# SELECTION OF FLUORESCENCE INDICES FOR THE PROXIMAL SENSING OF SINGLE AND MULTIPLE STRESSES IN SUGAR BEET

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## ABSTRACT

In our studies, we evaluated the suitability of fluorescence-based signals to detect and differentiate the occurrence of single and multiple stresses in sugar beet leaves. In the scope of greenhouse and field experiments we evaluated the impact of nitrogen supply, drought, and occurrence of pathogens on the characteristic fluorescence signature of selected sugar beet cultivars. Here, we present major outcomes using a commercial hand-held multiparametric sensor. In particular, we focus on the fluorescence indices 'Blue-to-Far Red fluorescence ratio' and 'Nitrogen Balance Index'. The advantages and limitations of these and other fluorescence signals and fluorescence indices are discussed.

**Keywords:** Drought, Pathogens, Nitrogen, detection, differentiation, fluorescence indices

## INTRODUCTION

The use of fluorescence indices for sensing the impact of abiotic and biotic stresses in agricultural crops is well documented in the literature. Pigment fluorescence gives a precise picture about the plant physiology and its changes following the occurrence of stresses. In general, alterations in such optical signals is caused either by the stress-induced accumulation of one or more fluorophores, or the degradation of specific molecules such as chlorophyll. Unfortunately, many stresses might influence the fluorescence signature of leaves and plants in a similar way. Thus, one of the biggest challenges aiming the practical use of optical sensors is not only to detect the occurrence of stresses, but also to differentiate between different stress types. With this background we conducted our experiments using potted plants allocated inside a polytunnel to evaluate the potential of several fluorescence indices for the detection and differentiation of abiotic and biotic stresses at leaf level.

## MATERIAL AND METHODS

### Plant material and fluorescence recordings

Experiments were conducted on selected sugar beet (*Beta vulgaris* L.) cultivars Pauletta, Cesira, Berenika and Mauricia (KWS Saat AG, Einbeck, Germany). Fluorescence signals were collected at leaf level with a multiparametric handheld fluorescence sensor (Multiplex®, Force-A, Orsay, France), as described elsewhere (Bürling *et al*., 2013; Leufen *et al*., 2013; Kautz *et al.*, 2014). Briefly, the fluorescence is excited by light-emitting-diodes (LED) by UV, green or red light. Fluorescence signals are measured in the blue (425-475 nm), red (680-690 nm) and far-red (720-755 nm) spectral regions. The fluorescence signals were always recorded at leaf level and an area of approximately 50 cm . Dataset comprises the absolute fluorescence intensities and a number of fluorescence ratios, including the 'Blue-to-Far Red ratio' (BFRR_UV) and the 'Nitrogen Balance Index' (NBI). Reference analysis such as osmotic potential, proline concentration and chlorophyll content were done according to standardized methods used in our group (Bürling *et al*., 2013; Leufen *et al*., 2013; Kautz *et al.*, 2014).

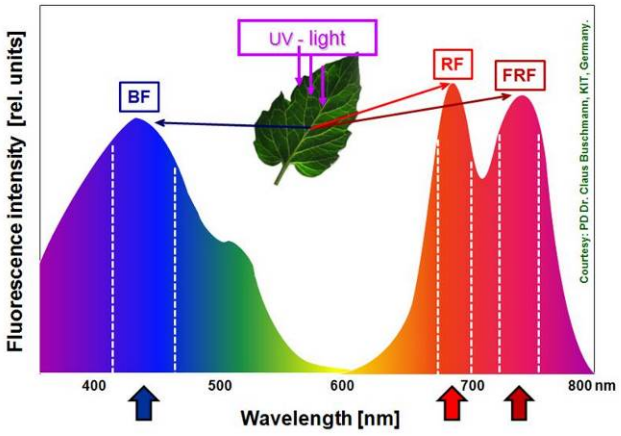



**Figure 1.** Characteristic fluorescence spectra of a green leaf when excited with UV light. Arrows indicate relevant spectral regions which were considered in the present study. Graph adapted and modified after Buschmann *et al*., 2000.

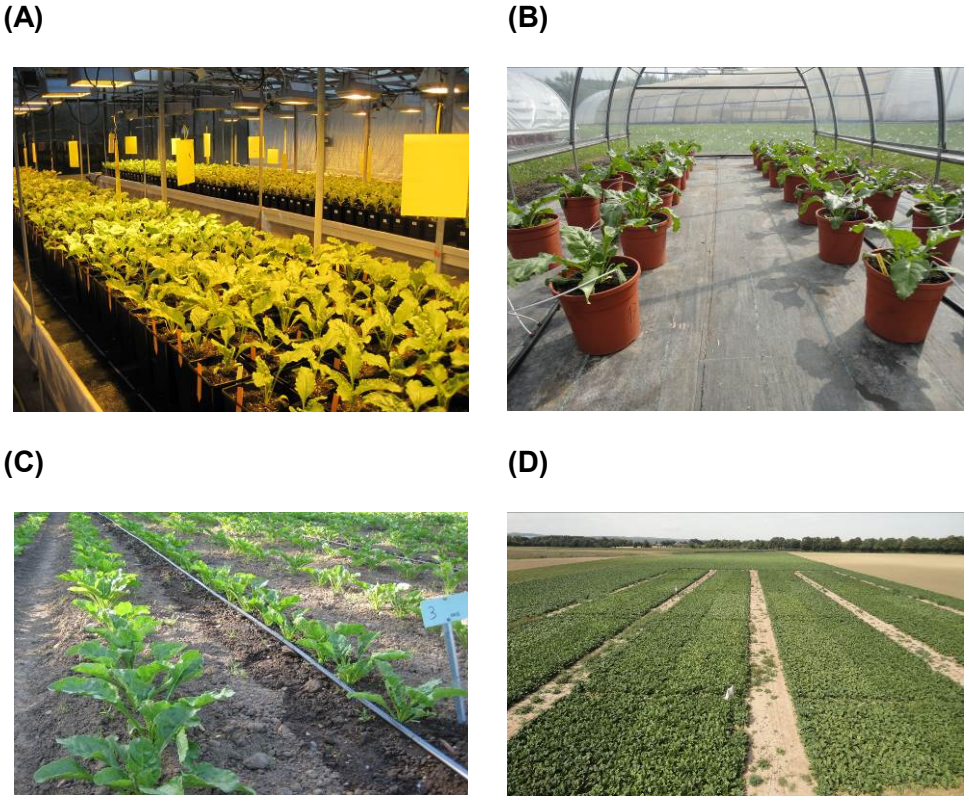



**Figure 2.** Experimental setup in closed greenhouses (a), partially open polytunnels (b) and field conditions (c, d). Copyright of photographs: Dr. Mauricio Hunsche (University of Bonn).

**Experiments**

*Trial 1: Impact of water deficit*

Experiments on the impact of water deficit were conducted under greenhouse and field conditions. In the greenhouse, uniform plants grown in 2 l plastic pots (0.20 m height, 0.10 m diameter) filled with a commercial peat substrate were selected for the experimental treatments. Plants (n = 8 per genotype and treatment) were placed at random on two benches with controlled nutrient supply and supplemental light (photoperiod 16 h) by using high-pressure sodium lamps (250-350 µmol $m^{-2}$ $s^{-1}$ PAR at the leaf level). The water deficit induced and maintained by withholding irrigation for a pre-defined period.

In the field, sugar beet plots were established and managed according to the local recommendations of good agricultural practice. Accordingly, seeds were sown in 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 m x 3 m) were randomized (n = 4 for each genotype and treatment) in the blocks. Water supply was ensured by drip

irrigation along the central row of plants. During the season, the soil moisture was monitored at 0.40 m depth with digital tensiometers (Blumat Digital BD2, LM-GL, Bambach GbR, Geisenheim, Germany) in nine representative 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.

*Trial 2: Impact of nitrogen supply*
A field experiment was set at the experimental station Campus Klein-Altendorf, University of Bonn. The sugar beet cultivars Pauletta, Cesira, Berenika, and Mauricia were cultivated according to the regional agricultural practices, except for the nitrogen fertilization. Nitrogen was provided either at 20 or 100 kg N $ha^{-1}$. The fluorescence signals were recorded from Mai to October in regular intervals.

*Trial 3: Impact of multiple stresses*
Experiments were done with the cultivars Pauletta and Cesira, which differ in their susceptibility to powdery mildew. Plants were grown in pots allocated in polytunnels. Abiotic and biotic stresses were applied individually or in combination, while control plants were not exposed to any stress. An overview of the experimental treatments is provided in Table 1.

**Table 1.** Experimental treatments evaluated in the study.

| Abbreviation | Treatment Description |
|---|---|
| C | Control plants (regular watering, 150 kg N $ha^{-1}$) |
| ND | Low N–supply (100 kg N $ha^{-1}$ ) |
| PM | Powdery mildew (inoculated at 29 DAS) |
| WD | Water deficit (start at 32 DAS) |
| PM-ND | Powdery mildew (inoculation at 29 DAS) + low N-supply |
| WD-ND | Water deficit + low N-supply |
| WD-PM | Water deficit + powdery mildew |
| WD-PM-ND | Water deficit + powdery mildew + low N-supply |

## RESULTS AND DISCUSSION

### Impact of drought stress
A short-term water deficit induced by interruption of the irrigation caused a significant decrease of the Blue-to-Far-Red fluorescence ratio (BFRR_UV). The four evaluated sugar beet cultivars underwent alterations in this parameter, whereas the cultivar ‘Pauletta’ was less affected. The observed modifications were strongly driven by alterations of the far-red fluorescence in a range of 10% (Pauletta) to 21% (Berenika). In parallel, the blue fluorescence changed in a range of -0.5% (Berenika) to 1.3% (Pauletta). Evaluations of the osmotic potential confirmed the stress situation, being the change of the values in the range of 24% (Mauricia) to 44% (Cesira).

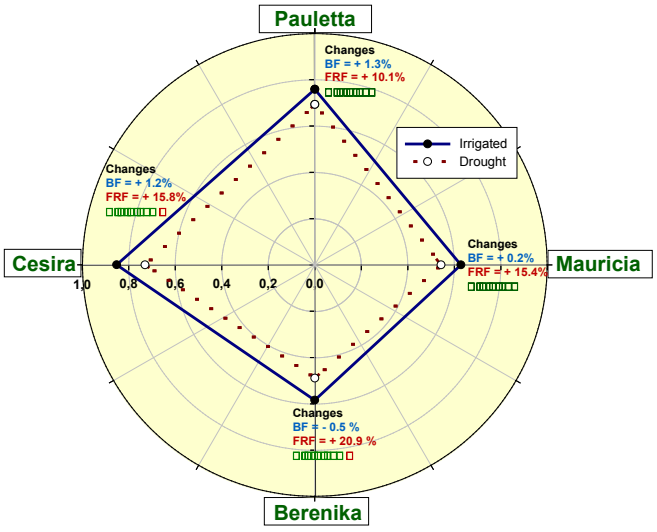



**Figure 3.** Impact of soil water availability on the BFRR_UV of sugar beet leaves. Plants were cultivated in field plots and drop-irrigated to avoid undesired drought; at about 100 DAS irrigation was stopped to induce water deficit stress. Alterations of the blue and red fluorescence, and the osmotic potential (given for each cultivar in the body of the graph) are expressed as percent of the control plants.

**Impact of nitrogen supply**

In a field experiment, we evaluated the impact of nitrogen fertilization (20 or 100 Kg N $ha^{-1}$) on the fluorescence indices 'Blue-to Far-Red Fluorescence' (BFRF_UV) and 'Nitrogen Balance Index' (NBI) in a timeframe of 90 to 180 days after sowing (DAS). In the four evaluated cultivars (Pauletta, Cesira, Berenika and Mauricia), BFRR raised in the timeframe of the experiment (Fig. 4). However, the increase from the first to the last measurement was less accentuated in the cultivar Mauricia. The BFRR indicated only sporadically differences induced by the nitrogen treatments; in the majority of cases, there was no significant difference between the nitrogen fertilization levels.

In contrary, the NBI revealed significant differences between the treatments and dynamic changes during the vegetation period (Fig. 4). In most cases, NBI was higher in those plants fertilized with 100 kg N $ha^{-1}$, although not always significantly. A detailed view confirms that NBI and its development in the time was specific for the evaluated species. While for the cv. Mauricia values remained more or less unchanged at a high level, values of Cesira remained constant at an intermediary level. In contrast, values of Pauletta decreased linearly from the first to the last evaluation date, while values of Berenika decreased from 80 to 160 DAS, and increased slightly thereafter.

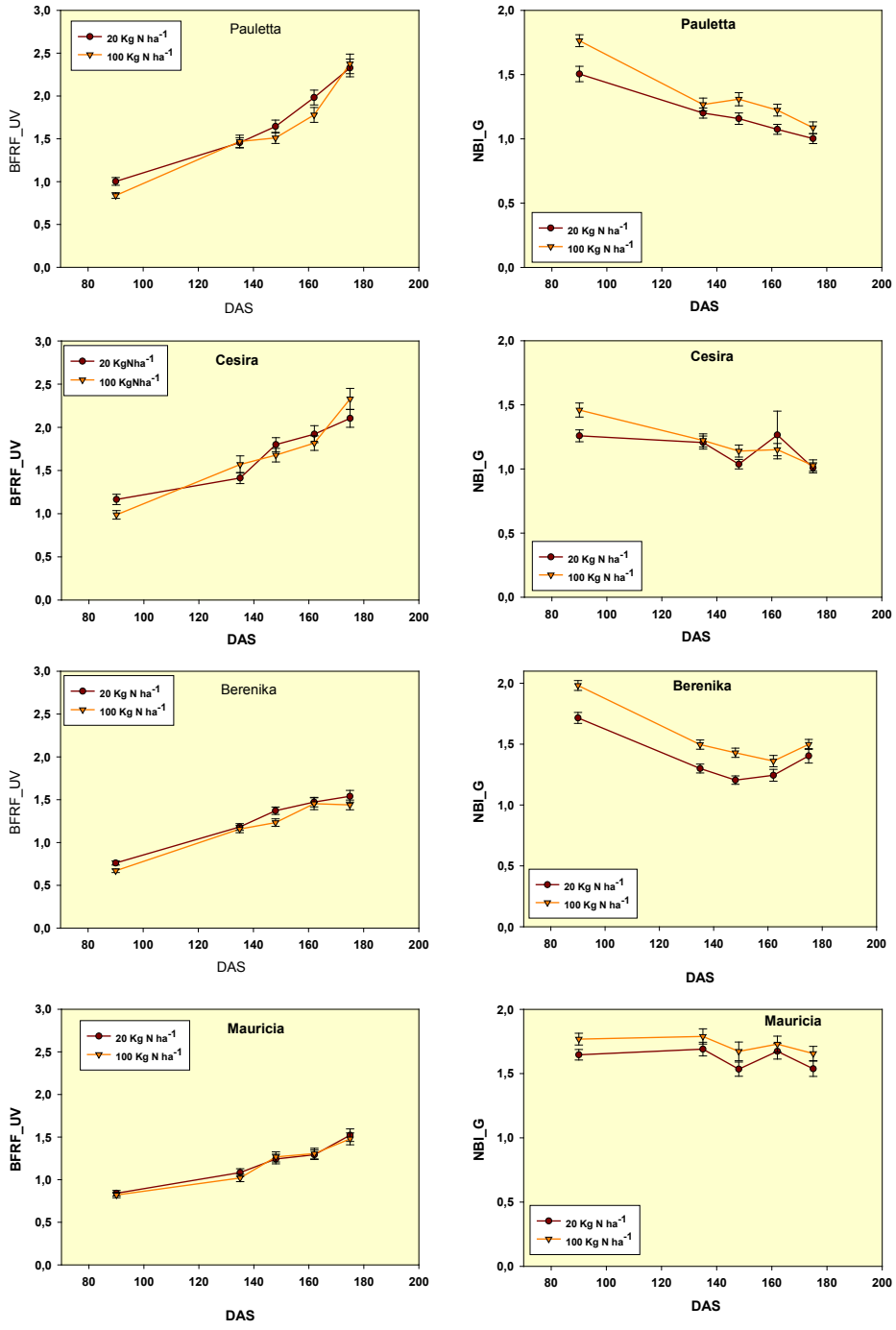
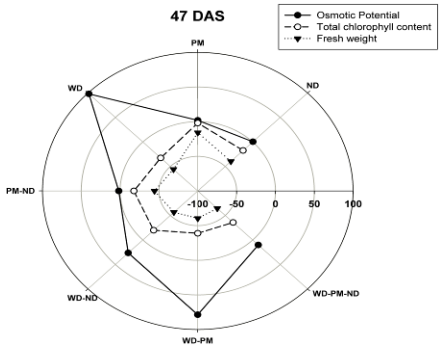



**Figure 4.** Influence of nitrogen supply (20 or 100 Kg N $ha^{-1}$) on the fluorescence indices BFRR_UV and NBI_G (Means ± SE, n > 15). Recordings were taken in the vegetation season 2012.

**Impact of multiple stresses**

For this study with potted plants the cultivars Pauletta and Cesira were selected for physiological and biochemical evaluations at 41 and 47 days after sowing. In general, both cultivars had a similar response pattern to the applied treatments, although the intensity of stress-induced modifications were genotype-specific. In both cultivars, the treatment WD+ND caused a strong (about 25%), and the treatments WD and WD + PM caused a very strong (75% to 90%), increase of the osmotic potential. With exception of the treatment PM, chlorophyll concentration decreased in 25% or more under the influence of water deficit, nitrogen deficiency, and the combined stresses (Fig. 5).

Pauletta




Cesira

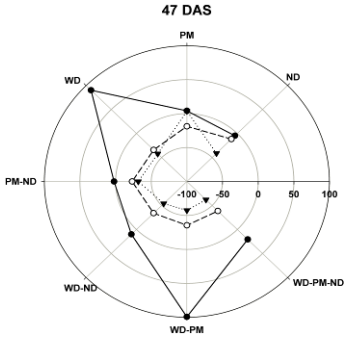



**Figure 5.** Impact of single and multiple stresses on the osmotic potential, chlorophyll content, and fresh-weight of leaves. Values in the range of -100% to +100% indicate the alteration of the respective values, as compared to the control treatment (0%).

Also in this study we focused on the BFRR_UV and NBI as indicative indices for stress sensing (Fig. 6). For BFRR, there was no clear trend concerning the impact of treatments and evaluation date. At 41 DAS highest values in Pauletta were observed in the treatment PM-ND while in Cesira there were no outstanding values, being the lowest values registered in the WD and the control treatments. At 47 DAS the trends changed somehow: in Pauletta a comparative increase in the treatments PM and WD, and in Cesira a comparative decrease in the treatment WD-ND, were recorded.

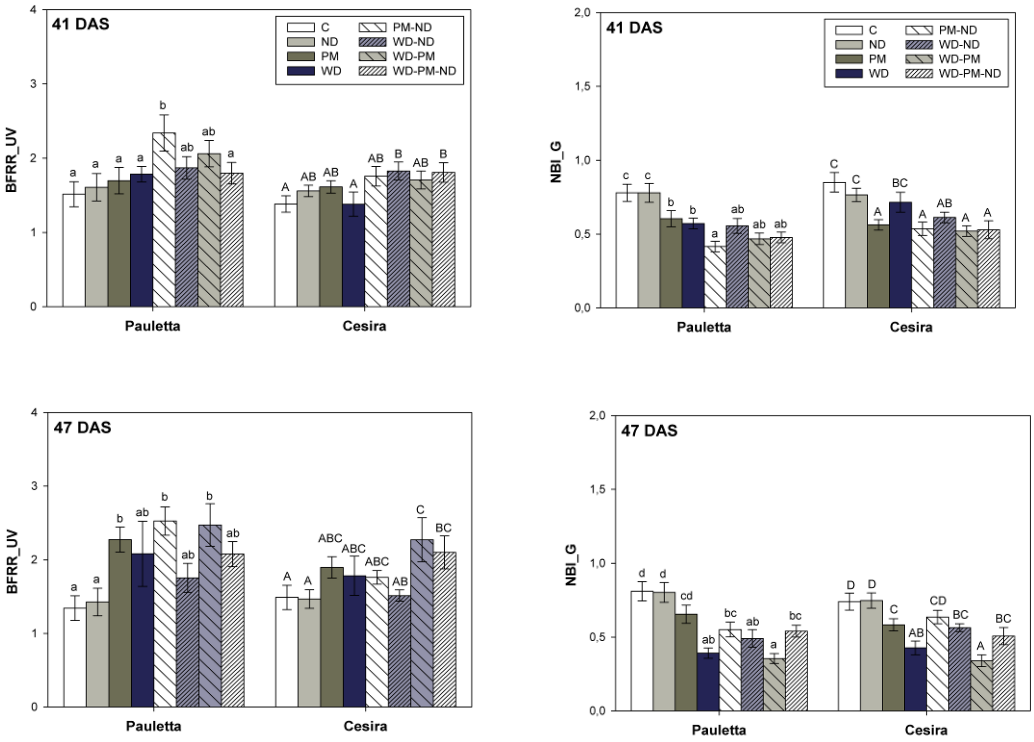



**Figure 6.** Impact of single and multiple stresses on the BFRR_UV and NBI of the sugar beet cultivars Pauleta and Cesira. Means followed by the same letter do not differ according to Duncan ($p \leq 0.05$). Data were originally published in Leufen *et al*., 2014.

At the same time, the NBI revealed a more homogeneous picture. At 41 DAS highest values were observed in the treatments C and ND in both cultivars; in Cesira, also the treatment WD induced significantly higher values. At 47 DAS the trends of higher values in C and WD were confirmed for both cultivars, contrasting the values of WD and WD+PM which were at lowest level.

## DISCUSSION AND CONCLUSIONS

Aim of this work was to evaluate the suitability of fluorescence-based signals to detect and differentiate the occurrence of single and multiple stresses in sugar beet leaves. For this purpose we selected nitrogen supply levels and water deficit as abiotic stresses, and powdery mildew as biotic stress, as representative factors impairing the physiology of the plants. As indicative parameters, we selected the fluorescence indices 'Blue-to-Far Red fluorescence ratio' and 'Nitrogen Balance Index' to evaluate stress-induced alterations of the plant physiology.

Fluorescence spectroscopy is widely used to evaluate the physiological response of plants to stresses under controlled and semi-controlled conditions.

Amongst others, the fast and easy-recordings, the non-destructive nature of the measurements, and the possibility to perform measurements also in the field can be mentioned as decisive aspects of its widely acceptance. Classically, the pulse-amplitude-modulated (PAM) chlorophyll fluorescence is used to estimate the photosynthetic performance of stressed and non-stressed plants. More recently, the spectrally resolved fluorescence systems using UV (Fig. 1) or other excitation sources (e.g., green or red light) have been developed, tested, and adjusted for different applications (e.g., Buschmann *et al*., 2000; Bürling *et al*., 2013; Leufen *et al*., 2013; Kautz *et al*., 2014). The literature on fluorescence spectroscopy is very rich, and more detailed background information can be found several excellent research and/or review articles (e.g., Cerovic *et al*., 1999; Buschmann and Lichtenthaler, 1998).

Although the absolute fluorescence intensities in the blue, green, red and far-red fluorescence might be used to evaluate the physiological state of the plants, their use under non-standardized conditions requires caution. While the blue and green fluorescence are strongly related to the amount and composition of specific fluorophores (Cerovic *et al*., 1999), the red and far-red intensities are related to both the quantity of chlorophyll and the functionality of photosynthetic steps (Buschmann *et al*., 2000).

The occurrence of drought, nitrogen deficiency or leaf diseases during or at specific phases of plant development might induce specific physiological changes (e.g., stomatal closure, change of the electron transport rate, etc.) and alterations of amount and composition of fluorescing pigments (e.g., synthesis and/or degradation of chlorophyll, accumulation of blue- or green fluorophores in epidermal cells). In addition, leaves may undergo structural changes (e.g., increase of number of cells per surface area due to drought stress). In the sum, such alterations lead to specific changes of the characteristic fluorescence signature. On the top, pathogens provoking foliar diseases might not only influence the results by impairing the plants, but they also contribute to the signal alteration due to their own auto-fluorescence.

When exposing sugar beet plants to a drought stress we observed a strong increase of the UV- excited far-red fluorescence, while the blue fluorescence was less affected (Fig. 3). Because the fact that absolute fluorescence intensities are susceptible to the interference of other factors (equipment setup, excitation intensity, distance between sensor and leaf, temperature, etc), the use of fluorescence indices which are based on at least two independent intensities is preferred. Therefore, we selected the indices BFRR_UV and NBI as indicative parameters (Ben Ghozlen *et al*., 2010). According to our evaluations in the greenhouse and field, the BFRR significantly responds to the occurrence of drought, while its impact due to nitrogen deficiency is not always significant (Figs. 3, 4, 6). BFRR also responds to powdery mildew, particularly when it happens together with other stresses.

On the other hand, the NBI is a complex fluorescence excitation-emission ratio (FRF_UV/RF_G) which depends on both epidermal phenolics and chlorophyll. NBI responds particularly to nitrogen nutrition of the plant and can be used directly in comparison studies if the light and the developmental stage are kept constant among samples (*Zoran Cerovic, personal communication*). In three of four cultivars we observed higher NBI in plants receiving more nitrogen fertilization (Fig. 4). On the other hand, N deficit did not cause a significant decrease of NBI (Fig. 6); this effect was observed for water deficit and multiple stresses.

In summary, we compared different sugar beet cultivars grown under several conditions and exposed to different abiotic and biotic stresses. The results show that specific fluorescence signals might be used for sensing single or multiple stresses in sugar beet. In many cases, there was a relationship to biochemical factors. However, an overall and robust differentiation of the stress factors by using only one fluorescence ratio could not be accomplished. In the sum, our results clearly demonstrate the potential, but also some limitations, for using specific fluorescence indices as diagnostic tool in precision farming.

## ACKNOWLEDGMENTS

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