, respectively. To

achieve more reliable information, the calculation of ratios of the peaks from the absolute intensities represents a sensible solution for comparisons of treatments (Cerovic et al. 1999).

Recent investigations demonstrate the potential of the ChlF based detection of WD in tomato plants (Mishra et al. 2012); although the potential of the multiparametric fluorescence technique particularly in horticultural crops remains widely underexplored. Particularly fluorescence ratios determined *in situ* under light conditions provide much faster information about the physiological status of the plant as the PAM technique requiring dark adaption of the plants. Therefore, the aim of the present study was to explore the influence of WD and the RW on the fluorescence emission of leaves of adult tomato (*Solanum lycopersicum*) plants.

In this context, we hypothesized that the fluorescence ratios, the BF to far-red fluorescence (FRF) ratio after UV-light excitation (BFRR_UV), the logarithm of the ratio FRF after red light excitation to the FRF after excitation with UV-light (FLAV), and the ratio of FRF after UV-light excitation to red fluorescence after red light excitation (NBI) of the multiple fluorescence technique reveal the onset and intensity of stress in plants exposed to long-term WD, as well as RW of the plants. As reference parameters of the PAM ChlF we choose the relative apparent electron transport rate (ETR) and the coefficient of photochemical quenching (qP). These records estimate the fraction of open centers of the PS II (Baker 2005).

Materials and Methods

Abbreviations

BF, blue fluorescence; BFRR_UV, blue-to-far-red fluorescence ratio; Chl, chlorophyll; ChlF, chlorophyll fluorescence; CT, control treatment; 'Cupido', *Solanum lycopersicum* L. F1 hybrid 'Cupido'; cvs, cultivars; d, days; DAT, days after treatment induction; DM, dry matter; ETR, relative apparent electron transport rate; FLAV, logarithm of the ratio of red-excited FRF to the UV-excited FRF; FM, fresh matter; Fm, maximum fluorescence; Fo, ground fluorescence; FRF, far-red fluorescence; 'Harzfeuer', *Solanum lycopersicum* L. F1 hybrid 'Harzfeuer'; NBI, ratio of UV-excited FRF to the red-excited red fluorescence; NS, nutrient solution; PAM, pulse-amplitude-modulated chlorophyll fluorescence; Pro, proline; PSII, photosystem II; qP, coefficient of photochemical quenching; RF, red fluorescence; 'Rio Grande', *Solanum lycopersicum* L. var. 'Rio Grande'; RW, re-watering; RWC, relative water content; TM, turgid mass; UV, ultra-violet; WD, water deficiency; ψ_π, osmotic potential

Genotypes and growth conditions

Seeds of the *Solanum lycopersicum* L. cultivars 'Cupido', 'Harzfeuer' (both Volmary GmbH, Münster, Germany) and 'Rio Grande' (donation of Dr. Mustafa Demirkaya, Erciyes University, Turkey) were used in the present study.

Experiments were conducted under greenhouse conditions. Seeds were steeped in the dark (20 °C for three days). Germinated seeds were transferred into rock wool trays and cultivated until the third leaf stage. As next, plantlets were transplanted into 10 l pots filled with perlite (Lerligran G, Knauf Perlite GmbH, Dortmund, Germany) and placed on two greenhouse tables. Fertigation was based on Kristallon™ Blau (Yara GmbH & Co. KG, Dülmen, Germany) and amended with calcium nitrate tetrahydrate ($Ca(NO_3)_2$) (99 % purity, AppliChem GmbH, Darmstadt, Germany). Excessive nutrient solution (NS) was drained off at the bottom of the pots after perlite saturation. NS and its spillover were kept separated from each other and NS was not recycled. The average day/night air temperature 40 cm above the tables was 28/18 °C with an air humidity of 50–70 %.

Treatments and sampling

Plants of each cultivar were grown for about 5 weeks and then divided into two treatments (n = 10 plants per treatment): T1, well watered control plants; T2, WD (20 % of the volume of the control plants). Starting at plant age of 33 d WD was induced and held for 46 d; thereafter all plants were fertigated in excess for 16 d enabling the plants to recover. Leaf samples were taken at day 46 and 62 after treatment induction. Freeze dried samples (Gamma 1–16 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) were ground for determinations of Chl and proline (Pro).

Analytical determinations and fluorescence recordings

The relative water content (% RWC) of the leaves was calculated as:

$$\% \text{ RWC} = \left(\frac{\text{FM} - \text{DM}}{\text{TM} - \text{DM}}\right) \times 100$$

Leaf disks (13 mm diameter) were punched out and the fresh matter (FM) was determined. To determine the turgid mass (TM), samples were immersed in deionized water for 24 h in the dark. As next, samples were oven dried (80 °C/48 h) to obtain the dry matter (DM).

About 4 g of leaf FM were squeezed; 200 µl of the extract was centrifuged at 10,000 rpm for 10 min. at 4 °C. Osmotic potential (ψ_π) of 15 µl supernatant was analyzed twice (Osmomat 030-D, Gonotec GmbH, Berlin, Germany). Means [osmol kg^{-1}] were multiplied by −2.437 (correction coefficient valid for 20 °C) to get ψ_π in MPa (Taiz and Zeiger 2007).

To determine the Pro concentration, 3 ml sulfosalicylic acid were added to 0.1 g ground DM and centrifuged at 4,200 rpm for 20 min. at 20 °C. Afterwards, 0.2 ml of the supernatant was filled up with 1.8 ml sulfosalicylic acid, 2 ml glacial acetic acid and 2 ml ninhydrine acid. The

mixture was boiled at 100 °C for 1 h in a hot water bath. After the samples cooled down to 20 °C, 4 ml toluene was added to the mixture. The upper, organic part was collected for spectrophotometric measurements (Lambda 35 UV/VIS Spectrophotometer, PerkinElmer, USA). The absorbance of extracts was evaluated at 520 nm.

The concentration of Chl a and b was analyzed from dried and ground samples (0.05 g DM) after extraction with 5 ml methanol and centrifugation at 4,000 rpm for 15 min. at 4 °C. The supernatant was transferred to 50 ml volumetric flasks. The extraction procedure with methanol was repeated three times; thereafter, the flasks were filled up to 50 ml with methanol. The absorbance of the extracts was determined at 647 nm and 664 nm with an UV-VIS spectrophotometer (Lambda 35 UV/VIS Spectrophotometer, PerkinElmer, USA).

Fluorescence measurements were performed under laboratory conditions on detached leaves, either immediately after sampling or after dark-adaptation to the room conditions. In order to minimize possible modifications after the sampling, batches of three leaves each were harvested for the sequential handling. Fluorescence determination in the time-course of the experiment was done on leaves of different physiological ages: the first measurements were conducted on the fourth leaf counted from bottom (cotyledons excluded), every 7–10 d a higher leaf level was selected according to the plant growth. Fluorescence readings were taken with two devices: the Imaging-PAM® (Heinz-Walz GmbH, Effeltrich, Germany) chlorophyll fluorometer and the multiparametric fluorescence excitation system Multiplex®3 (Force-A, Orsay Cedex, France), as described elsewhere (Leufen et al. 2013).

PAM ChlF parameters were recorded from the adaxial side of dark-adapted (30 min) leaves. After determining ground (Fo) and maximum fluorescence (Fm), specific parameters as related to the kinetic curves were evaluated over a period of 300 seconds. Based on the literature (Kramer et al. 2004), we selected the coefficient of photochemical quenching (qP; Imaging PAM, calculated as (Fm'–F)/(Fm'–Fo')) and the relative apparent electron transport rate (ETR; Imaging PAM, calculated as 0.5 × Yield × PAR × 0.84 μequivalents m^{-2} s^{-1}) as meaningful parameters.

The light source (0.5 μmol m^{-2} s^{-1} PAR) used for fluorescence excitation and actinic illumination contains 96 blue light diodes emitting at 470 nm. Fluorescence images were recorded by a black and white CCD (8.458 mm chip with 640 × 480 pixels) camera operated in 10-bit-mode at 30 frames per second, as described elsewhere (Bürling et al. 2010). Data evaluation was based on the recorded pictures. In each single image, three areas of interest (AOI) were selected: leaf edge, apex and center. Afterwards the mean of the three AOIs was calculated before running the statistical analysis.

For multiple fluorescence excitation and fluorescence ratios, BF, RF and FRF spectral bands, excited with UV, green and red light, were recorded with a hand-held multiparametric fluorescence sensor at the adaxial lamina of detached leaves. These fluorescence recordings were done immediately after harvesting the leaves. Leaves were fixed horizontally on a sample holder at a defined distance (10.5 cm) to the sensor body. A frontal cover plate having an aperture (6 cm diameter) was used to standardize the area to be measured. As indicative parameters we selected three fluorescence ratios, the BF to FRF ratio after UV-light excitation (BFRR_UV), the FLAV Index (FLAV) as expressed by the logarithm of the ratio of FRF after red light excitation to the FRF after excitation with UV-light, and the Nitrogen Balance Index (NBI) given by the ratio of FRF after UV-light excitation to RF after red light excitation.

Data analysis

Statistical analysis was done with IBM SPSS Statistics 20.0 (IBM Corporation, New York, USA). Means were compared by t-test ($P \leq 0.05$) and graphs (mean ± SE) were drawn using SigmaPlot 11.0 (Systat Software Inc., San Jose, CA, USA).

Results

Relative water content, osmotic potential, proline and chlorophyll

RWC was significantly reduced after 46 d of WD in comparison with leaves of the control treatment (CT) (Table 1). RWC was 20 % ('Harzfeuer') to 27 % ('Cupido') lower in those plants cultivated under WD. The ψ_π of the leaves revealed a significant decrease in all three cultivars (cvs) growing under WD as compared to control plants and was about 40 % lower than under well-watered conditions (Table 1). Pro contributed significantly to the adjustment of ψ_π in the leaves. Pro concentrations were significantly higher 46 d after treatment induction (DAT) as compared to the respective CT (Table 2). At the end of the WD phase, 'Rio Grande' grown under WD emerged followed by 'Harzfeuer' and 'Cupido'. After the RW period, plants of 'Harzfeuer' showed no significant differences between control and former WD treatment. In contrast, Pro concentrations in the previous WD treatments from 'Cupido' and 'Rio Grande' were significantly lower compared to the respective CT. Finally, the concentration of Chl a, Chl b and Chl a + b in leaves of the WD plants decreased significantly after 46 d compared with the CT (Table 2). During the RW period, the plants of the former WD treatment revealed a significantly higher concentration of Chl a, Chl b and Chl a + b in comparison with the fulltime well watered plants.

PAM parameters

The time curves of the ETR were distinct in plants with WD, evaluated 6 and 27 DAT, as compared to the well-

Table 1. Relative water content (% RWC), osmotic potential (ψ_π) and alteration in percent (%). Tomato leaves were harvested 46 d after inducing water deficiency (WD).

Cultivar	Treatments	RWC [%] [a]	%	ψ_π [a]	%
'Rio Grande'	Control	83.34 ± 1.40*		−1.34 ± 0.02*	
	WD	64.66 ± 0.89	−22.25 ± 1.62	−1.91 ± 0.02	−42.03 ± 1.64
'Harzfeuer'	Control	77.15 ± 0.89*		−1.39 ± 0.04*	
	WD	58.43 ± 0.40	−24.19 ± 0.83	−1.90 ± 0.01	−36.65 ± 1.31
'Cupido'	Control	78.33 ± 1.03*		−1.47 ± 0.08*	
	WD	56.67 ± 0.40	−27.56 ± 0.86	−2.05 ± 0.02	−39.87 ± 3.14

[a] Values are mean ± SE of five replicates. * significant difference between treatments (t-test, $P \leq 0.05$).

Table 2. Concentrations of proline, chlorophyll (Chl) a, Chl b, total Chl (Chl a + b) and the ratio of Chl a and Chl b of tomato leaves. The water deficit (WD) was conducted until 46 d of treatment (DAT); thereafter, plants were allowed to recover until 62 DAT when the final evaluation was done. Control plants (C) served as control.

Cultivar	Treatments	DAT	Concentration [mg g^{-1} DM][a]			
			Proline	Chl a	Chl b	Chl a + b
'Rio Grande'	C	46	5.08 ± 0.22*	14.87 ± 0.58*	2.15 ± 0.09*	17.02 ± 0.66*
	WD	46	9.15 ± 0.36	10.92 ± 0.32	1.55 ± 0.05	12.47 ± 0.36
	C	62	5.88 ± 0.47*	13.68 ± 0.51*	1.35 ± 0.10	15.03 ± 0.60*
	WD	62	4.07 ± 0.28	16.15 ± 0.19	1.60 ± 0.09	17.75 ± 0.27
'Harzfeuer'	C	46	5.28 ± 0.11*	14.88 ± 0.26*	2.24 ± 0.07*	17.12 ± 0.32*
	WD	46	8.60 ± 0.49	11.36 ± 0.52	1.66 ± 0.12	13.02 ± 0.64
	C	62	3.61 ± 0.24	13.76 ± 0.48*	1.39 ± 0.09*	15.15 ± 0.57*
	WD	62	3.37 ± 0.15	16.16 ± 0.77	1.96 ± 0.12	18.12 ± 0.89
'Cupido'	C	46	5.86 ± 0.24*	14.18 ± 0.77*	1.96 ± 0.12*	16.14 ± 0.89*
	WD	46	7.35 ± 0.69	10.34 ± 0.57	1.53 ± 0.07	11.87 ± 0.64
	C	62	6.29 ± 0.26*	11.48 ± 0.59*	1.29 ± 0.06*	12.77 ± 0.65*
	WD	62	3.97 ± 0.19	14.42 ± 0.34	1.57 ± 0.05	15.99 ± 0.39

[a] Values are mean ± SE of five replicates. * significant difference between treatments (t-test; $P \leq 0.05$) at each day after treatment.

watered CT (Fig. 1). The strongest influence of water shortage on the ETR was observed for 'Rio Grande'. Despite the distinct pattern of the curves, the biggest numerical difference between leaves of control and WD was observed in the timeframe of 100 to 150 s after start of the measurement. After the RW phase, the ETR of the former WD treatment was slightly higher than of the control plants irrespective of the cultivar (Fig. 1C, F, I).

The time course analysis of the qP demonstrated that qP was strongly affected by WD 6 DAT (Fig. 2A, D, G). The most pronounced difference was noticed in 'Rio Grande', particularly on day 6 at 100 s after first illumination (Fig. 2A). Further, the PAM-images clearly demonstrate the spatial variability of the values over individual leaves after 80 and 300 s of first illumination (Fig. 3). During the RW phase, an approximation of the curves between the former WD and the respective CT was observed (Fig. 2C, F, I).

Multiparametric fluorescence ratios

The BFRR_UV revealed WD induced changes in 'Rio Grande' and 'Harzfeuer' at 6 DAT. In general, this parameter was significantly higher in plants of the WD treat-

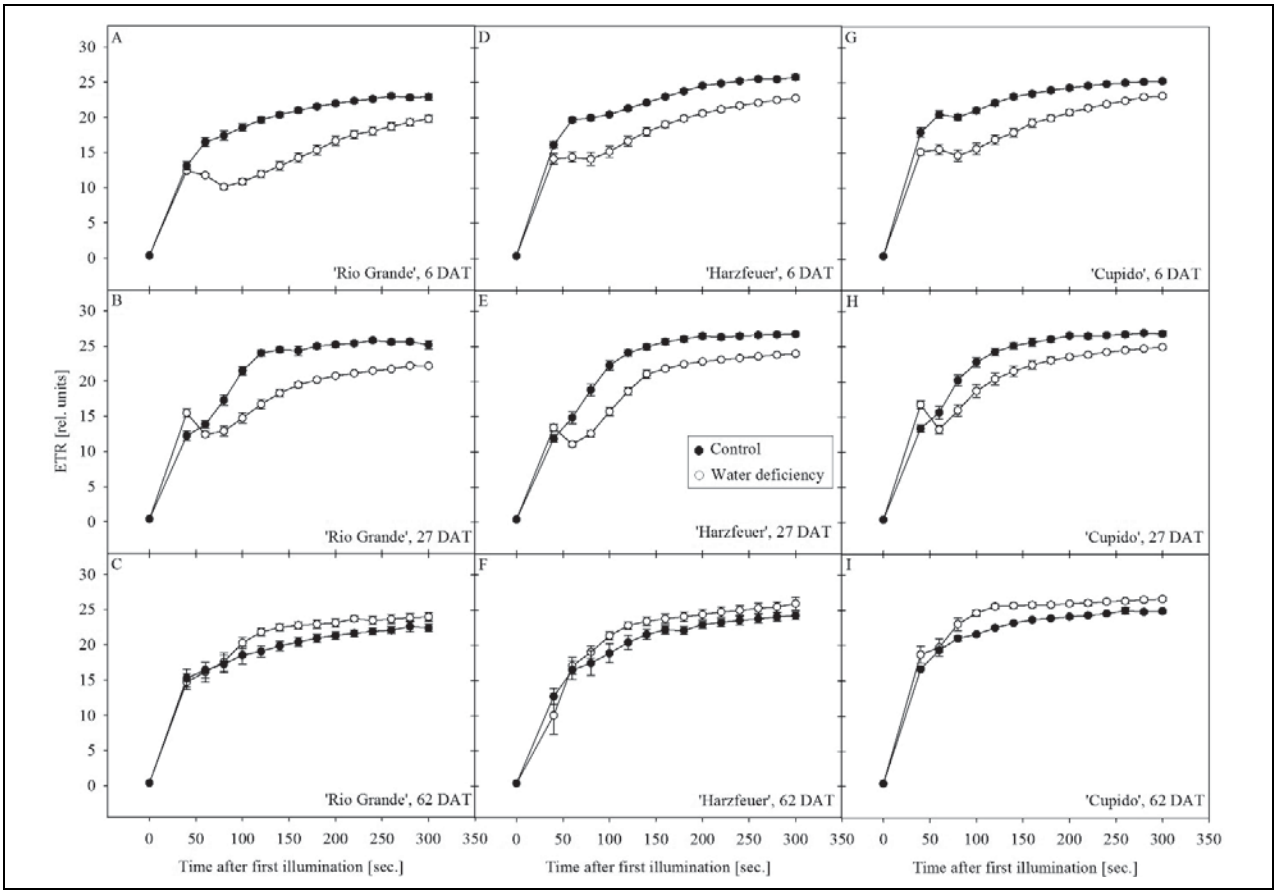


Fig. 1. Time curves of the relative apparent electron transport rate (ETR). ETR was measured on tomato leaves from the cultivars 'Rio Grande' (A–C), 'Harzfeuer' (D–F) and 'Cupido' (G–I) on day 6 (A, D, G), 27 (B, E, H) and 62 (C, F, I) of the experiment. Values represent the mean ± SE (standard error) of ten (until day 44) or five (from day 48) samples.

ment. The significant differences between control and WD plants lasted until the DAT 46, when the RW phase was initiated. Then, the significant differences registered in the previous phase were not evident anymore in 'Rio Grande' and 'Harzfeuer' (Fig. 4A, B). In contrast, the BFRR_UV in 'Cupido' remained high (Fig. 4C).

The FLAV, which is related to the accumulation of epidermal flavonols in the leaves, indicates a clear impact of the WD at 6 DAT (Fig. 4). Drought exposed plants had higher FLAV values than well-watered plants. 'Rio Grande' responded within 7 d to the RW phase (Fig. 4D); in 'Harzfeuer' the approaching of the FLAV values was observed after 11 d (Fig. 4E). Values of 'Cupido' decreased, too, but remained significantly higher than the control plants until the end of the experiment.

The NBI_R of the WD exposed plants were lower than the values of the CT. However, this parameter responded with some delay to the stress situation. Reliable significant differences between the treatments were observed from 37 DAT in 'Rio Grande' and 'Harzfeuer'. In 'Cupido', significant changes were observed starting at 23 DAT. By trend, the RW of the plants led the values to approach the normal values, but this was more evident for 'Rio Grande' and 'Harzfeuer' (Fig. 5A, B).

Discussion

Here, we proof the suitability of specific ratios of the multiparametric fluorescence technique to reveal the onset and intensity of stress in different tomato genotypes exposed to long-term WD, as well as during RW. Thereby, we selected traditional parameters (e.g., RWC, Chl a, Pro) for the monitoring of the plant's physiological status.

Plants growing under WD can undergo several anatomical, morphological, physiological, biochemical and molecular adaptations in order to maintain a positive turgor. It is evident that stomata close progressively with increased drought stress, followed by reduced net photosynthesis rates. Additionally, it is well known that a good correlation between leaf water potential and stomatal conductance exists, even under water shortage (Reddy et al. 2004). In our trial, plants exposed to WD had significantly lower RWC and ψ_π as well as higher Pro concentra-

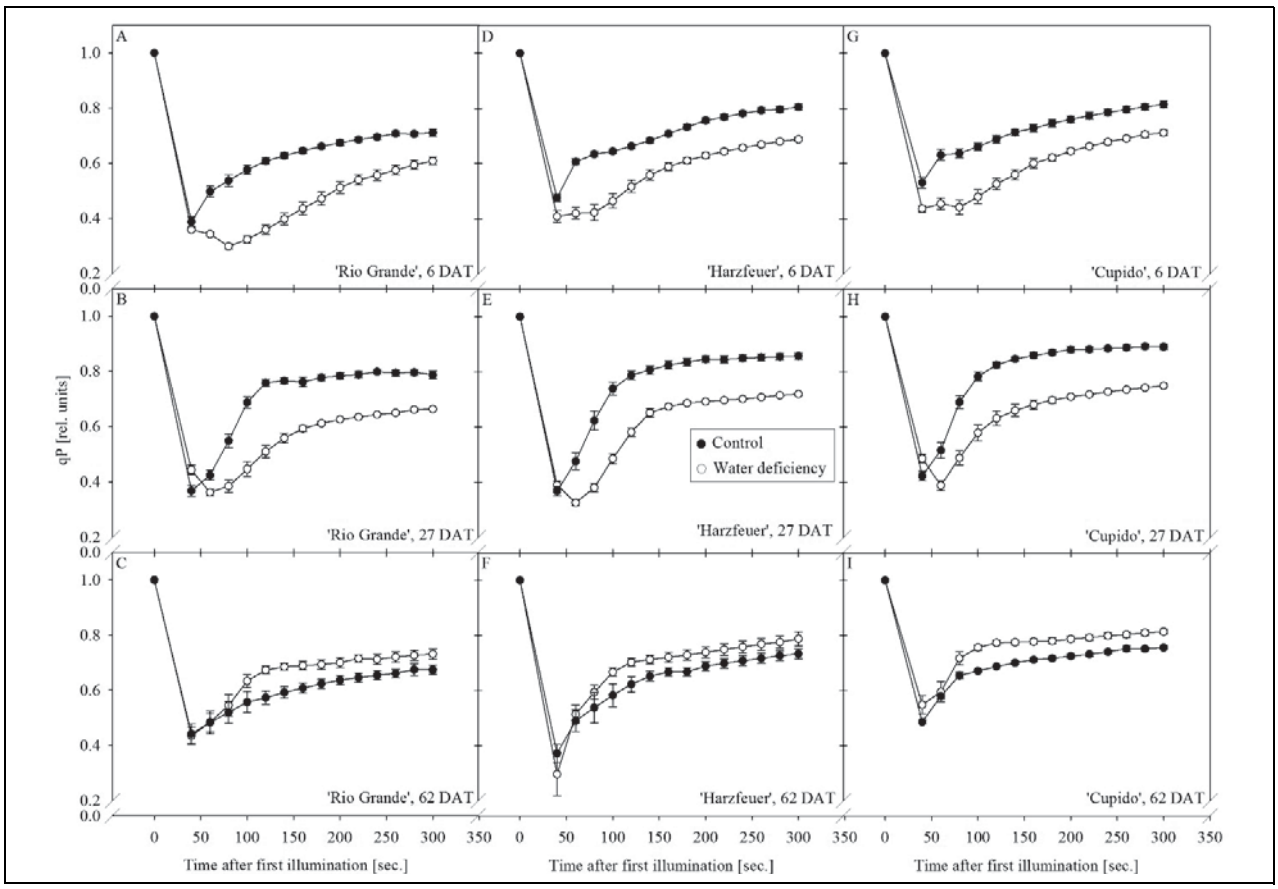


Fig. 2. Time curves of the coefficient of photochemical quenching (qP). The parameter was measured on tomato leaves from the cultivars 'Rio Grande' (A–C), 'Harzfeuer' (D–F) and 'Cupido' (G–I) on day 6 (A, D, G), 27 (B, E, H) and 62 (C, F, I) of the experiment. Values represent the mean ± SE (standard error) of ten (until day 44) and five (from day 48) samples.

tion (Table 1). Previously, these parameters were highlighted as reliable indicators of drought stress and resistance, respectively (Parry et al. 2005). To prevent water loss due to drought stress, plants accumulate osmolites such as proline, amongst others to support the osmotic adjustment in the cells (Morant-Manceau et al. 2004) or act as stabilizer of subcellular structures (Sánchez-Rodríguez et al. 2010). WD induced a significant increase in Pro concentration (Table 2), which is in line with other publications (Kishor and Sreenivasulu 2014).

Furthermore, changes in the Chl concentration are frequently used as stress indicator (Matile and Hörtensteiner 1999). In our experiment WD significantly decreased the Chl a + b concentration in the three evaluated genotypes. The Chl degradation was accompanied by disfunction in the functionality of the photosynthetic apparatus (Tuba et al. 1996). This was also confirmed by us in the three genotypes at 6 and 27 DAT taking the ETR and qP as indicative parameters. Based on this results and findings of Tuba et al. (1996) the three used cvs revealed a rather indicate to be poikilochlorophyllous behaviour with significant decrease of chlorophyll content in the WD phase followed by an increase in response to RW. In our study, the Chl a + b concentration was 23 % ('Harzfeuer') to 26 % ('Rio Grande' and 'Cupido') lower during WD and about 18/19 % ('Rio Grande'/'Harzfeuer') to 25 % ('Cupido') higher after RW than in fulltime well-watered plants. The fast increase of the Chl a + b concentration after RW provides another evidence that the three cvs are better classified as poikilochlorophyllous.

Further support for this opinion is the ETR, which reflects the stomatal limitations imposed on photosynthesis and thus, the activity of CO_2 assimilation (Baker and Rosenqvist 2004). Consequently, the decrease of the ETR under WD 6 as well as 27 DAT stands for a lower CO_2 assimilation activity. Complementary, the adjustment of the ETR shows an effect of RW (Fig. 1). Further, the qP gives supplementary information about the functionality of PSII concerning the photosynthetic quantum conversion and open PSII reaction centers or more specifically, the fraction of Q_A in its oxidized state (Kramer et al. 2004). Briefly, qP = 1 stands for the probability by which excitons in the PSII antenna system will initiate a photochemical reaction, hence the probability is zero when

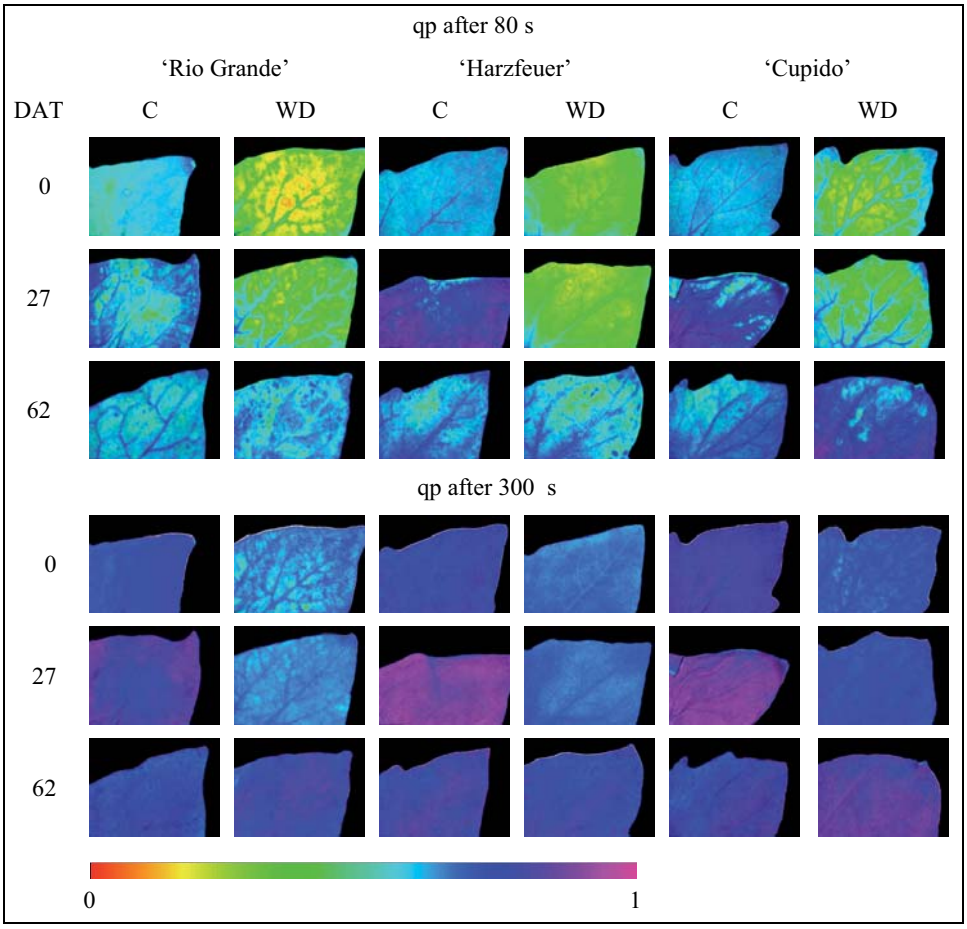


Fig. 3. Time course of the coefficient of photochemical quenching (qP). The images of the parameter were recorded 80 and 300 sec. after the first illumination of the tomato leaves from the cultivars 'Rio Grande', 'Harzfeuer' and 'Cupido' at day 6, 27 and 62 of the experiment (DAT). C = Control plants; WD = plants treated with water deficiency.

q = 0 (Krause and Jahns 2004). Thus, low qP in the WD plants reveals a strong impact on the PSII, i.e. a reduction of the open PSII reaction centers (Fig. 2). The immediate effect of WD as well as the plant adaptation during the WD and re-watering phases is highlighted also by the spatially resolved fluorescence pictures (Fig. 3). The approximation of the values after 16 d of normal water supply confirms those results observed for ETR measurements. Nevertheless, the changes in Chl a + b concentrations during the stress and RW phases might have influenced these results.

The BFRR_UV and the FLAV indices, recorded by the multiple fluorescence excitation technique, revealed early physiological changes in the WD exposed plants (Fig. 4). Here, we observed a cultivar-dependent response to the WD, which was more pronounced in 'Cupido' than in 'Rio Grande' and 'Harzfeuer'. Further, the BFRR_UV of 'Rio Grande' and 'Harzfeuer' showed significant effects of RW after a few days of full water supply as compared with 'Cupido'. Analysis of the raw data demonstrate that the increase of the BFRR_UV is explained by a strong ('Harzfeuer') and a slight ('Cupido', 'Rio Grande') increase of the absolute intensities of BF, and by a very strong ('Cupido') and a moderate ('Harzfeuer', 'Rio Grande') decrease of ChlF in the far-red band, respectively (data not shown). While the decrease of FRF might be associated with the reduction of the Chl content and the shielding of the excitation light by epidermal UV-absorbing compounds, the increase of the BF is directly related to the accumulation of blue-fluorescing compounds. It is well known that phenolic compounds are the major substances contributing for the BF (Lichtenthaler and Schweiger 1998). As shown in Fig. 4, a significant increase of phenols as well as of flavonoids due to drought stress might be expected in tomato leaves (Sánchez-Rodríguez et al. 2010). Furthermore, the decrease of the Chl a + b concentration and lower quantity of carotenoids, which normally re-absorb BF and green fluorescence emission, might intensify this effect (Szigeti 2008).

The accumulation of flavonols in the leaf epidermis can be monitored by the fluorescence screening technique (Bilger et al. 1997), in our study provided by the FLAV index. The FLAV recordings in the time-course enable to recognize a strong and early influence of WD on 'Harzfeuer' and a delayed response of 'Rio Grande' and 'Cupido' (Fig. 4). The re-establishment of water supply revealed a fast response of 'Rio Grande' and 'Harzfeuer', but not of 'Cupido'. The pronounced FLAV increase during the WD phase, and its decrease after RW, confirms previous observations that the FLAV Index is a reliable indicator of drought-induced stress (Bürling et al. 2013). Alterations of the FLAV might arise due to changes in the synthesis and

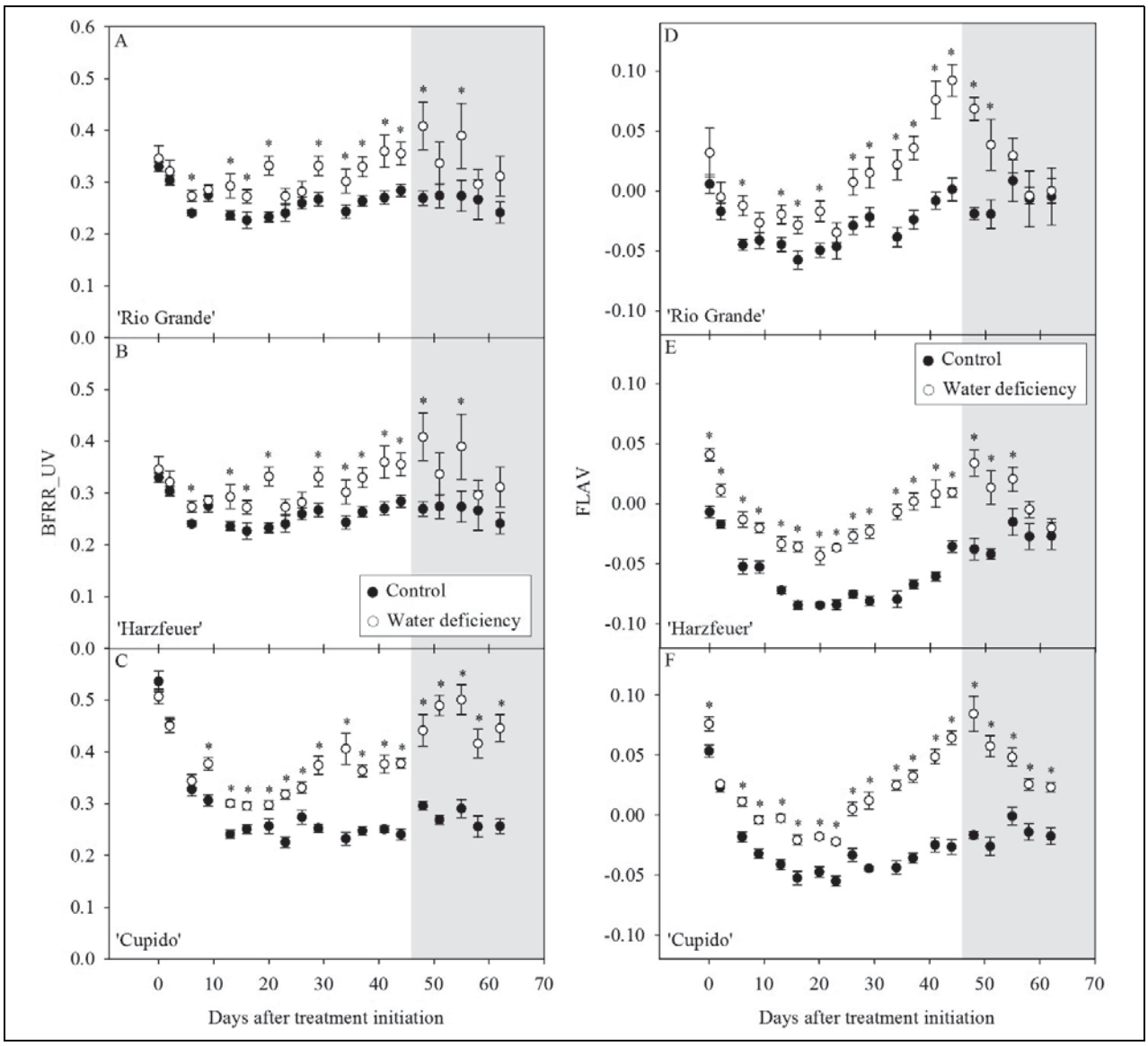


Fig. 4. Blue-to-far-red fluorescence ratio after excitation with UV-light (BFRR_UV) (A–C) and the FLAV- Index expressing the logarithm of the ratio of far-red fluorescence after red light excitation and the far-red fluorescence after excitation with UV- light (D–F). Values were recorded on tomato leaves from the cultivars 'Rio Grande' (A, D), 'Harzfeuer' (B, E) and 'Cupido' (C, F). The grey regions represent the re-watering time without water deficiency. Values represent the means ± SE (standard error) of ten (until day 44) and five (from day 48) samples. * Significant differences ($P \leq 0.05$) between control and water deficiency treatment for each cultivar and measuring day assessed by t-test.

accumulation of flavonoids in the tissue, particularly the epidermal flavonols, as well as the 'apparent' concentration of these compounds per leaf area as driven by the water loss and the size-reduction of single cells. In a RW phase cells absorb huge amounts of water, inducing a 'dilution effect', as observed e.g., for the osmolites (Table 2) and also other cellular compounds. This explains the almost total approximation of the FLAV values, except for 'Cupido'.

The NBI_R, which depends on the content of Chl and epidermal phenolics, provides a promising ratio for fast and non-invasive sensing of changes of the Chl and flavonoid concentration (Tremblay et al. 2012). We show a significant decrease of the ChlF ratio during WD e.g., at 6 DAT in 'Rio Grande' and 'Cupido'. In contrast, 'Harzfeuer' demonstrates a delayed response (Fig. 5). Further, the general course of adaptation of 'Rio Grande' and 'Harzfeuer' to RW was quite similar, while 'Rio Grande' responded significantly within 5 d, 'Harzfeuer' approached this level within 16 d. In 'Cupido' the values did not show any approximation after RW. Due to the fact that the NBI depends on both Chl and epidermal phenolics, and taking into account that the difference in the Chl concentration

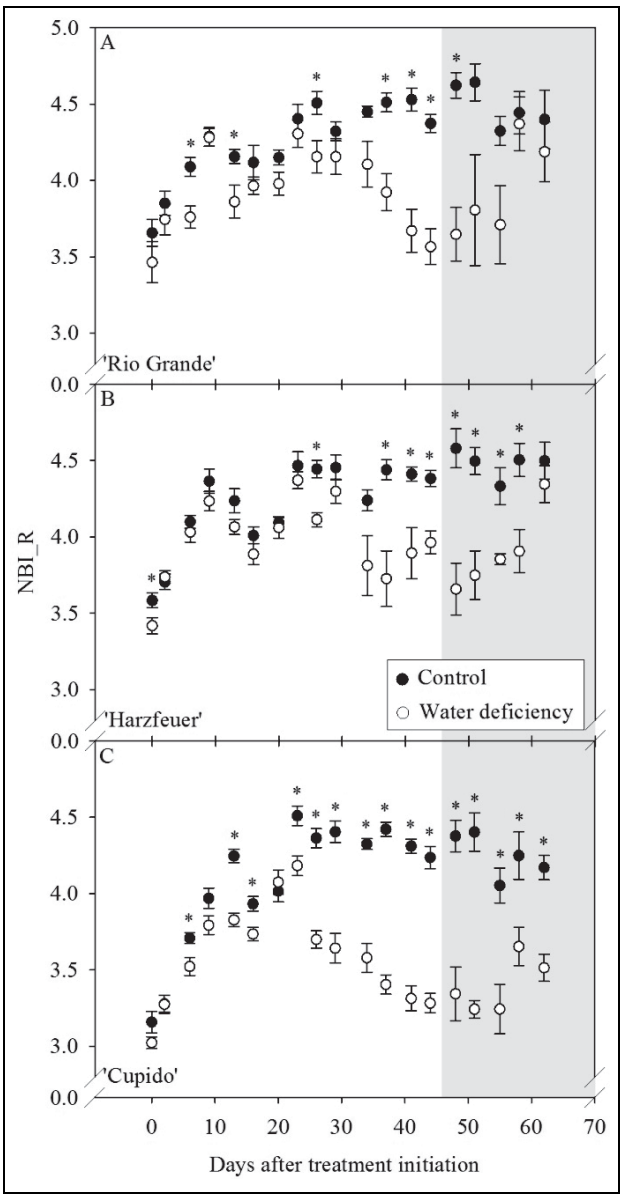


Fig. 5. Time course of the ratio of FRF after UV-light excitation to RF after red light excitation (NBI_R). NBI was recorded on the cultivars 'Rio Grande' (A), 'Harzfeuer' (B) and 'Cupido' (C). The grey regions represent the re-watering time without water deficiency. Values represent the mean ± SE (standard error) of ten (until day 44) and five (from day 48) samples. * Significant differences ($P \leq 0.05$) between control and water deficiency treatment for each cultivar and measuring day assessed by t-test.

of control and stress did not differ appreciable between the genotypes, our trails indicate a stronger relevance of the synthesis of phenolic compounds in 'Cupido', also confirming the trend observed for FLAV. Irrespective of the similar response of the three cvs to the PAM ChlF (Figs. 1–3), the fluorescence indices BFRR_UV, FLAV and NBI indicate a stronger drought-induced activation of the secondary metabolism in 'Cupido'.

Conclusions

We demonstrate that the ChlF provides reliable parameters for sensing water deficit and RW processes in adult tomato plants. Thereby, the BFRR_UV, FLAV and NBI_R ratios of the multiparametric fluorescence enable a more effective and faster sensing of WD stress without the need of dark-adaption as required for the PAM recordings. Fluorescence emissions of 'Cupido' revealed the strongest changes to WD and also the slowest approximation after RW. In contrast, 'Harzfeuer' and 'Rio Grande' showed less influence of WD and faster response to RW. Our results indicate that the three cvs were similarly impaired in their primary metabolism while 'Cupido' was comparatively stronger influenced in its secondary metabolism. On this basis, we also highlight the potential of the multiparametric fluorescence ratios for the fast screening of horticultural genotypes. Nevertheless, further studies are needed to analyze the accumulation pattern of compounds at cell level leading to the alterations of the fluorescence signals.

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