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ORIGINAL PAPER

Physiological response of sugar beet (*Beta vulgaris*) genotypes to a temporary water deficit, as evaluated with a multiparameter fluorescence sensor

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Abstract Greenhouse and field experiments were carried out to evaluate the potential of specific fluorescence emission parameters for the detection of a temporary water deficit in selected sugar beet (*Beta vulgaris* L.) genotypes. Changes in the plant physiology due to reduced water availability were recorded with a multiparameter fluorescence sensor in addition to destructive and non-invasive reference analysis. Our results show that an insufficient water supply is followed by only slight changes of the UV-excited blue fluorescence. However, significant alterations due to desiccation were detected in several chlorophyll fluorescence parameters measured after excitation with UV, green and red light. In the scope of our activities, the relevance of the green light source for the fluorescence excitation became evident and enabled to characterize cultivar-specific reactions during dehydration and re-watering period. A field experiment was conducted to validate the data collected in the greenhouse. As proven, several days of low water supply led to effects similar to those observed in the greenhouse study. Our results indicate that the far-red fluorescence, as well as the simple and complex fluorescence ratios having the chlorophyll fluorescence as basis, is the appropriate parameter to evaluate physiological responses of sugar beet plants exposed to a short-term, temporary water deficit.

Keywords Blue fluorescence · Chlorophyll *a* fluorescence · Drought stress · Stress physiology · Greenhouse study · Field experiment

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Introduction

Agricultural productivity is limited worldwide by various biotic and abiotic stresses. Drought is of particular importance, since it is the main abiotic stress factor which causes the highest yield losses (Boyer 1982). Recently, strong efforts have been made to improve drought tolerance of commercial varieties aiming to maintain high production level under adverse conditions (Ober et al. 2004, 2005). Conventional breeding programs are long-term and cost-intensive projects, which comprise the evaluation of several hundreds of crossing-lines. Hence, there is a rising demand for precise and objective evaluation methods to support a selection of promising genotypes. As proven, photosynthesis is highly sensitive to drought, since its efficiency decreases with increasing water deficit (Bloch et al. 2006). Thereby, the reduced soil water content triggers the stomatal closure leading to a lower internal CO_2 concentration, which consequently limits photosynthesis (Cornic and Masacci 1996). Furthermore, specific metabolic impairments may limit photosynthesis during drought (Flexas and Medrano 2002). Gas exchange measurements can contribute to elucidate the physiological mechanisms underlying the drought tolerance. However, this method is not practical for comparing a large number of genotypes due to its sensitiveness to external environmental influences.

In contrast, several studies point out the potential of optical non-invasive methods as reliable tools to characterize plant physiological changes under stress situations (Berger et al. 2010; Chaerle and Van Der Straeten 2001; Jones and Schofield 2008). Especially, the pulse-amplitude modulated (PAM) chlorophyll fluorescence (ChlF) has become a more and more frequently used method to determine the effects of adverse conditions on plant photosynthesis. Thereby, chlorophyll *a* fluorescence readings indicate that the photosystem II may react insensitively to water deficit (Havaux 1992). When recorded with spectral resolution, the chlorophyll fluorescence has two peak emission maxima, i.e., in the red (680 nm) to far-red (730 nm) spectral region. Both emission maxima are related to the chlorophyll *a* fluorescence (Lichtenthaler et al. 1997). Progressive desiccation of plant leaves can lead to an increase of the red fluorescence (Lichtenthaler and Rinderle 1988). Furthermore, excitation of green leaves by UV-light enables the recording of the species-characteristic (Lichtenthaler and Schweiger 1998; Stober and Lichtenthaler 1993a, b) blue and green fluorescence (BGF). This fluorescence signal is primarily emitted by various phenolic compounds mainly located in the cell walls (Cerovic et al. 1999; Lang et al. 1991). In this context it has been shown that plants exposed to water deficit may accumulate more phenolic compounds such as ferulic acid, which is reflected in higher fluorescence intensity (Hura et al. 2007, 2009a, b; Lichtenthaler and Schweiger 1998).

In general, the fluorescence intensity is strongly influenced by the technical equipment and measuring conditions (Cerovic et al. 1994; Morales et al. 1998), and therefore the use of fluorescence ratios is highly recommended (Cerovic et al. 1999; Schweiger et al. 1996). One example is the ratio *F690/F735*, which is a good in vivo estimator of chlorophyll content (D'Ambrosio et al. 1992; Hák et al. 1990). Others are the blue-to-red or blue-to-far-red fluorescence ratios, which may rise up with increasing drought stress (Buschmann et al. 2000; Cerovic et al. 1999). In contrast to the BGF, which needs to be excited by UV-light, the selection of the excitation source is of relevance for the chlorophyll fluorescence readings. Amongst others the light colour impacts the penetration profile of the light in the leaf tissue and enables to collect information either from more superficial or deeper tissue layers (Brodersen and Vogelmann 2010).

Investigations of BGF and ChlF for an in vivo detection of drought susceptibility in sugar beet plants are scarce. In our studies we hypothesized that the multiparameter fluorescence sensing is a suitable method to characterize genotype-specific responses of sugar beet to temporary water deficit conditions. Furthermore, we expected that with increasing desiccation photosynthesis is reduced and the secondary plant metabolism raised, both together influencing the fluorescence signals used to sense the plant response to water deficit. In order to prove this, greenhouse experiments under semi-controlled conditions were carried out using sugar beet genotypes with unknown drought susceptibility. The obtained data were subsequently validated in a field experiment to prove whether the method used is an effective screening technique or not.

Materials and methods

Greenhouse experiment

The experiments were carried out from October 2010 to January 2011 in a heated greenhouse. Seeds of the sugar beet (*Beta vulgaris* L.) cultivars Pauletta (a), Berenika (b), Cesira (c) and Mauricia (d) differing in leaf morphology and performance under common agricultural practices were provided by the company KWS Saat AG (Einbeck, Germany). Pelleted seeds of each genotype were sown in trays filled with sand as growing medium. One week after germination, uniform plants were transplanted into 2 l plastic pots (0.20 m height, 0.10 m diameter) filled with a commercial peat substrate (Typ 5, Brill, Georgsdorf, Germany). Plants ($n = 8$ per genotype and treatment) were placed at random on two benches (10.5 × 1.65 m) with controlled nutrient supply (pH 6.5 and an EC 180 mS cm^{-1}) at 20/16 °C day/night temperature and 16 h photoperiod. Plants received supplemental light by using high-pressure sodium lamps (Philips SON-T Agro 400W) providing 250–350 µmol m^{-2} s^{-1} PAR at the leaf level. The water deficit was accomplished by withholding the nutrition solution and was induced on the same plants in two consecutive phases. Between the two drought periods plants were allowed to recover under full irrigation for 29 days.

Field experiment

The field experiment was conducted at the Institute of Crop Science and Resource Conservation (INRES), Department of Horticultural Science, University of Bonn. Crop establishment and management during plant's development followed recommendations of good practice. Accordingly, seeds were sown in the spring when soil temperature was on average higher than 5 °C. Seeds were sown by using a commercial three row plot drill targeting a density of 90,000 plants per hectare (0.50 m distance between rows, 0.18 m between plants within the row). The plots (9.8 × 3 m) were randomized ($n = 4$ for each genotype and treatment) in the blocks. Water supply was assured by a drip irrigation system along the central row of plants. During the season, the soil moisture was measured at

0.40 m depth with digital tensiometers (Blumat Digital BD2, LM-GL, Bambach GbR, Geisenheim, Germany) in five non-irrigated plots and four irrigated plots. Fluorescence measurements and sampling for destructive reference analysis were performed at random along the central row of each plot on the youngest fully expanded leaves.

Non-destructive measurements

Fluorescence measurements

The hand-held optical fluorescence sensor Multiplex® 3 (Force-A, Orsay, France) was used to record the auto fluorescence of leaves under ambient light conditions, as described by Ben Ghozlen et al. (2010). Briefly, the fluorescence is excited by light-emitting-diodes (LED) in spectral ranges about 375, 518, and 630 nm. Fluorescence signals are measured in the blue (425–475 nm), red (680–690 nm) and far-red (720–755 nm) spectral regions. A grid in front of the sensor enabled a constant distance of 0.10 m between sensor and leaves. The fluorescence signals were always recorded at leaf level and an area of approximately 50 cm^2. In the greenhouse study, at day 61 after sowing, two upper, fully-expanded opposing leaves of each plant were labelled, and fluorescence readings were taken up to 105 days after sowing (DAS).

Gas exchange measurements

Gas exchange measurements were conducted during the first experimental phase with a portable infrared gas analyzer (CIRAS-1, PP Systems, United Kingdom) equipped with a leaf cuvette (PLC B, PP Systems, United Kingdom) covering an area of 2.5 cm^2. Net photosynthetic rate (Pn), stomatal conductance (G), internal CO_2 partial pressure (CI) and transpiration rate (E) were measured at the leaf tip by avoiding major veins. In order to standardize the measurement conditions and to minimize the effect of the environment, plants were taken to a defined measuring site established in the greenhouse. For the measurements, CO_2 concentration was set to 350 ± 5 ppm, light irradiation on the leaf surface was about 250–350 µmol m^{-2} s^{-1} PAR, and the air flow entering the chamber was 200 ± 5 ml min^{-1}.

Analysis of reference constituents

Sampling methodology

The osmotic potential from leaves of the greenhouse experiment, as well as their chlorophyll and ferulic acid concentration, were determined at day 100 after sowing. From each sugar beet plant, one of the labelled leaves was harvested from which two leaf sections (1 cm^2 each) were punched out from the apex. Of these samples, chlorophyll and ferulic acid concentration were determined. The remaining part of the harvested leaf was used for determination of the osmotic potential. Analysis of chlorophyll concentration and osmotic potential of sugar beet plants grown in the field were carried out at 104 and 108 DAS, following a procedure similar to the greenhouse study. Thereby, eight leaves were randomly collected from all plots, including each genotype and treatment.

Chlorophyll concentration

For the determination of the chlorophyll concentration leaf disks were transferred into 10 ml centrifugal glasses filled with 5 ml dimethyl sulfoxide (DMSO). The glasses were closed and dark-stored for 24 h under laboratory conditions. The chlorophyll concentration was determined with a UV–VIS spectrophotometer (Perkin-Elmer, Lambda 5, MA, USA) by measuring the absorbance of extracts at 665 nm (A_{665}) and 647 nm (A_{647}). Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and *total* chlorophyll (Chl *t*) concentrations were calculated according to the following equations:

$$\text{Chl } a = 12.7 \times A_{665} - 2.79 \times A_{647}$$

$$\text{Chl } b = 20.7 \times A_{647} - 4.64 \times A_{665}$$

$$\text{Chl } t = \text{Chl } a + \text{Chl } b$$

Ferulic acid

Ferulic acid concentration was determined according to the method described by Morales et al. (1996), with specific modifications. Before analysing with a UV–VIS spectrophotometer (Perkin-Elmer, Lambda 5, MA, USA), ferulic acid was extracted with 5 ml of methanol/water (4:1, v/v), the mixture was shaken for 1 min. Ferulic acid (4 hydroxy-3 methoxycinnamic acid, Merck, Hohenbrunn, Germany) at a purity of ≥ 98 % was used as standard.

Osmotic potential

In both greenhouse and field studies, leaf disks were used for the determination of osmotic potential serving as reference for the intensity of drought stress. The samples were placed in bags (Bioreba, Switzerland) and extruded with a hand homogenizer. Thereafter, a volume of 1.5 ml was filled and the cell sap centrifuged (Eppendorf, Centrifuge 5417 R, Hamburg, Germany) for 10 min at 25,000 min^{-1} at 4 °C. From the supernatant, 15 µl was pipetted into tubes and the osmolality measured with a freezing-point depression osmometer (Osmomat 030-D, Genotec GmbH, Berlin, Germany). At the beginning of the measurements, the osmometer was calibrated using preformed Genotec

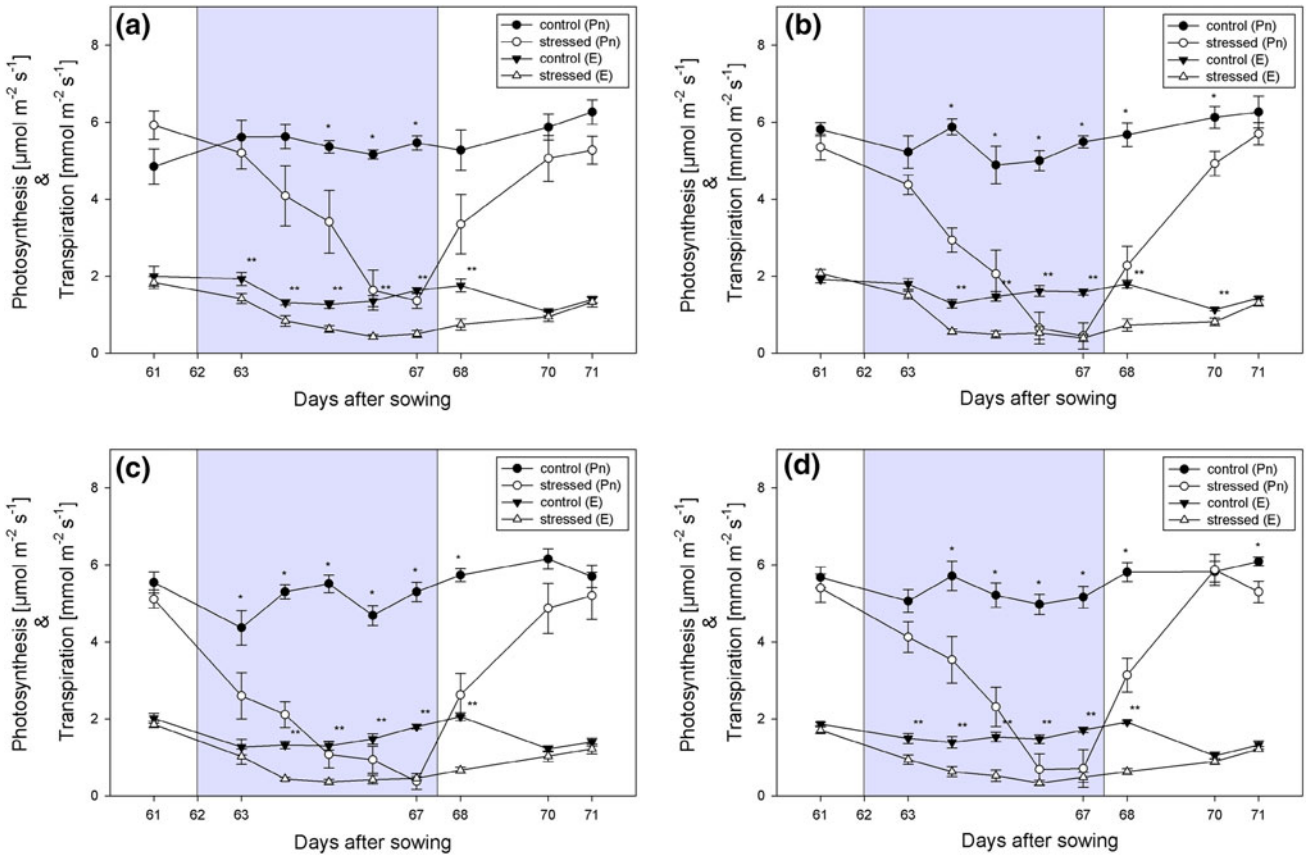

Fig. 1 Development of net photosynthesis (µmol m^{-2} s^{-1}) and transpiration (mmol m^{-2} s^{-1}) for the sugar beet cultivars Pauletta (**a**), Berenika (**b**), Cesira (**c**) and Mauricia (**d**) influenced by water supply. Measurements took place under semi-controlled conditions in the greenhouse between 61 and 71 DAS on leaves of irrigated (control) and temporarily non-irrigated (stressed) sugar beet plants; water withholding for the 'stressed' plants was between 62 and 67 DAS. *Asterisks* indicate significant differences (*t* test, $P \leq 0.05$) between irrigated and temporarily non-irrigated plants: *significant differences for photosynthesis (Pn), **for transpiration (*E*). Mean ± SE ($n = 8$) (colour figure online)

vials (850 mmol kg^{-1} H_2O) and distilled water (0 mmol kg^{-1} H_2O).

Statistical analysis

Data were statistically analysed with SPSS statistic software (PASW statistics version 19.0, SPSS Inc., Chicago, USA). For each genotype and evaluation date, means of well-watered and water-deficit plants were compared by analysis of variance and paired *t* test or Mann–Whitney *U* test ($P \leq 0.05$).

Results

Gas exchange

Gas exchange measurements were conducted under defined conditions in the greenhouse for the first experimental period between 61 and 71 DAS. Therein focus was on Pn and E of irrigated (control) and temporarily non-irrigated (stressed) plants. Immediately after the withholding of water supply to the non-irrigated plants, E continuously decreased up to day 67 (Fig. 1). Significant differences between both treatments could be detected for the cultivars Pauletta and Mauricia already 1 day after the water supply was stopped (63 DAS). As soon as the stressed treatment groups were re-watered, E progressively recovered. The effects of the temporary water shortage followed a similar trend for Pn, whereas here changes were even more accentuated in non-irrigated plants as compared with control plants. Similarly, Pn reduction was significantly impaired on the first or second day of the water deficit; thereby, the smallest decline of Pn was observed in the cultivar Pauletta. The recovery phase allowed Pn and E to reach nearly the normal level some days after re-watering of plants.

Fluorescence emission

Chlorophyll fluorescence and 'Simple Fluorescence Ratio'

With the portable sensor for multiple fluorescence excitation, the fluorescence intensity in three spectral regions was

determined. Based on the fluorescence intensities, several independent or correlated fluorescence parameters (fluorescence ratios) were calculated. The development of selected chlorophyll fluorescence readings at leaf level is illustrated for each cultivar, treatment and experimental stage (59–71 DAS and 94–105 DAS, respectively) in Figs. 2, 3, 4. Especially during the first experimental period a very late and small increase of the red fluorescence emission for non-irrigated plants of the cultivar Pauletta could be determined (Fig. 2a), whereas the red fluorescence of the cultivars Cesira (Fig. 2c) and Berenika (Fig. 2b) rose shortly after the water supply was stopped. A similar trend could be observed during the second experimental phase. Here, the red fluorescence emission of all water deficit plants immediately increased when the water supply was withheld. Especially for the genotype Pauletta, the values recovered much earlier during and after the second stress phase as compared with the other cultivars (Fig. 2a). In contrast, the stressed plants of Cesira could not recover during the experimental phase due to strong damage. In general, similar trends could be established for all cultivars when recording the far-red fluorescence (Supplementary Figure S1) over the whole experimental period.

Water withholding from 62 to 67 DAS, and 96 to 100 DAS, caused significant alterations in the 'Simple Fluorescence Ratio' (SFR, far-red emission divided by the red emission) measured after green (G) or red (R) excitation (Fig. 3). Comparisons indicate that the most pronounced differences in the absolute values between irrigated and non-irrigated plants were obtained when using green excitation light. Temporary water withholding led to a reduction of the SFR in the affected plants. Concerning the differences between genotypes during the first experimental phase, the cultivars Pauletta and Mauricia showed a less pronounced susceptibility to the water shortage than the cultivars Cesira and Berenika (Fig. 3).

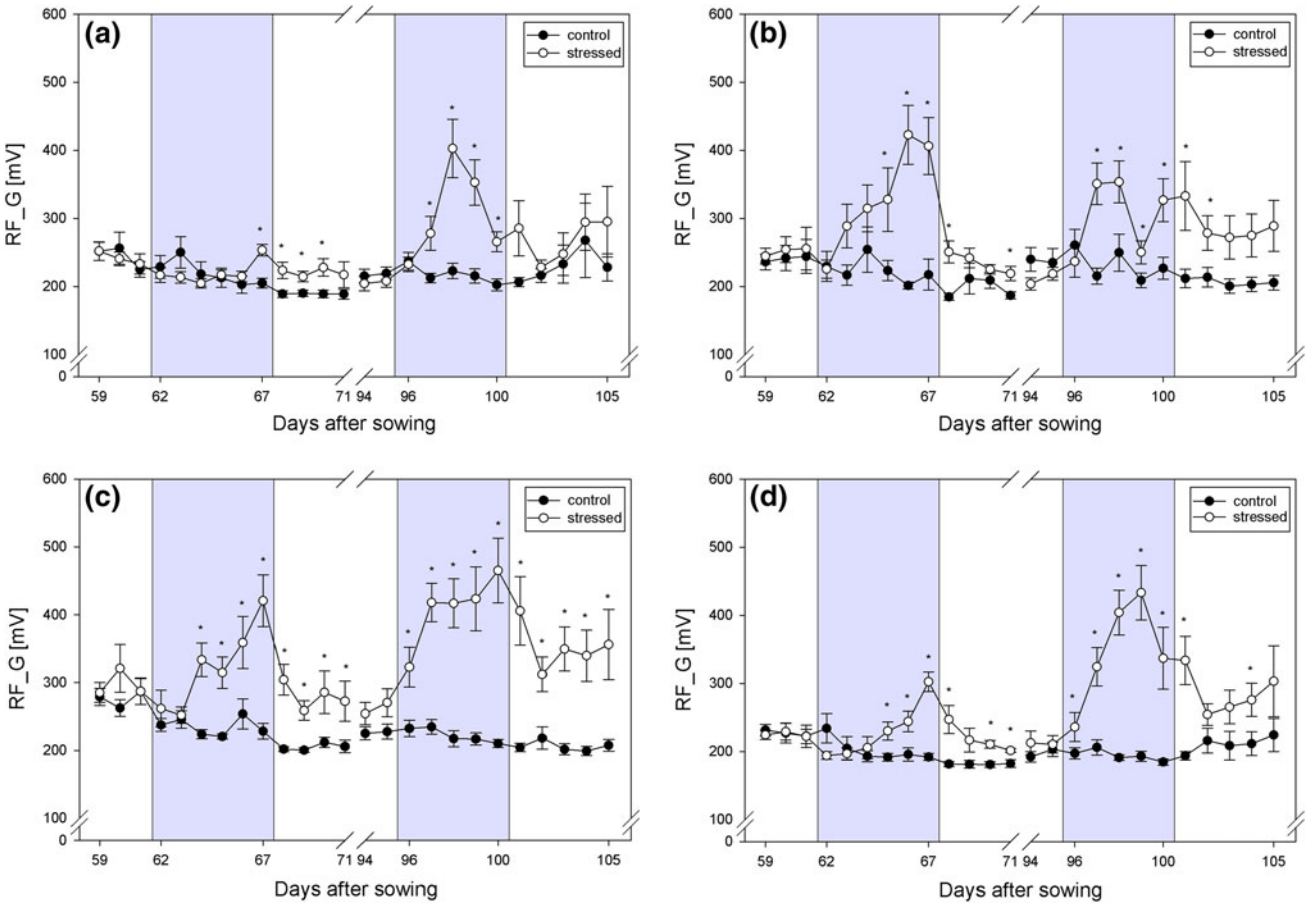

Fig. 2 Influence of water supply on the red fluorescence (RF) recorded after green excitation light (G) on the sugar beet cultivars Pauletta (**a**), Berenika (**b**), Cesira (**c**) and Mauricia (**d**) cultivated in the greenhouse. Measurements were regularly taken on marked leaves between 59 and 105 DAS. *Grey regions* in the *graphs* indicate the periods where water supply in the water deficit treatment group was stopped. Values indicate mean ± SE ($n \geq 8$). *Asterisks* indicate significant differences with a $P \leq 0.05$ (t test) between leaves of irrigated (control) and seasonal non-irrigated (stressed) plants for each cultivar and measuring day (colour figure online)

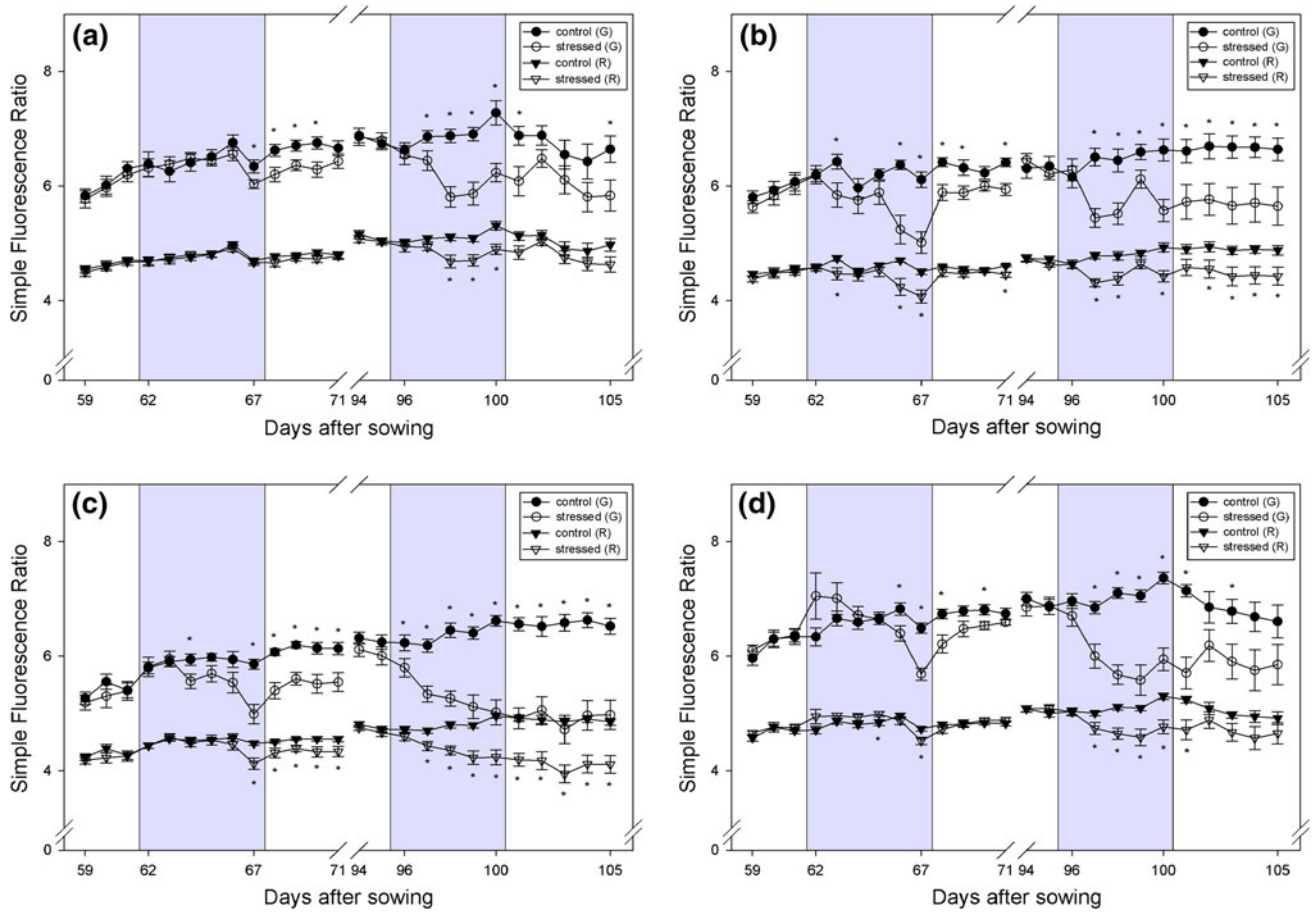

Fig. 3 Influence of water supply on the 'Simple Fluorescence Ratio' (SFR) on the sugar beet genotypes Pauletta (**a**), Berenika (**b**), Cesira (**c**) and Mauricia (**d**) measured after excitation with *green* (G) and *red* light (R). Fluorescence recordings were taken in greenhouse plants at leaf level between 50 and 105 DAS. *Grey regions* in the *graph* illustrate the periods where water supply was stopped for the stress treatment. Values indicate mean ± SE ($n \geq 8$). *Asterisks* indicate significant values with a $P \leq 0.05$ (t test) between leaves of irrigated (control) and seasonal non-irrigated (stressed) plants for each cultivar and measuring day (colour figure online)

Blue fluorescence

Changes in the BGF intensity were marginal during the experimental periods. Of all tested cultivars, significant differences between the treatments could be ascertained only for Pauletta (Fig. 4). Thereby, a slight increase of the BGF of temporarily non-irrigated plants in comparison with well watered plants could be identified between 64 up to 71 DAS. Furthermore, all cultivars show a significantly higher BGF during the second experimental period than in the first one.

Blue-to-far-red fluorescence ratio

Based on the blue fluorescence (BGF) and the far-red fluorescence, the parameter blue-to-far-red fluorescence emission ratio (BFRR_UV) was calculated. Similar to the 'Simple Fluorescence Ratios', in all cultivars the BFRR_UV decreased when the water supply was stopped in the first or second experimental phase. Thereby, the BFRR_UV was more strongly affected in the cultivar Mauricia and less affected in leaves of the cultivar Pauletta (Fig. 5). At the beginning of the second experimental phase, significant differences between the treatments could be ascertained for the cultivar Mauricia. This suggests that the recovery period of 29 days (67–96 DAS) was insufficient for a complete regeneration of this cultivar.

Destructive reference analysis of greenhouse plants

Changes in the concentration of chlorophyll *a*, *total* chlorophyll and ferulic acid, as well as osmotic potential were analysed at 100 DAS, as displayed in Table 1. As comparisons show, the osmotic potential of the stressed plants was significantly higher than that of the respective control plants. Osmotic adjustment in the cultivar Pauletta was less accentuated as compared with the other genotypes. Considering the behaviour of each cultivar, no differences

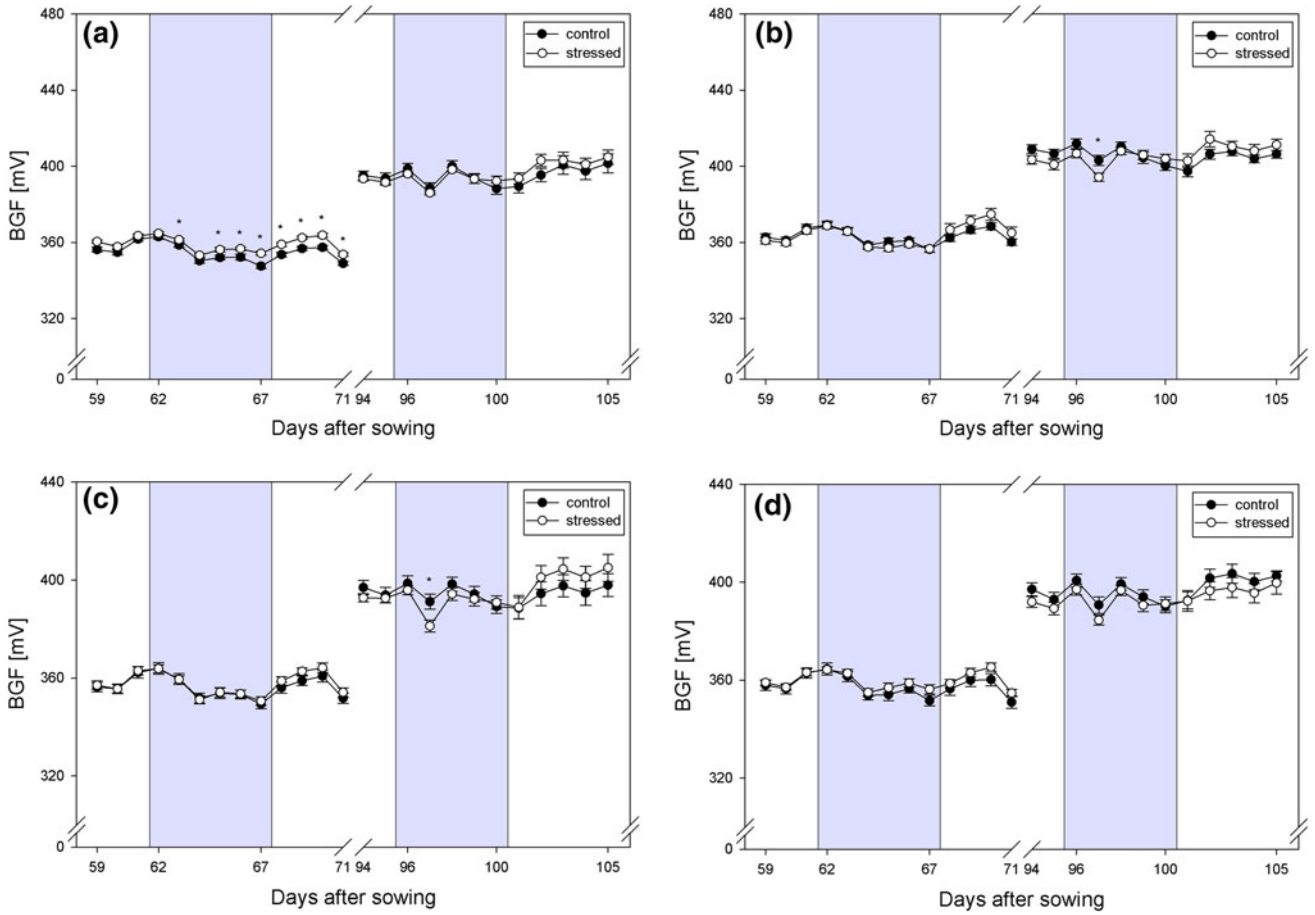

Fig. 4 Influence of water supply on the blue fluorescence (BGF) recorded after UV excitation light on the sugar beet cultivars Pauletta (**a**), Berenika (**b**), Cesira (**c**) and Mauricia (**d**) cultivated in the greenhouse. Measurements were regularly taken on marked leaves between 59 and 105 DAS. *Grey regions* in the *graphs* illustrate the periods where water supply was stopped in the case of the non-irrigated plants. Values indicate mean ± SE ($n \geq 8$). *Asterisks* indicate significant differences with a $P \leq 0.05$ (t test) between leaves of irrigated (control) and seasonal non-irrigated (stressed) plants for each cultivar and measuring day (colour figure online)

between the treatments could be observed when referring to the concentrations of chlorophyll *a*, total chlorophyll and ferulic acid. However, irrespective of the treatments, the cultivar Cesira revealed significantly lower chlorophyll *a*, total chlorophyll and ferulic acid concentrations than the other cultivars (Table 1).

Field experiment

In the field experiment, a longer rainless period resulted in an increase of tensiometer readings to about 750 mbar (maximum value) in all rain fed plots, whereas the soil moisture tension of the irrigated plots reached 50 mbar between 103 and 108 DAS (data not shown). Similar fluorescence parameters were used in the field, to validate the results of the greenhouse study. Relative changes to control plants showed that soil desiccation led to an increase of absolute fluorescence readings and a decrease of fluorescence ratios at 104–108 DAS (Table 2). However, in the case of the cultivar Pauletta, all fluorescence parameters showed no significant differences between irrigated and rain fed plots even during the rainless period. A completely different trend was observed for 'Berenika', where all fluorescence parameters of both treatments were significantly different at 104 DAS, already. When comparing the four cultivars, variations of the BGF intensity were relatively small. Nevertheless, the drought under field conditions led to significant differences for 'Berenika' and 'Mauricia' at 104 DAS, and for 'Pauletta' and 'Cesira' at 108 DAS, respectively (Supplementary Figure S2).

The analytical determination of chlorophyll *a*, *total* chlorophyll, as well as osmotic potential provided reference parameters for the fluorescence readings. The rainless period showed only slight effects on chlorophyll *a* and total chlorophyll concentration (Table 3). A significant decline of both parameters in the rainfed treatments could be determined at 108 DAS for the cultivars Berenika and Cesira. In contrast, irrigated and rainfed plants of all tested

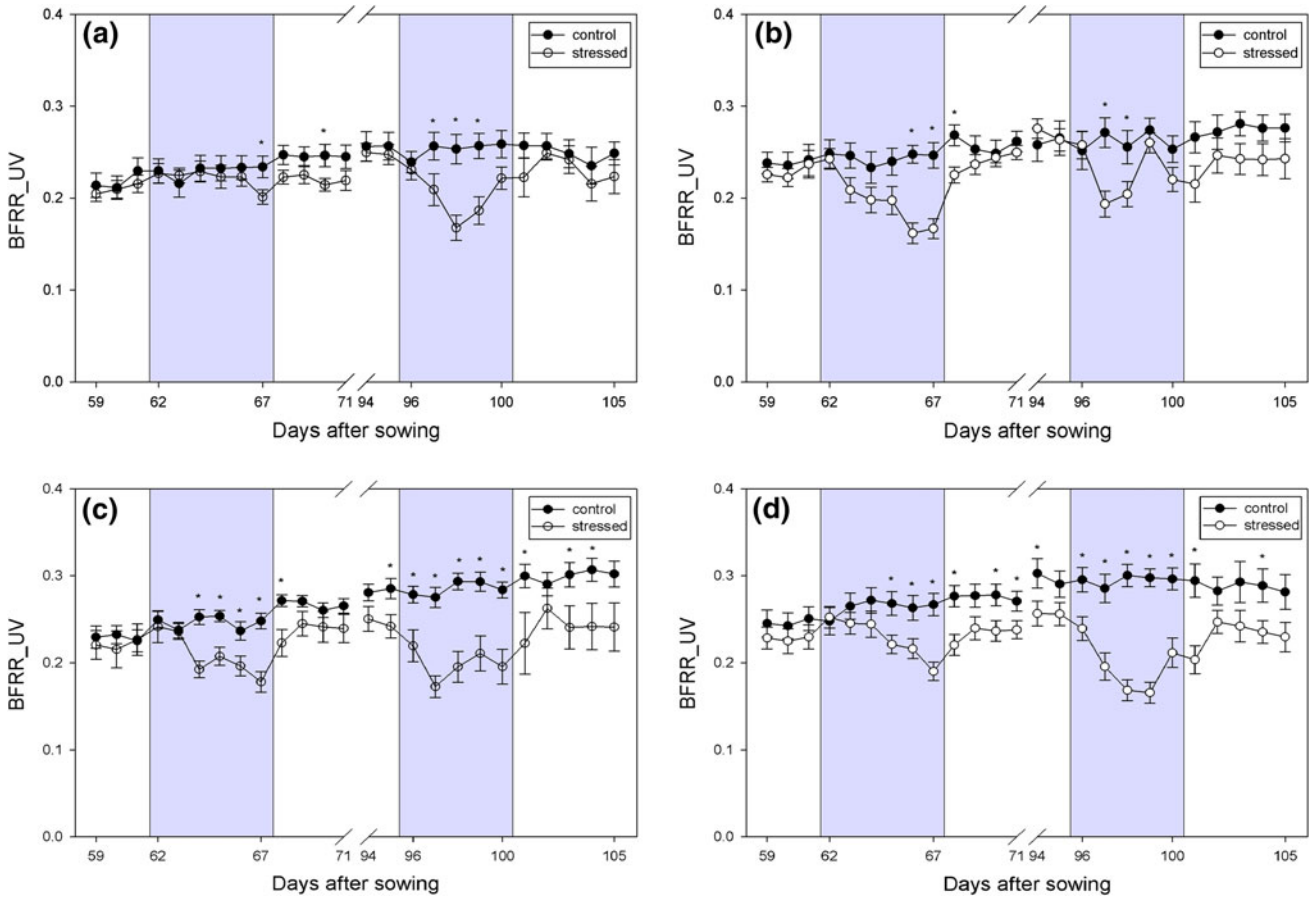

Fig. 5 Influence of water supply on the blue-to-far-red fluorescence ratio (BFRR_UV) recorded on the sugar beet cultivars Pauletta (**a**), Berenika (**b**), Cesira (**c**) and Mauricia (**d**) cultivated in greenhouse. Measurements were regularly taken on marked leaves between 59 and 105 DAS. *Grey regions* in the *graphs* illustrate the periods where water supply was stopped in the case of the non-irrigated plants. Values indicate mean ± SE ($n \geq 8$). *Asterisks* indicate significant differences with a $P \leq 0.05$ (t test) between leaves of irrigated (control) and seasonal non-irrigated (stressed) plants for each cultivar and measuring day (colour figure online)

Table 1 Osmotic potential (MPa), chlorophyll *a*, total chlorophyll and ferulic acid concentration [μg cm^{-2}] in leaves of irrigated (control) and temporarily non-irrigated (stressed) sugar beet cultivars grown under greenhouse conditions and sampled at 100 DAS

Sugar beet cultivars	Treatment	Osmotic potential (MPa)	Chlorophyll *a* (μg cm^{-2})	Total chlorophyll (μg cm^{-2})	Ferulic acid (μg cm^{-2}, 10^{-4})
Pauletta	Control	0.99 ± 0.04*	9.89 ± 0.7	12.83 ± 0.8	0.83 ± 0.07
	Stressed	1.42 ± 0.07	9.24 ± 0.8	12.01 ± 1.0	0.83 ± 0.07
Berenika	Control	1.16 ± 0.02*	8.91 ± 0.4	11.69 ± 0.4	0.70 ± 0.04
	Stressed	1.80 ± 0.03	9.73 ± 1.1	12.61 ± 1.4	0.71 ± 0.10
Cesira	Control	1.09 ± 0.02*	8.34 ± 0.9	10.91 ± 1.1	0.51 ± 0.05
	Stressed	1.77 ± 0.07	8.50 ± 0.8	10.81 ± 1.0	0.53 ± 0.07
Mauricia	Control	0.98 ± 0.02*	9.73 ± 0.5	12.51 ± 0.6	0.93 ± 0.14
	Stressed	1.57 ± 0.05	10.4 ± 0.6	13.54 ± 0.7	0.86 ± 0.06

* Significant differences (t test, $P \leq 0.05$) between the water supply treatments for each cultivar, Mean ± SE ($n = 8$)

cultivars displayed differences in the rate of osmotic potential as early as 104 DAS. Increasing soil desiccation led to a progressive decline of osmotic potential in all rainfed plants between both sampling dates.

Discussion

The present paper aims to elucidate the impact of drought on the fluorescence signature of four sugar beet genotypes.

Table 2 Influence of water supply on selected fluorescence parameters measured 104 and 108 DAS in sugar beet leaves developed under field conditions

Sugar beet cultivars	Days after sowing	Modification of fluorescence parameters (% of control)				
		RF_G	FRF_G	BFRR_UV	SFR_G	SFR_R
Pauletta	104	4.9	2.6	−8.7	−1.9	−0.9
	108	5.8	5.2	−12.2	−2	−1
Berenika	104	16.1*	10.1*	−8.7*	−4.4*	−1.3
	108	25.6*	15.7*	−16.3*	−7.4*	−4.2*
Cesira	104	9.1	3.7	2.4	−3.6*	−2.2*
	108	9.6*	5.5*	−14.3	−4.1*	−3.0*
Mauricia	104	2.6*	0.6	0.1	−3.0*	−1.7
	108	14.9*	10.1*	−13.6*	−3.7*	−1.8*

The following data represent relative changes (%) of control plants

* Significant differences (fluorescence ratios analysed by *t* test, $P \leq 0.05$, absolute fluorescence parameters by Mann–Whitney *U* test, $P \leq 0.05$) between irrigated and rainfed plants for each cultivar and measuring day ($n = 96$)

Table 3 Chlorophyll *a*, *total* chlorophyll concentration (μg cm^{-2}) and osmotic potential (MPa) analysed 104 and 108 DAS in sugar beet leaves developed under temporary water deficit and field conditions

Sugar beet cultivar	Treatment	104 DAS			108 DAS		
		Chlorophyll *a* (μg cm^{-2})	Total chlorophyll (μg cm^{-2})	Osmotic potential (MPa)	Chlorophyll *a* (μg cm^{-2})	Total chlorophyll (μg cm^{-2})	Osmotic potential (MPa)
Pauletta	Control	42.8 ± 1.3	49.8 ± 2.4	1.05 ± 0.02*	32.8 ± 0.9	42.5 ± 1.1	1.04 ± 0.03*
	Stressed	44.0 ± 1.0	49.4 ± 1.2	1.21 ± 0.03	34.2 ± 1.0	43.9 ± 1.3	1.30 ± 0.05
Berenika	Control	43.1 ± 0.9	47.7 ± 1.0	1.03 ± 0.03*	37.9 ± 1.3*	49.1 ± 1.5*	1.10 ± 0.03*
	Stressed	42.3 ± 1.1	47.4 ± 1.3	1.42 ± 0.02	33.9 ± 1.1	45.0 ± 1.3	1.48 ± 0.05
Cesira	Control	35.9 ± 0.9	40.4 ± 1.1	1.03 ± 0.03*	30.5 ± 0.9*	40.2 ± 1.1*	1.00 ± 0.03*
	Stressed	35.9 ± 1.9	40.4 ± 2.2	1.31 ± 0.04	28.0 ± 0.8	36.3 ± 1.0	1.44 ± 0.05
Mauricia	Control	44.3 ± 0.9	50.6 ± 1.0	1.03 ± 0.05*	36.0 ± 1.1	46.3 ± 1.4	1.12 ± 0.03*
	Stressed	42.5 ± 1.2	48.6 ± 1.8	1.29 ± 0.03	38.6 ± 1.1	49.5 ± 1.4	1.39 ± 0.02

* Significant differences (*t* test, $P \leq 0.05$) between irrigated and rainfed (control) plants for each cultivar and measurement day (Mean ± SE, $n = 32$)

Thereby, our hypothesis was that multiparameter fluorescence sensing is an appropriate method to characterize genotype-specific responses to temporary water deficit conditions. Here, we demonstrate that this technique provides reliable information about plant physiological constitutions resulting from transient shortage of water supply. Further, essential fluorescence parameters for detection of drought-induced stress in sugar beet plants were identified.

In contrast to Clover et al. (1999), our results clearly show that chlorophyll fluorescence is strongly affected by drought. In our independent experiments under greenhouse and field conditions the values of F690 and F730 rose with increasing water deficit. A number of studies on drought stress point out that changes in chlorophyll fluorescence intensities originate mainly from disruptions in the photosynthetic performance, resulting in damage to the photosystems and light-harvesting complexes (Buschmann and Lichtenthaler 1998; Lang et al. 1996; Schweiger et al. 1996). Thereby, inhibited carbon metabolism and reduced utilization of light-phase products are responsible for the damage to PSII, since the harvested radiation cannot be converted into chemical energy (Cornic and Masacci 1996). In our trials, increasing chlorophyll fluorescence suggest that the light utilization by the stressed plants was temporarily lower than in the well-watered plants, which was confirmed by the leaf gas exchange measurements. Soil moisture deficit also changed the 'Simple Fluorescence Ratio' due to a strong decline of its values in non-irrigated sugar beet plants. These decreases were caused by higher far-red fluorescence rather than by the decrease of red fluorescence. Higher far-red fluorescence intensity (Supplementary Figure S1) is subjected to a strong re-absorption of the ChlF near 690 nm, since in vivo chlorophyll *b* and carotenoids transmit the absorbed energy

to chlorophyll *a* (Cerovic et al. 1999). As previous studies pointed out, the FR/R ratio corresponding to the SFR might be used to estimate the chlorophyll content in vivo (D'Ambrosio et al. 1992; Hák et al. 1990). However, our findings indicate that changes in the SFR do not necessarily correlate with changes of the chlorophyll content. This effect becomes obvious for all tested genotypes in the greenhouse study where both treatments did not display any significant differences in the chlorophyll *a* and *total* chlorophyll concentration. Better matching between SFR and destructive chlorophyll values could be determined under field conditions, indicating that this ratio is influenced by the cultivation system and environmental conditions. Further, the selection of the right excitation light source was of particular relevance for the data quality. Green excitation light provides significantly better information about the ChlF than red excitation light. As compared with red, green excitation light is less absorbed by chlorophylls and penetrates into lower leaf layers before the light is absorbed and transferred into red fluorescence (Buschmann and Lichtenthaler 1998).

In order to prove the sensitivity of the tested fluorescence technique, gas exchange measurements were carried out during the first drought period in the greenhouse study. Similar to the fluorescence readings, water withdrawal negatively affected all gas exchange parameters. As soon as the water supply was stopped, a continuous decrease in Pn became obvious. This effect is explained by a reduced CO_2 diffusion caused by a reduction in stomatal conductance, since root-sourced abscisic acid lead to stomatal closure (Davies and Zhang 1991). However, Pn of 'Pauletta' was characterized by a lower sensitivity to changing water supply conditions. The differences between the cultivars regarding their photosynthesis during desiccation and re-watering periods highlight that drought-tolerant species can efficiently control their stomatal function and permit some carbon fixation at drought stress (Yordanov et al. 2003). Despite the fact that both fluorescence and gas exchange devices have different work principles, they indicated the same genotype-specific responses to drought. Consequently, the present knowledge can be used in further water deficit studies with fluorescence measurement as a potential substitute of gas exchange measurements, which are frequently limited by environmental influences.

Calculations of leaf osmotic potential (OP) are an additional important parameter to estimate plant-specific responses to desiccation. All temporarily non-irrigated genotypes, independent of the cultivation system, were characterized by lower OP than control plants. This follows the osmotic adjustment which leads to the accumulation of solutes to maintain a constantly high turgor (Bagatta et al. 2008; Chimenti et al. 2002). Our results indicate genotype-specific differences, as non-irrigated plants of 'Pauletta' always have the lowest OP. As indicated before, the blue fluorescence (BF) provides relevant information to characterize cultivar-specific responses to unfavourable conditions (Cerovic et al. 1999; Hura et al. 2009a; Lichtenthaler et al. 1997). In the greenhouse trial, differences only occurred for 'Pauletta' during the first experimental period. This might be explained by the pronounced accumulation of phenolic compounds in plant leaves under water deficit. From a physiological point-of-view, these phenolics operate as a light-filter for the photosynthetic apparatus (Schweiger et al. 1996). Thereby, the harmful radiation is transferred into blue-green fluorescence, and in this way PSII is protected (Buschmann and Lichtenthaler 1998). Due to the fact that ferulic acid is the main component responsible for the blue fluorescence in sugar beet leaves (Morales et al. 1996, 1998), an increase of its emission could be expected in temporarily non-irrigated plants. Unfortunately, destructive reference analysis of ferulic acid concentrations were only performed 100 DAS. Our results confirm that the evaluated genotypes differ in their ferulic acid concentration. 'Cesira' and 'Berenika', which reacted more sensitively in their ChlF to temporary water deficit, were characterized by a considerable lower ferulic acid concentration than 'Pauletta' and 'Mauricia'. Nevertheless, similar to the BF, there were no significant differences between the treatments. This effect can be associated with genotype as well as growth stage dependence of the BF (Dahn et al. 1999; Meyer et al. 2003). As our results displayed, the B/FR ratio is also an appropriate parameter. Thereby, changes in the blue-to-far-red fluorescence ratio were mainly caused by the increase of the ChlF. Thus, we proved in this study that ChlF by using green excitation light was the predominant parameter to identify unfavourable conditions in sugar beet plants.

Conclusion

Specific parameters of the multispectral fluorescence signature, and especially those indices based on the far-red chlorophyll fluorescence, are reliable indicators for sensing a temporary water deficiency stress in sugar beet. The four evaluated cultivars had distinct responses concerning the extent of the changes during the stress and re-watering induced recovery phases. These findings were confirmed by gas exchange and destructive reference measurements. Regardless of whether the genotypes were cultivated in the field or under greenhouse conditions, the most promising fluorescence parameter was red fluorescence excited by using green light. Perspectively, our results support the potential of the multiparametric fluorescence technique for the objective screening of sugar beet genotypes to drought stress tolerance.

Author contribution GN was responsible for acquisition of funds (subproject S2, CROPSENSe.net) and research planning. GL carried out the research and wrote the manuscript under the guidance and supervision of MH.

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