

Influence of water shortage on apple seedling growth under different radiation composition

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Abstract

Abiotic stresses strongly impair plant development and might impose detrimental effects particularly on seedlings. Irradiance and water deficit are relevant factors, which affect performance of young plants under controlled conditions. In our study, we investigated the influence of water shortage combined with different radiation sources - light emitting diodes (LED) and compact fluorescence lamps (CFL) - on physiological and biochemical parameters of young apple plants. Stress responses were assessed by fluorescence-based indices, while relative water, chlorophyll (Chl), and proline content served as reference parameters. The watering regime had a higher impact on biochemical indicators than the radiation sources. Lower Chl content was determined in plants grown under LED both in control and in water deficit plants. Nitrogen balance index and nitrogen balance index with red radiation excitation showed similar patterns regarding leaf Chl results in relation to the radiation source, being higher under CFL. In contrast, the flavonol index was higher in plants cultivated under LED. Stomatal conductance and maximal photochemical efficiency emphasised a radiation quality effect with higher values for CFL. In conclusion, fluorescence indices related to nitrogen status and flavonol content are promising parameters to sense physiological impairments under the given conditions. However, discrepancies compared to previous studies might be related to the different plant species, the nature of dehydration, and the measuring conditions.

Keywords: compact fluorescence lamps, light emitting diodes, portable fluorescence sensors, water deficit.

Introduction

Improper radiation sources and limitation of water supply can significantly impair the development of seedlings, where is a high susceptibility of lipids, proteins, and nucleic acids to damage, resulting in various biochemical and physiological disorders, and consequently in yield losses (Osakabe *et al.* 2014, Nouri *et al.* 2015).

In controlled (climate-chamber) and semi-controlled (greenhouse) conditions, a broad range of artificial radiation, *e.g.*, sodium vapour lamps, compact fluorescence

lamps (CFL), and light emitting diodes (LED), are commonly used to reach optimal photosynthetic active radiation. Most LED systems provide mainly red and blue radiation, which in comparison to green radiation is the most absorbed part of spectrum by photosynthetic pigments and provides most energy for photosynthesis (Kim *et al.* 2005, Massa *et al.* 2008, Astolfi *et al.* 2012). Nevertheless, under strong white radiation, green radiation might drive leaf photosynthesis even more efficiently than red radiation (Terashima *et al.* 2009). Excessive blue radiation might initiate a chloroplast avoidance response

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Abbreviations: CFL - compact fluorescence lamps; CFL_WD - compact fluorescence lamps and water deficit; Chl - chlorophyll; Chl INDEX - chlorophyll index; DAS - days after sowing; Flav Dx - flavonol index (*Dualex*[®]); Flav Mx - flavonol index (*Multiplex*[®]); F_v/F_m - variable to maximum Chl fluorescence (maximum quantum efficiency of PS II); g_s - stomatal conductance; LED - light emitting diodes; LED_WD - led emitting diodes and water deficit; NBI - nitrogen balance index; NBI R - nitrogen balance index with red excitation; PAM - pulse amplitude modulation; PPFD - photosynthetic photon flux density; PS - photosystem; RWC - leaf relative water content; SFR R - simple fluorescence ratio with red excitation.

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as well as a decrease in mesophyll conductance resulting in decreased photosynthetic efficiency (Loreto *et al.* 2009). In summary, these studies suggest that a fine balance in choosing the right radiation spectrum for plant cultivation is essential.

Radiation quality might also support plants in protecting them against water shortage by mediating stomatal aperture and controlling transpiration (Zeiger and Field 1982, Chen *et al.* 2012). On these terms, Hoffmann *et al.* (2015) demonstrated that blue radiation caused structural and functional acclimations at pepper plants at leaf and cellular levels. In the same study, morphological acclimations contributed to different water requirements, while photosynthetic acclimations, affected by the composition of chloroplasts, reduced susceptibility to a short-term water deficit (Hoffmann *et al.* 2015). Generally, wide-spread information on the interaction between radiation quality and physiological responses of plants to water deficit is still missing. A more precise knowledge of such interactions could contribute to promote a new outlook for purposeful applications of radiation quality to reduce negative impacts of stress conditions on ornamental and horticultural plants.

Methods to assess plant responses to the effect of radiation and water supply mostly depend on destructive and time-consuming determination of biochemical indicators, such as chlorophyll (Chl), nitrogen, and proline content, and physiological indicators, such as water and osmotic potentials, as well as relative water content (RWC). However, non-destructive sensing methods have also been applied in plant breeding systems to minimise these limitations on evaluating stress responses. Among others, the use of fluorescence approaches with pulse-amplitude-modulation (PAM) has gained attention (Roháček *et al.* 2008, Lichtenthaler *et al.* 2013).

Fluorescence of Chl molecules, emitted in the red (R) and far-red (FR) spectral regions, is the basis of a widely applied technique with the purpose to evaluate impacts of adverse environmental conditions on plant physiology (Bürling *et al.* 2013, Lichtenthaler *et al.* 2013, Kautz *et al.* 2014). Chlorophyll *a* and *b* emits fractions of absorbed radiation energy as fluorescence (Buschmann and Lichtenthaler 1999). Besides traditional methods based on the PAM method, Chl fluorescence can also be detected through multi-indices fluorescence excitation approaches (Tremblay *et al.* 2012). Variations in the fluorescence signature caused by alterations in the amount and composition of fluorescing pigments besides Chl can be used as additional indicators to broaden the assessment of the environmental impact on plant physiology (Gitelson *et al.* 1999). Portable fluorescence sensors have been developed to facilitate the assessment of secondary metabolites, such as phenolic compounds in the leaf epidermis. Their synthesis is frequently triggered as a protective mechanism against environmental stresses as imbalance reaction to the primary metabolism. Different ratios and indices can be calculated depending on the incident radiation excitation and collected fluorescence signals to estimate a plant physiological status by means of Chl, flavonol, and nitrogen content (Ghozlen *et al.* 2010,

Cerovic *et al.* 2012, Tremblay *et al.* 2012).

In addition, studies with different species are important to understand how, for example, the morphological structure will influence fluorescence measurements. Species, such as sweet peppers, with a shorter growing phase, low lignified stems, and thicker leaf layer, might have a different fluorescence signature than species with a longer growing phase, more lignified stem, and thinner leaves, such as *Malus domestica* (Kautz *et al.* 2015, Hamann *et al.* 2018). Our study hypothesises that specific radiation-mediated acclimations at plant and leaf levels reduce the vulnerability of apple seedlings to water deficit. In particular, the aim of this study was to investigate the physiological responses of apple seedlings cultivated either under CFL or under LEDs and additionally subjected to a period of water shortage.

Materials and methods

Plants, growth conditions, and experimental setup: The trial was conducted in a custom-built climate chamber. Seeds of *Malus domestica* Borkh. cv. Golden Delicious were stratified at 4 °C in the dark for 28 d. Subsequently, they were sown in sterilized trays filled with a mixture of peat (60 %), sand (20 %), and Perlite (20 %) and allocated under white compact fluorescence lamps (CFL) with main radiation peaks at 435, 545, and 612 nm (MASTER PL-L 4P, Cool Daylight, 30 °C, Philips, Amsterdam, The Netherlands) (Hoffmann *et al.* 2015). The photosynthetic photon flux density (PPFD) of CFL was set to 95 $\mu\text{mol m}^{-2} \text{s}^{-1}$ whereby 14 % of the radiation energy was provided by blue, 40 % by green, and 46 % by red radiation.

Five weeks after sowing, seedlings were transplanted into pots (11 cm, Lamprecht-Verpackungen, Göttingen, Germany) filled with 250 g of the above described peat-sand-Perlite mixture and cultivated under the same environmental conditions for three additional weeks. Next, half of the plants were kept under CFL while the remaining plants were allocated under LED modules (a prototype optimised for our research purposes; Ushio Lighting, Tokyo, Japan). The LED-modules were characterized by a 2:1 combination of red and blue LEDs with single peaks at 665 and 445 nm, respectively. The PPFD of the LED modules was also set to $95 \pm 5 \mu\text{mol m}^{-2} \text{s}^{-1}$, whereby 36 % of the energy was provided by blue radiation and 64 % by red radiation. Plants were cultivated under a 12-h photoperiod, day/night temperatures of 21/20 °C, and relative humidity of 80 %. One week after the plants were assigned to the respective radiation source (CFL or LED), the water supply treatments were initiated (63 d after sowing; DAS). After the substrate was saturated with a Hoagland nutrient solution (pH 6.2, electrical conductivity 1.4 mS cm^{-1}), drought stress was induced by withholding water and nutrient solution for 15 d (63 - 78 DAS), followed by a recovery period for 8 d (78 - 86 DAS). Control plants were irrigated daily according to their needs throughout the experiment. The experimental treatments were identified as follows: CFL - compact fluorescence lamps (control), CFL_WD - CFL + water deficit, LED -

light-emitting diodes (control), LED_WD - LED + water deficit. The experiment was separated into three different watering phases: well-watered - when all plants were completely watered to soil saturation, water deficit - when watering of the plants was withheld, and recovery - when all plants were re-watered to pot saturation.

Physiological and biochemical indicators: The so-called reference parameters were assessed in a destructive way to compare with, and validate against, the parameters assessed by fluorescence devices. Leaves were harvested and sampled on 71, 76, and 83 DAS to evaluate leaf relative water content, Chl content, and proline content.

Relative water content (RWC), as a measure of leaf hydration status (Mullan and Pietragalla 2012), was measured according to the method described by Barrs and Weatherley (1962) with few adaptations. Disc of 2.10 cm² was excised from leaves with a sharp cork borer to avoid leaf veins. Discs were then weighed to obtain leaf fresh mass (FM), after which the samples were hydrated in Petri dishes overnight to obtain fully saturated mass (FSM). Samples were then oven dried at 80 °C overnight to determine dry mass (DM). The RWC was calculated as described by González and González-Vilar (2001).

Chlorophyll content was assessed by applying the method described by Strobl and Türk (1990). Three fully expanded leaves of each seedling on each measurement day were collected and cold-transported to the lab, immediately frozen (-20 °C ± 2), freeze-dried, ground for 1 min using a ball mill (MM 2000, Retsch, Haan, Germany) and 5 cm³ of methanol were added to 50 mg of the dried and ground sample, mixed, and centrifuged at 4000 rpm for 15 min (Varifuge 3 OR, Heraeus Sepatech, Hanau, Germany). Absorbance of the extracts was quantified by a UV-VIS spectrophotometer (Lambda 35, Perkin-Elmer, Waltham, MA, USA) at 650 and 665 nm. Chlorophyll content was calculated using formulas given in Hoffmann *et al.* (2015).

Proline content was determined colourimetrically according to the method described by Abrahám *et al.* (2010) with slight modifications. Briefly, 3 cm³ of sulfosalicylic acid (3 %, m/v) were added to 0.1 g of dried and ground leaf material, and the mixture was homogenized and centrifuged at 4000 rpm for 15 min (Varifuge 3.0R). Next, 0.2 cm³ of the supernatant was added to 1.8 cm³ of sulfosalicylic acid, 2 cm³ of glacial acetic acid, and 2 cm³ of ninhydrine acid and incubated in a hot water bath (100 °C) for 1 h. After cooling to 20 °C, 4 cm³ of toluene was added and mixed. The absorbance of the supernatant was measured at 520 nm with a UV-spectrophotometer (Lambda 35). Proline content was calculated from a standard curve.

Fluorescence measurements: Fluorescence indices were assessed by two portable fluorescence sensors, Dualux®4 Scientific and Multiplex®3 (Force-A, Orsay, France). They were adopted to record conditions during the experiment. These devices can be used for instant evaluation of the plant status both under natural irradiance in the field or under artificial irradiance in controlled chambers. Three

different leaves per plant distributed in the lower, middle, and upper part of the seedling were selected and recordings were taken on 63, 66, 69, 71, 73, 76, 78, 83, and 86 DAS.

The hand-held sensor Dualux®4 Scientific combines the use of fluorescence and radiation transmission of a leaf (Cerovic *et al.* 2012). It determines the absorbance of the leaf epidermis in the ultraviolet (UV) range through the differential measurement of the Chl fluorescence and can also estimate the Chl content of the leaf using different wavelengths in red (R) and in far-red (FR) regions. In this study, Chl index (Chl INDEX), flavonol index (Flav Dx), and nitrogen balance index (NBI) were measured. The calculation details of these parameters can be found in the literature (Cerovic *et al.* 2012, Tremblay *et al.* 2012, and briefly mentioned in Table 1 Suppl.).

The portable multi-parametric fluorescence sensor Multiplex®3 uses LED which excite the plant material at three excitation channels in spectral regions of 375 nm (UV), 518 nm (green), and 630 nm (red). The plant fluorescence was detected in red (RF 680 - 690 nm) and far-red (FRF 720 - 755 nm) spectral regions. A disc with an aperture of 4 cm was used in front of the optical unit to enable the measurement of an area of approximately 12.5 cm² by maintaining a constant distance of 10 cm between the radiation source in the device and the measured leaf surface. The analysed indices obtained by Multiplex®3 were SFR R, NBI R, and Flav Mx. The description of these parameters and their calculations can be found in Bürling *et al.* (2011), Zhang *et al.* (2012), Leufen *et al.* (2014), Hoffmann *et al.* (2015), and in Table 1 Suppl.

Maximum quantum efficiency of PS (photosystem) II (F_v/F_m) was recorded once a week using an imaging pulse-amplitude-modulated fluorometer (Imaging PAM, Heinz-Walz, Effeltrich, Germany). Fluorescence images (640 × 480 pixels) were taken by a black and white CCD camera on fully expanded leaves at the third leaf insertion level. To standardize measuring conditions and to make sure that all PS II reaction centres were open when the maximal photochemical quenching was determined, plants were dark-adapted for 30 min prior to the evaluations (see the review of Maxwell and Johnson 2000). After recording the ground fluorescence (F_0), a radiation saturation pulse was given to determine the maximum fluorescence yield (F_m). Measurements were performed on 63, 71, 76, and 83 DAS.

Correlation between maximum quantum efficiency of PS II (F_v/F_m) and Chl fluorescence indices, represented by the fluorescence indices (Chl INDEX and SFR R), were portrayed in diagrams with linear regression and coefficient of determination (R^2).

Stomatal conductance (g_s) was recorded with a portable infrared gas analyser (CIRAS-1, PP Systems, Amesbury, USA) equipped with a standard 2.5 cm² leaf cuvette (PLC B, PP Systems). Measurements were carried out under the corresponding CFL and LED irradiance with adopted standardised settings: CO₂ concentration 350 ± 5 µmol mol⁻¹, PPFD of 100 ± 5 µmol m⁻² s⁻¹, a boundary layer resistance (R_b) of 0.27 m² s mol⁻¹, and a leaf chamber air flow rate of 200 cm³ min⁻¹.

Statistics: Statistical procedure was performed with the SPSS statistic software (PASW statistics version 25.0, SPSS, Chicago, USA). For this, a two-way ANOVA ($P\alpha = 0.05$), determining the impact of radiation quality and water supply as well as the interaction between both,

was performed. A linear relation with the coefficient of determination (R^2) between Chl-based fluorescence indices, maximum photochemical efficiency of PS II, and the leaf Chl content was also performed.

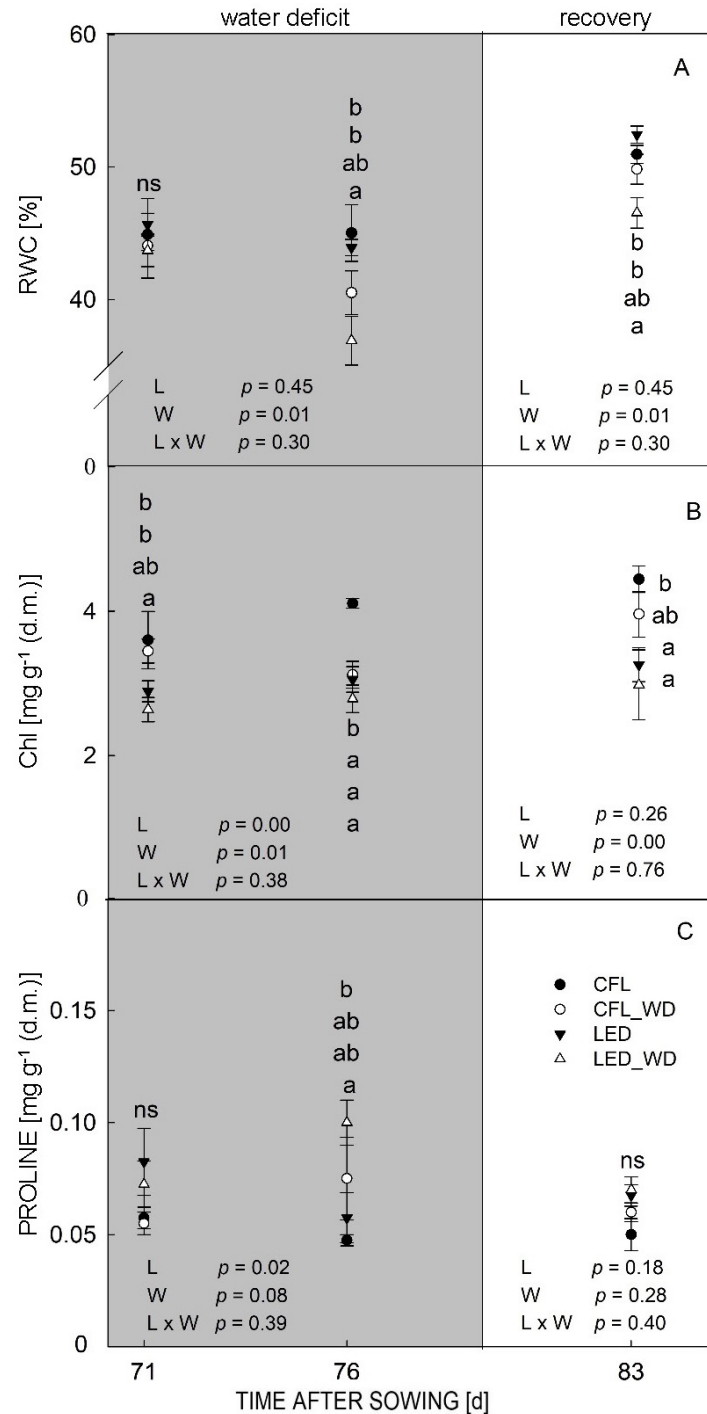


Fig. 1. A - leaf relative water content (RWC), B - leaf chlorophyll content (Chl), and C - leaf proline content of apple seedlings cultivated in a climate chamber under different radiation sources (CFL - compact fluorescence lamps and LED - light emitting diodes) during progressive water stress (71 and 76) and recovery (83 d). Treatments: CFL - control plants grown under CFL, CFL_WD - water deficit plants grown under CFL, LED - control plants grown under LED, and LED_WD - water deficit plants grown under LED. Means \pm SEs, $n = 15$, different letters indicate significant differences between treatments (two-way ANOVA, L - irradiance, W - watering, L x W - interaction).

Results

Mean values of RWC showed no significant differences during the early water deficit phase (71 DAS). A higher RWC was seen in well-watered plants on 76 DAS compared to the water deficit treatments. During the recovery phase (83 DAS), water restricted plants subjected to LED treatment (LED_WD) presented lower RWC mean values than other treatments (Fig. 1A).

Radiation quality and watering regimes significantly affected total leaf Chl content during water deficit and recovery phases. Lowest leaf Chl content was measured in LED_WD plants, followed by their well-watered control plants. In contrast, the highest values were seen in CFL well-watered plants during the course of the experiment; Chl content only slightly decreased on 76 DAS for CFL_WD plants (Fig. 1B).

The lowest proline content was recorded for CFL plants throughout the experimental period (Fig. 1C). Even though there were no significant differences, LED and LED_WD showed a higher proline content on 71 DAS. On 76 DAS, higher proline content was seen in both water deficit treatments. These differences faded at 83 DAS (recovery period).

Chlorophyll estimation by Chl INDEX and SFR R show increasing values throughout the treatment period. In addition, the lowest Chl INDEX values were recorded in CFL well-watered plants during the whole course of the experiment (Fig. 2A). In contrast, significantly higher Chl INDEX was recorded for LED_WD plants on 76 DAS and for LED well-watered plants on 78 DAS as well as on 83 and 86 DAS.

Interestingly, the SFR R index showed more pronounced differences between the treatments. Already at

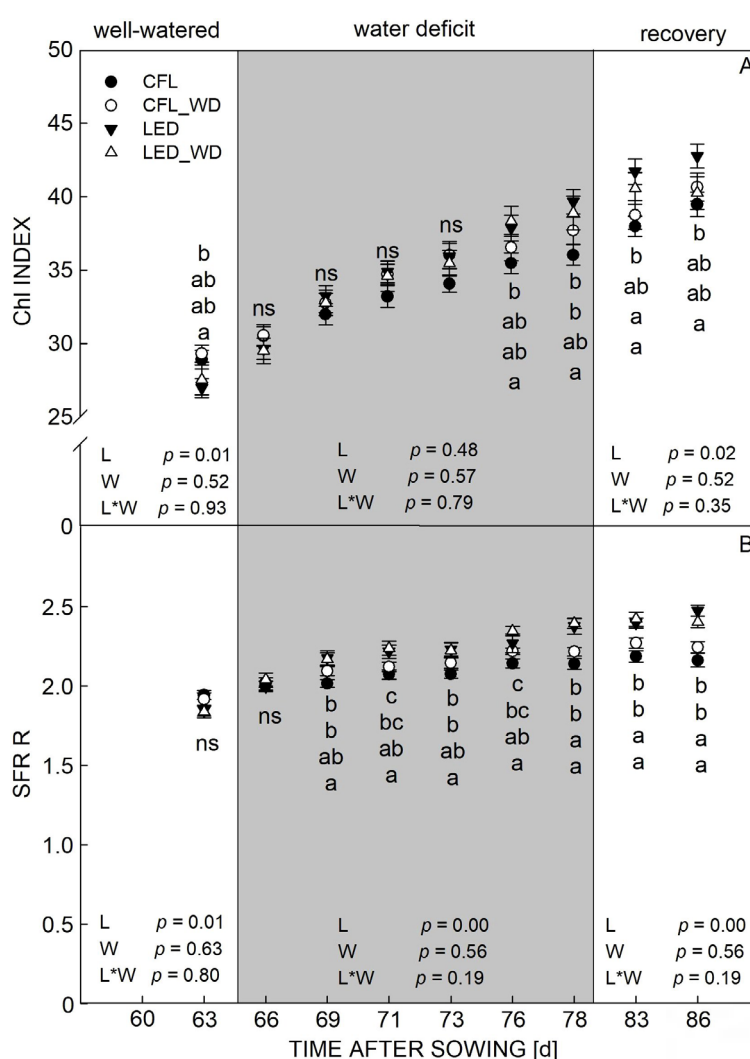


Fig. 2. Chlorophyll fluorescence indices Chl Index (A) and SFR R (B) assessed by Dualex® and Multiplex®, respectively, in apple seedlings cultivated in a climate chamber under different radiation sources (CFL - compact fluorescence lamps and LED - light emitting diodes) during well-watered phase (63 d), progressive water stress 66 - 78 d and recovery (83 and 86 d). Treatments: CFL - control plants grown under CFL, CFL_WD - water deficit plants grown under CFL, LED - control plants grown under LED, and LED_WD water deficit plants grown under LED. Means \pm SEs, $n = 60$, different letters indicate significant differences between treatments (two-way ANOVA, L - irradiance, W - watering, L*W - interaction).

69 DAS, significantly higher values were recorded in both water deficit treatments (CFL_WD and LED_WD) when compared to the well-watered plants (Fig. 2B). On 76 DAS, increased values were seen in both LED treatments and this trend continued throughout the recovery phase. Significantly lower values were recorded for CFL plants on 78, 83, and 86 DAS.

Nitrogen balance status was assessed by two indices, NBI and NBI R, as a factor of the Chl content in mesophyll and the content of phenolic compounds in leaf epidermis (Fig. 3). Irrespectively of the watering regime, higher values for both indices were recorded for plants grown under CFL than under LED. For NBI, this effect was seen

from 66 DAS onwards (Fig. 3A), whereas NBI R recorded significant differences already on 63 DAS (Fig. 3B). The NBI was not able to identify significant differences between the control and water deficient plants (Fig. 3A). In contrast, the NBI R showed highest values for CFL_WD plants (significant on 78 DAS). On this day, LED_WD plants also showed significantly higher values when compared with respective well-watered plants. This effect faded during the recovery phase.

In Fig. 4, the fluorescence indices estimating the epidermal flavonols are displayed. In contrast to the NBI indices, significantly highest values (both Flav Dx, Fig. 4A and Flav Mx, Fig. 4B) were recorded for plants grown

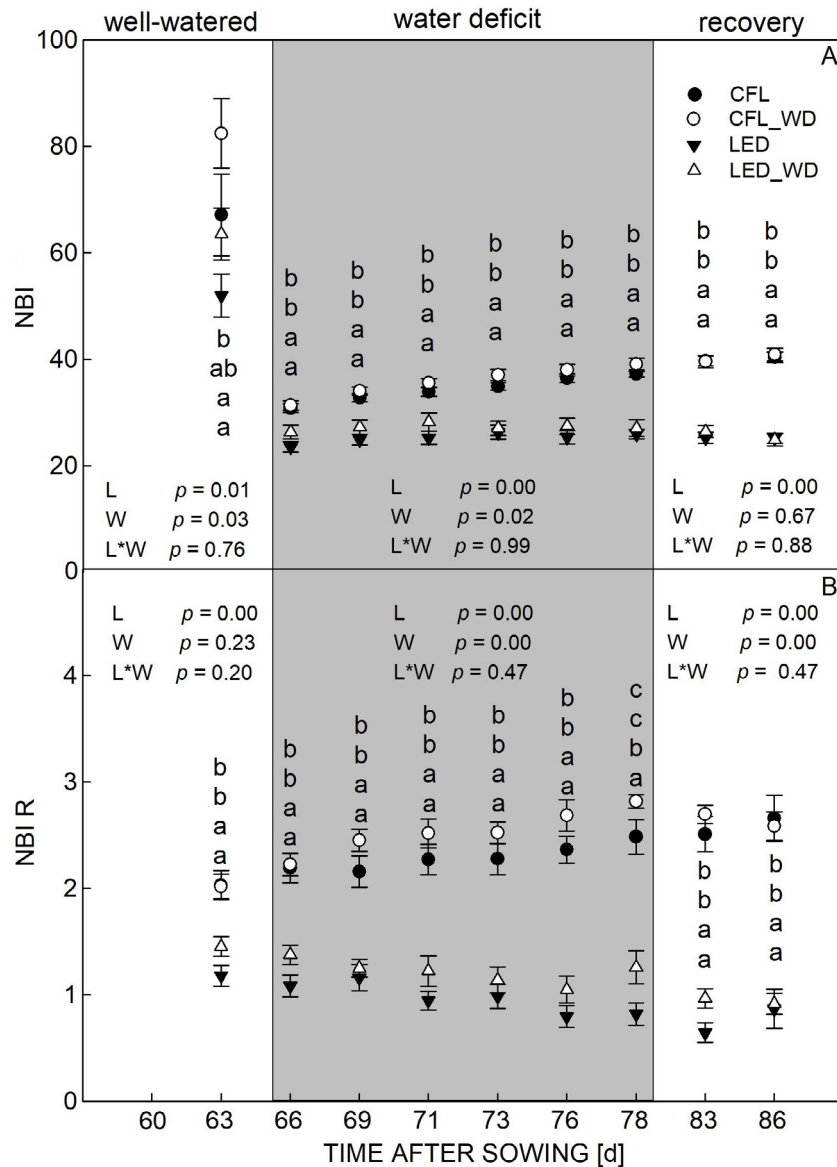


Fig. 3. Nitrogen balance indices NBI (A) and NBI R (B) assessed by Dualex® and Multiplex®, respectively, in apple seedlings cultivated in a climate chamber under different radiation sources (CFL - compact fluorescence lamps and LED - light emitting diodes) during well-watered phase (63 d), progressive water stress (66 - 78 d) and recovery (83 and 86 d). Treatments: CFL - control plants grown under CFL, CFL_WD - water deficit plants grown under CFL, LED - control plants grown under LED, and LED_WD - water deficit plants grown under LED. Means \pm SEs, $n = 60$, different letters indicate significant differences between treatments (two-way ANOVA, L - irradiance, W - watering, L*W - interaction).

under LED from 66 DAS onwards. Interestingly, Flav Dx values for both LED treatments increased until the end of the experimental course, whereas values from CFL plants remained stable (Fig. 4A). The Flav Mx index behaved similarly, even though there was a dip in values on 78 DAS for LED_WD plants (Fig. 4B).

Significant differences in g_s were seen especially during the water deficit phase (Fig. 5A), with a lower g_s for water stressed plants grown under LED on 71 DAS. On 76 DAS, both CFL and LED grown plants which were subjected to water deficit further decreased their stomatal conductance. During the recovery period, there were no

significant differences between the treatments.

The fluorescence parameter F_v/F_m indicates the maximum quantum yield of PS II when all reaction centres are open (Fig. 5B). The highest F_v/F_m ratio was seen in well-watered plants grown under CFL. There were no significant differences between all four treatments during the water deficit phase. However, LED_WD plants showed lowest values on 83 DAS (recovery period).

In Fig. 6, there is a comparison of F_v/F_m with the performance of Chl fluorescence indices (Chl INDEX and SFR R). On 63 DAS, the treatments cluster around a lower Chl INDEX and low to medium F_v/F_m value. Coefficient

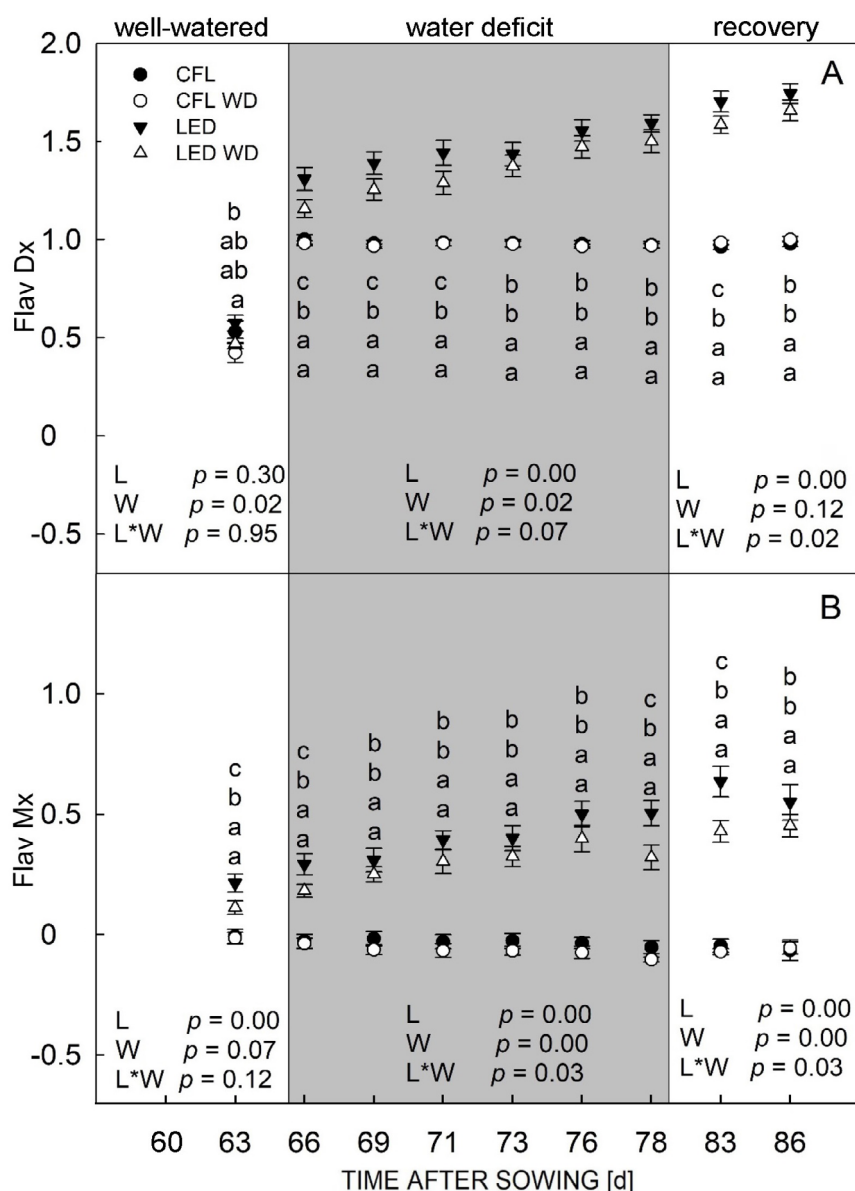


Fig. 4. Flavonol indices Flav Dx (A) and Flav Mx (B) assessed by Dualex® and Multiplex®, respectively, in apple seedlings cultivated in a climate chamber under different radiation sources (CFL - compact fluorescence lamps and LED - light emitting diodes) during well-watered phase (63 d), progressive water stress (66 - 78 d) and recovery (83 and 86 d). Treatments: CFL - control plants grown under CFL, CFL_WD - water deficit plants grown under CFL, LED - control plants grown under LED, and LED_WD - water deficit plants grown under LED. Means \pm SEs, $n = 60$, different letters indicate significant differences between treatments (two-way ANOVA, L - irradiance, W - watering, L*W - interaction).

of determination shows a high to very high correlation between F_v/F_m and Chl INDEX and F_v/F_m and SFR R (Fig. 6A,E). In contrast, higher Chl INDEX values are seen for all treatments on 71 DAS (Fig. 6B) and F_v/F_m values increase as well on 76 DAS (Fig. 6C), though a lower R^2 was observed. No clear correlation was seen between F_v/F_m and SFR_R during the water deficit phase (Fig. 6F,G). During the recovery period (83 DAS), all treatments show high Chl INDEX and SFR R values. Interestingly, both CFL treatments also have a high F_v/F_m ratio (Fig. 6D,H).

Discussion

In this study, physiological and biochemical responses of apple seedlings grown under different radiation sources and watering regimes were determined. The hypothesis of this study was that specific radiation conditions might mediate acclimation of apple seedlings to reduce their vulnerability to water deficit.

Radiation quality and watering regimes influenced physiological and biochemical indicators to different extent (Fig. 1). Relative water content is generally viewed as a good index for leaf water status (Bolat *et al.* 2014). In this study, RWC decreased during water stress (76 DAS) and increased during re-watering (83 DAS), independently

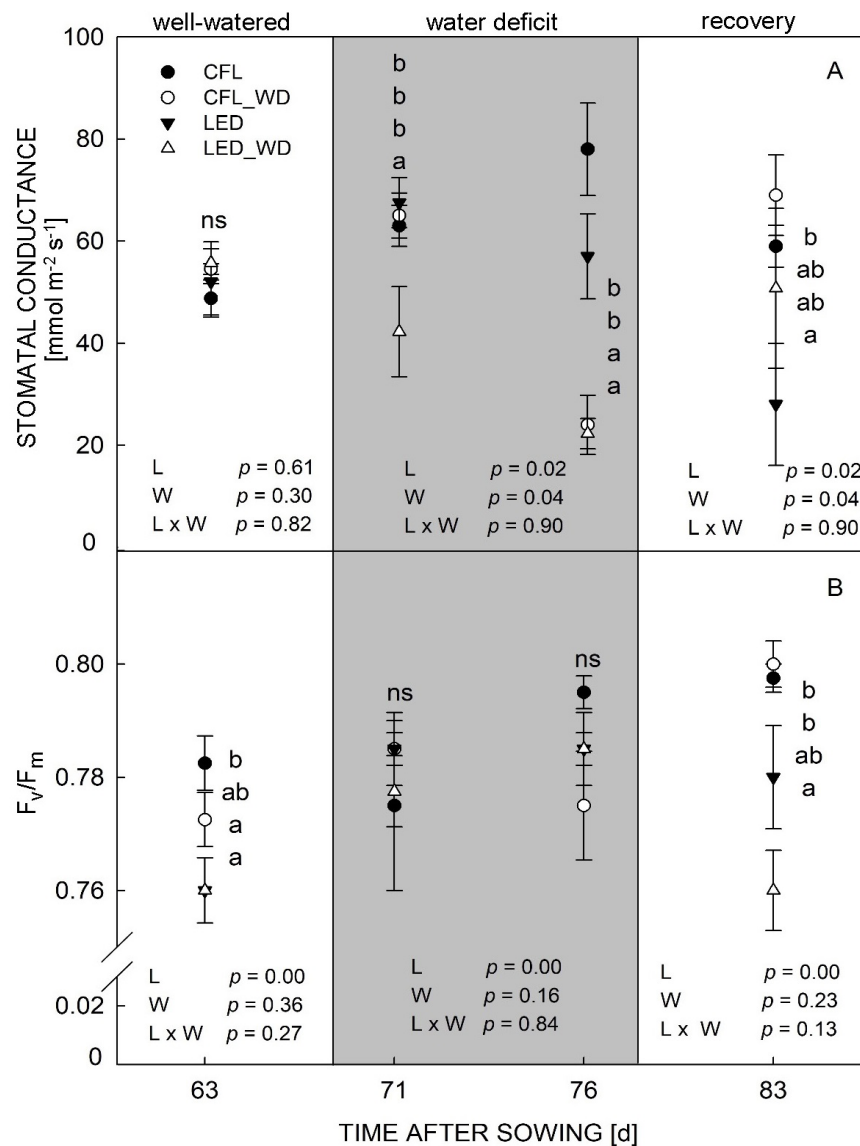


Fig. 5. Stomatal conductance (A) and maximal photochemical efficiency of photosystem II (F_v/F_m) (B) of apple seedlings cultivated in a climate chamber under different radiation sources (CFL - compact fluorescence lamps and LED - light emitting diodes) during well-watered phase (63 d), progressive water stress (71 and 76 d) and recovery (83 d). Treatments: CFL - control plants grown under CFL, CFL_WD - water deficit plants grown under CFL, LED - control plants grown under LED, and LED_WD - water deficit plants grown under LED. Means \pm SEs, $n = 60$, different letters indicate significant differences between treatments (two-way ANOVA, L - irradiance, W - watering, L \times W - interaction).

of the radiation source. However, the source of radiation affected Chl and proline content. Plants cultivated under LED showed lower Chl content and higher proline content

during the advanced water deficit period than those grown under CFL. However, this pattern was not followed in the early water deficit and recovery phases, so that further

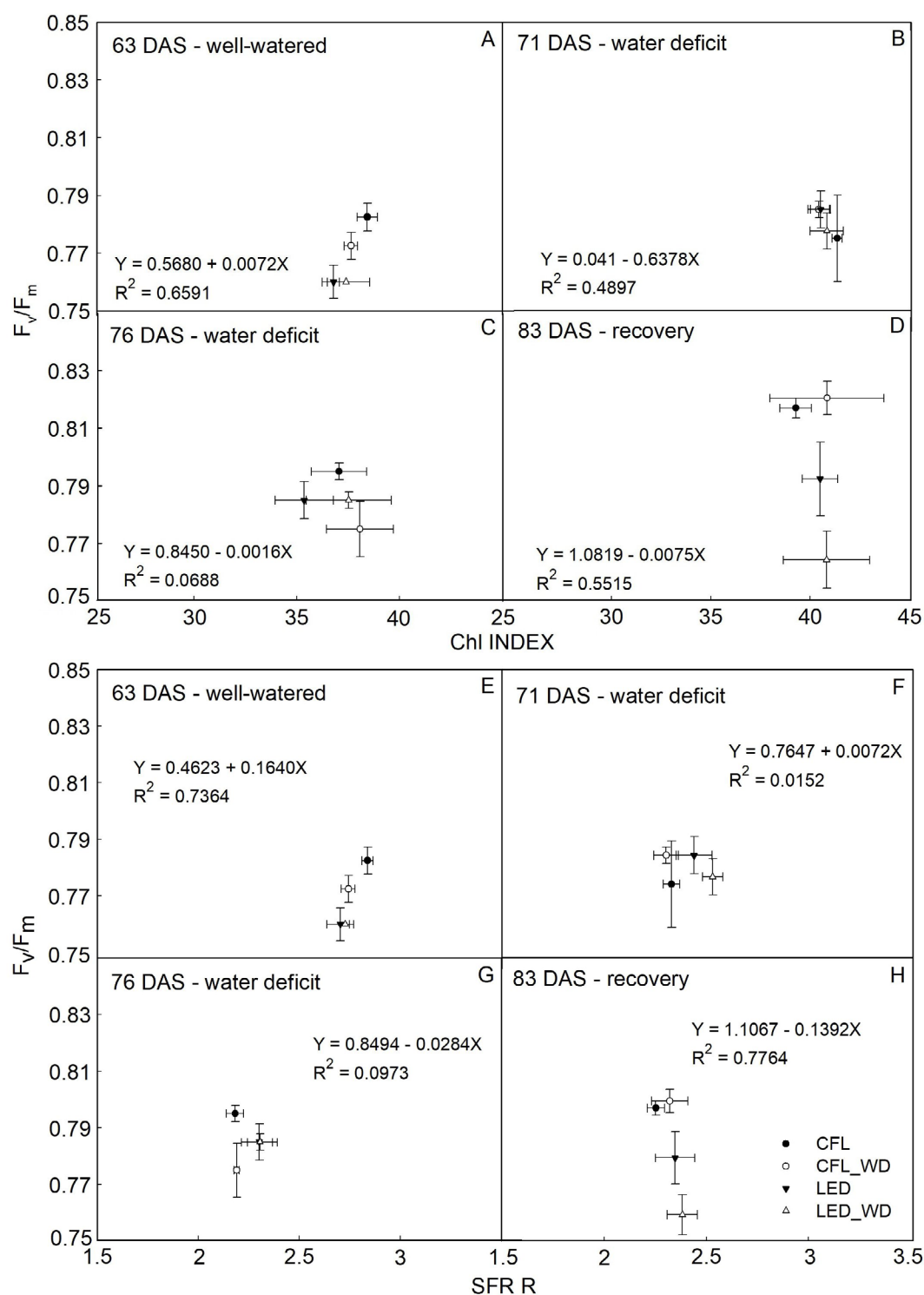


Fig. 6. Comparison between maximal photochemical efficiency (F_v/F_m) and chlorophyll index (Chl INDEX, A-D) or simple fluorescence ratio with red excitation (SFR R, E-H) of apple seedlings cultivated in a climate chamber under different radiation sources (CFL - compact fluorescence lamps and LED - light emitting diodes) during well-watered phase (63 DAS), progressive water stress (71 and 76 DAS) and recovery (83 DAS). Treatments: CFL - control plants grown under CFL, CFL_WD - water deficit plants grown under CFL, LED - control plants grown under LED, and LED_WD - water deficit plants grown under LED. Means \pm SEs, $n = 16$ (two-way ANOVA).

studies with longer water deficit periods are necessary. Wang *et al.* (2015), working with *Houttuynia cordata* seedlings, observed high RWC and Chl content in plants grown under fCFL and red LED. In general, proline accumulates in plants at a low water potential caused by drought, describing it as a suitable biochemical indicator of water stress in plants (Hayat *et al.* 2012). However, blue- and red-radiation sources were also found to regulate proline accumulation, especially when combined with low water potential under drought and salinity (Kovács *et al.* 2019).

The F_v/F_m reflects the maximal photochemical efficiency of the active centre of PS II in the dark (Krause and Weis 1991, Kalaji and Guo 2008). In this study, slightly higher photosynthetic efficiency was seen in CFL plants during the well-watered conditions and recovery phases (Fig. 5B). Wang *et al.* (2015) verified higher F_v/F_m for plants grown under CFL and blue radiation modules, supporting higher radiation absorption in treatments with blue than with red LED. This might explain the lower performance of plants under LED in the present study, since blue radiation, according to the proportion R:B (2:1), was provided to a lesser extent. Wang *et al.* (2015) assume that the different absorption might influence CO₂ assimilation rates of plants grown under radiation with distinct wavelengths.

Additionally, radiation quality and watering regime also impacted g_s (Fig. 5A). It is known that drought stress impairs stomatal conductance and ultimately results in stomatal closure to avoid water loss and simultaneously limit gas exchange (Jaleel *et al.* 2009). Blue radiation has been found to be more effective than red radiation in causing stomatal opening or preventing stomatal closure (Farquhar and Sharkey 1982, Shimazaki *et al.* 2007). This could explain the lower stomatal conductance in plants grown under LED during the early water deficit phase, though it apparently did not directly affect maximal photochemical efficiency.

Indices obtained by portable fluorescence sensors related to Chl estimation (Chl INDEX and SFR R) expressed increasing values throughout the experimental period (Fig. 2). The increase of Chl INDEX and SFR R in plants grown under LED occurred due to blue and red radiation absorption by the Chl molecules promoting more dark green leaves and accumulation of secondary metabolites (Runkle 2015). However, no correlation of Chl fluorescence indices was given with Chl content. Compared with F_v/F_m , both Chl INDEX and SFR R revealed an opposite disposition (Fig. 6) since the *Imaging-PAM* parameter reproduced the tendency of the wet-chemically assessed Chl content.

However, clear differences were verified regarding the nitrogen balance indices NBI and NBI R (Fig. 3). Both nitrogen indices showed higher mean values for plants grown under CFL lamps, similarly to the pattern of leaf Chl content. Nitrogen is part of the Chl molecule (Tremblay *et al.* 2012) and therefore has a direct relation with the amount of Chl accumulated in the mesophyll. Cerovic *et al.* (2012) described a direct relation between nitrogen and Chl by analysing the efficiency of Chl fluorescence to estimate the nitrogen status in crops by using the same

leaf clip used in the present work. Moreover, Abdallah *et al.* (2016) reported the practical usage of fluorescence indices, indicating the ratio Chl content and phenolic compounds (Chl/Flav) as an initial basis to estimate leaf nitrogen balance.

Increased mean values of Flav Dx and Flav Mx were recorded in plants grown under LED radiation source from 66 DAS onwards (Fig. 4). These indices are related to the accumulation of phenolic compounds in leaf epidermis, including flavonoids and flavonols. The latter increased with higher sun light in *Ginkgo biloba* seedling leaves (Xu *et al.* 2014). This could be UV or, as in the present study, LED radiation, which has more red and blue radiation in its spectrum than CFL lamps. Bantis *et al.* (2016) showed a significant increase in total phenolic content in *Ocimum basilicum* cultivated under the combination of high blue and green, high red:far-red (R/FR), and 1 % UV. It is known that the accumulation of flavonols and further secondary metabolites in plant cells is triggered in order to overcome stressful conditions. In addition, their synthesis can change due to environmental, physiological, and genetic factors (Zhao *et al.* 2005), with irradiance being one of the most influential factors (Kopsell *et al.* 2004, Kopsell and Sams 2013).

Conclusions

Here, we show that LED with their specific spectral regions were not able to efficiently shield plants from water stress. In contrast, plants grown under a wider spectral range (CFL) performed better during and after a short period of water withdrawal, corroborated by fluorescence indices related to the nitrogen balance (NBI and NBI R) and the Chl content. Phenolic compounds accumulation in leaf epidermis could also be well reproduced by flavonol indices (Flav Dx and Flav Mx), showing their higher content both in well-watered and water restricted plants set under LED. Hence, fluorescence indices related to nitrogen balance and flavonol accumulation promise to be a useful non-destructive tool to estimate physiological status of apple seedlings under different radiation sources and watering regimes. However, further studies should focus on different PPFD and spectra of LED lamps, and whether set up changes during the early cultivation phase of tree species would maximise a possible protective LED effect against water restrictions in older plants.

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