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Research article

Minimum magnesium concentrations for photosynthetic efficiency in wheat and sunflower seedlings

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ABSTRACT

Photosynthetic processes in the chloroplast depend on the abundance of magnesium (Mg) in relatively high amounts; hence chloroplasts might react more sensitive to Mg-deficiency than other physiological processes within other organelles. Most authors suggest a critical Mg concentration to be 1.5 mg g^{-1} DM for biomass and yield formation. However, it is not yet elucidated whether this value also applies to photosynthetic processes. The present study focused on the response of photosynthetic processes to different Mg tissue concentrations. Wheat (*Triticum aestivum*) and sunflower (*Helianthus annuus*) plants were grown hydroponically for 10 days with 8 different levels of Mg supply (1.0, 0.5, 0.25, 0.1, 0.075, 0.05, 0.025, 0.01 mM Mg). Specific leaf mass, SPAD values, assimilation rate, F_v/F_m, electron transport rate and photochemical and non-photochemical quenching parameters were determined on youngest mature leaves.

Tissue Mg concentrations decreased with lowering Mg supply to lowest concentrations of 0.7 mg g^{-1} DM in wheat leaves, but photosynthetic capacity was not affected. In sunflower leaves, lowest Mg concentrations of 0.56 mg g^{-1} DM were achieved and a diminished photosynthetic capacity was observed. The study shows that a Mg tissue concentration of 1.5 mg g^{-1} DM did not induce a negative effect on the photosynthetic capacity of wheat and sunflower leaves under our experimental conditions and hence, the critical Mg concentration for photosynthetic processes might be lower than for biomass and yield formation.

1. Introduction

Magnesium (Mg), one of the 17 plant nutrients, is well known to play essential roles in numerous processes in plant metabolism. Particularly, in photosynthesis and related processes Mg is of major importance. Concentrations of Mg in plant cells are highest in chloroplasts (Karley and White, 2009), the cell organelle where photosynthesis takes place. 15–35% of total plant Mg is bound to chloroplasts (Chen et al., 2018) and Mg concentrations in the chloroplast are reported to reach 5 mM (Grzebisz, 2015). A large share of Mg, which can be up to 35%, is bound to chlorophyll molecules, depending on Mg status of the plant (Cakmak and Kirkby, 2008). Illumination was shown to influence Mg distribution in Mg-deficient poplar leaves (Dorenstouter et al., 1985). Leaves acclimated to lower light intensities had up to 57% of Mg bound to chlorophyll, whereas in high-light acclimated leaves, the proportion was only up to 37%. A frequently reported response to Mg deficiency is the reduction of chlorophyll concentrations (Mengutay et al., 2013; Faust and Schubert, 2016; Tränkner et al., 2016).

During illumination, Mg is transported from the thylakoid lumen to the stroma due to the H^+ transport across the thylakoid membrane (Ishijima et al., 2003). Hence, Mg concentrations in the stroma increase. In spinach chloroplasts, which were kept in dark, the internal Mg^{2+} concentration was estimated to be 0.50 mM, and illumination caused an increase in Mg^{2+} concentration to 2.0 mM in the stroma (Ishijima et al., 2003). Several enzymes in the stroma are activated by free Mg^{2+} concentrations such as fructose-1,6-bisphosphatase, which was shown to be activated by 1–2 mM free Mg^{2+} (Ashton, 1998). The activation of Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) might require a considerable part of stromal Mg as this enzyme is present in very high concentrations (Dorenstouter et al., 1985). Low Mg concentrations induced an increase in the rate of deactivation of purified spinach Rubisco (Kim and Portis, 2006) and in citrus, Rubisco activity was decreased under Mg deficiency (Tang et al., 2012). Similarly, carbon dioxide (CO_2) assimilation rates were reported to decrease under deficient Mg supply (Lasa et al., 2000; Jezek et al., 2015; Tränkner et al., 2016). Limited CO_2 fixation is associated with carbohydrate accumulation in Mg-deficient source leaves due to impaired phloem loading (Cakmak and Kirkby, 2008). Accumulation of soluble sugars (sucrose, fructose, glucose) was observed in *Sulla carnosa* plants

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Abbreviations

Abs	Absorptivity
A_n	CO_2 net assimilation rate
CO_2	Carbon dioxide
DM	Dry matter
ETR	Electron transport rate
F_0	Minimum fluorescence of dark adaptation
$F_{0'}$	Minimal fluorescence yield of illumination
F_m	Maximum fluorescence of dark adaptation
$F_{m'}$	Maximum fluorescence yield of illumination
F_t	Fluorescence yield of light adaptation
Φ_{NO}	Quantum yield of nonregulated energy dissipation
Φ_{NPQ}	Quantum yield of regulated energy dissipation
Φ_{PSII}	Effective PSII quantum yield
F_v/F_m	Maximum PSII quantum efficiency
LA	Leaf area
LHCII	Light harvesting complex of photosystem II
Mg	Magnesium
NPQ	Non-photochemical quenching
PPFD	Photosynthetic photon flux density
PSII	Photosystem II
qL	Fraction of open PSII reaction centres
qP	Coefficient of photochemical quenching
RC	Reaction centre
ROS	Reactive Oxygen Species
Rubisco	Ribulose-1, 5-bisphosphate carboxylase/oxygenase
SLM	Specific leaf mass

when plants were supplied with 0.01 mM or 0 mM Mg (Farhat et al., 2014). Besides sugars, starch accumulation in the chloroplast is well documented by electron microscopy images, iodine staining or by chemical analysis (Vesk et al., 1966; Hall et al., 1972; Hermans et al., 2005; Farhat et al., 2014). The accumulation of non-structural carbohydrates, particularly starch, can increase specific leaf mass (SLM) by affecting leaf cell density (Britz and Adamse, 1994). In cucumber, the accumulation of starch was suggested to be the cause for increased SLM because subtracting the non-structural carbohydrate component and thereby expressing SLM on the residual dry matter (DM) basis, the increase in SLM was reduced (Britz and Adamse, 1994).

Reduced energy consumption in the light-independent processes of photosynthesis is suggested to lead to an over-reduction of the electron acceptors in the light-dependent reactions of photosynthesis (Cakmak and Kirkby, 2008). This leads to increased production of reactive oxygen species (ROS), which damage proteins, in particular the D1 protein of photosystem II (PSII). If the rate of damage exceeds the rate of repair of D1 protein, photoinhibition occurs. In combination with qI, photoinhibition can be assessed by the chlorophyll fluorescence parameter maximum PSII quantum efficiency (F_v/F_m), which was shown to be decreased under Mg-deficiency in citrus (Yang et al., 2012), sugar beet (Hermans et al., 2004) and *Sulla carnosa* (Farhat et al., 2015). Besides F_v/F_m, chlorophyll fluorescence allows to study the quantum efficiency of PSII, which gives insight about the redox state of PSII reaction centres. Light energy reaching the thylakoid membranes can either be used in biochemical reactions of photosynthesis (described by the chlorophyll fluorescence parameter Φ_{PSII}) or non-photochemically quenched and dissipated as heat (Φ_{NPQ}). Hence, assessing both photochemical and non-photochemical quenching provides an estimate of photosynthetic efficiency at PSII.

Typically studies on Mg deficiency either apply very low Mg concentrations or even no Mg, known to certainly induce Mg deficiency, and/or results are not related to the tissue Mg concentration. Furthermore, the concept of critical concentration is rarely applied to Mg studies. The critical nutrient concentration is defined as the "single point within the bend of the curve where the plant nutrient status shifts from deficient to adequate" (Dow and Roberts, 1982), and other definitions are mostly relating critical concentration to growth or yield formation (Dow and Roberts, 1982). For Mg it was reported that concentrations of 0.7 mg Mg g^{-1} leaf DM may be required to achieve 90% of maximum yield (Smith et al., 1985). It is commonly suggested that critical Mg tissue concentration is < 1.5 mg g^{-1} DM (Cakmak and Kirkby, 2008), but critical concentrations seem to vary among physiological processes. Recently, critical leaf Mg concentrations for CO_2 net assimilation were suggested to be higher than those for dry weight production (Hauer-Jákli and Tränkner, 2019). Hence, chloroplastic processes might react more sensitive to Mg concentrations. Furthermore, a meta-analysis reported on lower critical Mg concentrations for CO_2 net assimilation in monocots than in dicots (Hauer-Jákli and Tränkner, 2019). In the present study, eight different Mg supply levels were chosen to induce a gradient in Mg tissue concentrations in sunflower (dicot) and wheat (monocot) and photosynthetic processes were analysed. The objective of this study was to identify the Mg concentration at which photosynthesis and related processes are affected and to determine whether the analysed processes respond differently at different concentrations.

2. Materials and methods

2.1. Plant culture

Plant growth was performed in the greenhouse with a day/night light cycle of 14/10 h and a photosynthetic photon flux density of approx. 350 µmol m^{-2} s^{-1} at canopy height. Seeds of wheat (*Triticum aestivum* L. cv. Cornetto) and sunflower (*Helianthus annuus* L. cv. Delfie) were germinated in paper rolls in 1 mM $CaSO_4$. The germination solution for sunflower seeds also contained 20 µM H_3BO_3. After germination, seedlings were transferred into 5 L pots (2 plants per pot) containing a nutrient solution. Seedlings were grown for four days in half-strength nutrient solution, then 26 days in 100% of full-strength nutrient solution. The full-strength nutrient solution of control treatments contained: 1.75 mM $Ca(NO_3)_2$*4 H_2O, 1 mM K_2SO_4, 1 mM $MgSO_4$*7 H_2O, 0.25 mM NH_4NO_3, 0.2 mM $Ca(H_2PO_4)_2$* H_2O, 0.05 mM $CaCl_2$*2 H_2O, 0.1 mM $C_{10}H_{12}FeN_2NaO_8$, 1 µM or 20 µM H_3BO_3 for wheat and sunflower, respectively, 1 µM $ZnSO_4$*7 H_2O, 1 µM $MnSO_4$*H_2O, 0.2 µM $CuSO_4$*5 H_2O, 0.1 µM $H_{24}Mo_7N_6O_{24}$*4 H_2O. Seven different treatments of Mg-deficiency were established at the day of transplanting seedlings into nutrient solution (= 0 days after treatment start (DAT)). The concentrations of the Mg-treatments were 0.5 mM, 0.25 mM, 0.1 mM, 0.075 mM, 0.05 mM, 0.025 mM and 0.1 mM $MgSO_4$*7 H_2O. Nutrient solutions were constantly aerated and exchanged every three to four days depending on plant water consumption. Each treatment was replicated four times. Measurements were performed on youngest fully expanded leaves. For the purpose of increasing comparability, all measurements were done on the same leaf of the plant at 10 DAT.

2.2. Leaf SPAD measurements

Relative leaf chlorophyll concentrations were estimated *in vivo* using a SPAD-502 (Konica-Minolta, Japan) prior to measurements of chlorophyll *a* fluorescence on the same leaves; hence four replicates per treatment were measured. One replicate consisted of three measurements per leaf which were averaged directly by device option. Values are expressed as SPAD units.

2.3. Chlorophyll a fluorescence

Chlorophyll *a* fluorescence was determined using a PAM-

fluorometer (Imaging-PAM Maxi, Heinz Walz GmbH, Germany). Leaves were dark-adapted for 20 min prior to measurements by placing the whole plant in a large box (1.8 m^3) which allowed a photosynthetic photon flux density (PPFD) of only 10 µmol $m^{-2} s^{-1}$. Maximum PSII quantum yield [$F_v/F_m = (F_m–F_o)/F_m$] (Maxwell and Johnson, 2000) was measured and then, actinic light was switched on at a PPFD of 461 µmol m^{-2} s^{-1} and once per minute a saturation light pulse was applied at a PPFD of 2700 µmol $m^{-2} s^{-1}$ for 800 ms. The following quenching parameters were determined after 15 min: effective PSII quantum yield [$\Phi_{PSII} = (F_m'–F_t)/F_m'$] (Genty et al., 1989), quantum yield of regulated energy dissipation [$\Phi_{NPQ} = 1/\Phi_{PSII} –1/NPQ+1 + qL (F_m/F_o–1))$], quantum yield of non-regulated energy dissipation [$\Phi_{NO} = 1/NPQ+1 + qL (F_m/F_o–1))$], coefficient of photochemical quenching ($qP=(F_m'–F)/(F_m'–F_o')$ and NPQ ($NPQ=(F_m–F_m')/F_m'$) (Kramer et al., 2004). F_m and F_o denote, respectively, the maximum and minimum fluorescence of dark-adapted samples. F_t is the fluorescence yield of light-adapted samples and F_m' the maximum fluorescence yield in the light following a saturation pulse, and $F_o' = F_o/(F_v/F_m + F_o/F_m')$ (Oxborough and Baker, 1997). ETR was determined by calculating ETR = Φ_{PSII} *0.5*Absorptivity*461 µmol m^{-2} s^{-1}. Absorptivity is calculated as Abs. = 1-NR/NIR, where R is remission at 660 nm and NIR is remission at 780 nm. A circular and a rectangular area of interest were selected for sunflower and wheat leaves, respectively, avoiding the edges of the leaves and considering the centre of the leaf the most representative leaf area. Fluorescence values of all pixels within this area were averaged automatically by the devices software ImagingWin v2.41a (Heinz Walz GmbH, Germany). Measurements were performed with four replications per treatment.

2.4. Measurement of CO_2 net assimilation rates

CO_2 net assimilation rates (A_n) were determined by measuring leaf gas exchange (GFS-3000, Heinz Walz GmbH, Germany) on 4 cm^2 of non-chlorotic leaf area. Cuvette conditions were set as follows: 22 °C, 55% relative humidity, 380 ppm CO_2, PPFD of 1000 µmol m^{-2} s^{-1}. After A_n had stabilized, values were averaged over 5 min. Measurements were performed between 9 a.m. and 5 p.m. during the experimental period with four replications per treatment.

2.5. Calculating specific leaf mass and determination of Mg concentrations

To calculate specific leaf mass, leaves were cut after measurements and were photographed using a digital single-lens reflex camera (Canon EOS 600D, Canon Inc., Japan). The area of green pixels in each picture was calculated using ImageJ software (Rasband, 1997). Leaves were dried at 60 °C to weight constancy and dry weight was determined. Specific leaf mass (SLM) was calculated by dividing dry weight per obtained leaf area.

Determination of magnesium concentrations was performed with modifiations as in Hansen et al. (2009). Using a high-accuracy balance, 100 mg of dried and powdered leaf material was transferred to a Teflon digestion tube. The digestion medium consisted of 4 ml concentrated HNO_3 and 2 ml 30% H_2O_2 and micowave-digestion was performed at 200 °C at 15 bar for 75 min (Ethos.lab, MLS, Germany). After digestion, samples were diluted in 25 ml double-distilled H_2O. In each batch of microwave digestion, a certified reference material (apple leaf, SRM 1515, National Insitute of Standards and Technology, USA) was also digested. Magnesium concentrations were measured at 279.078 nm by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (Vista RL, CCD simultaneous ICP-OES, Varian Inc., USA) equipped with a Quarz Torch Low Flow with a 1.4 mm injector and a Sea Spray nebulizer with sample uptake of 2 ml min^{-1}. Calibration was achieved by a multielement standard solution purchased from Bernd Kraft, Germany. After approx. each 20 samples, measurement of the certified reference material is included to ensure accuracy of measurements. Both, SLM and Mg concentrations were analysed in four eplications per treatment.

2.6. Statistical analyses

Data and statistical analyses were performed using the software RStudio (R Core Team, 2017) and the R packages *agricolae* (de Mendiburu, 2017), *plyr* (Wickham, 2011), *fasttime* (Urbanek, 2016), *matrixStats* (Bengtsson, 2017), *data. table* (Dowle and Srinivasan, 2017), *tidyr* (Wickham and Henry, 2018), and *reshape* (Wickham, 2007). Analysis of variance (ANOVA) was performed to determine whether effects of treatments on the respective factor were significant, followed by Duncan's post-hoc test ($\alpha = 0.05$) where ANOVA indicated a significance. To determine significant differences in the decrease of Mg concentrations between control and Mg concentrations of the respective treatment, a *t*-test was performed ($\alpha = 0.05$). Non-linear regressions were fitted with the *nls* function implemented in R, using the model equation of $y = ax^{-1}+b$, which fitted best to the data set. Adjusted R^2 for non-linear regressions was obtained by the package *soilphysics* (da Silva and de Lima, 2017) and according to Spiess and Neumeyer (2010).

3. Results

3.1. Mg concentrations in leaves

Reducing the Mg supply significantly reduced the Mg concentrations both in wheat and sunflower (Fig. 1 and Table 1). Leaves of wheat that were supplied with 1 mM Mg (control) contained 2.7 ± 0.2 mg Mg g^{-1} DM. Tissue concentrations below the critical value of 1.5 mg Mg g^{-1} DM were achieved with a supply of 0.05 (1.2 mg ± 0.1 Mg g^{-1}

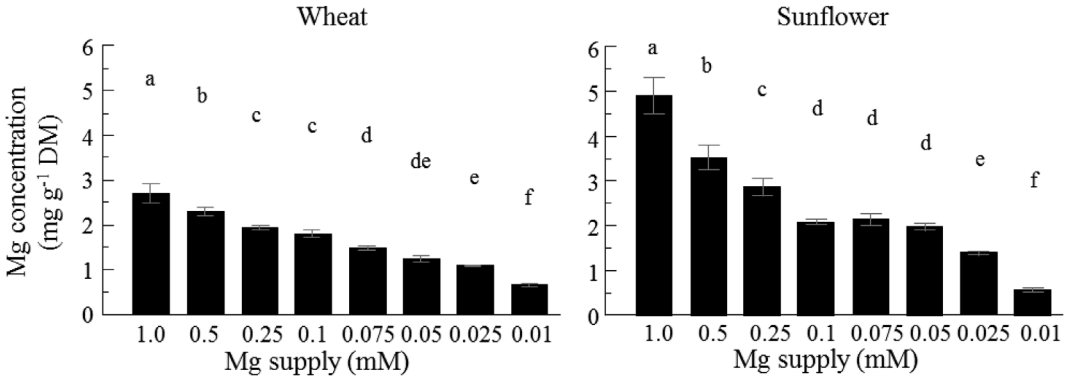

Fig. 1. Magnesium concentrations in latest fully expanded leaves of wheat and sunflower supplied with different magnesium concentrations. Mean values ± SE are shown (n = 4). Different letters indicate significant differences within the plant species ($p \leq 0.05$).

Table 1
Percentage of Mg concentrations in latest fully expanded leaves of wheat and sunflower supplied with different magnesium concentrations compared to control leaves (100%), which were supplied with 1 mM Mg. '*', '**', '***' indicate significance levels at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ respectively, ns is non-significant compared to control.

Mg supply	Wheat		Sunflower	
mM	%		%	
0.5	86.6 ± 8.0	ns	72.1 ± 3.3	**
0.25	72.9 ± 4.6	**	58.7 ± 1.1	***
0.1	67.9 ± 5.8	*	43.3 ± 3.6	***
0.075	56.2 ± 5.6	**	43.8 ± 1.6	***
0.05	46.2 ± 4.4	**	40.9 ± 1.8	***
0.025	40.8 ± 3.1	***	29.1 ± 3.0	***
0.01	24.9 ± 0.9	***	11.8 ± 1.7	***

DM), 0.025 (1.1 mg ± 0.0 Mg g^{-1} DM) and 0.01 mM Mg (0.7 ± 0.0 mg Mg g^{-1} DM). In wheat leaves, the Mg concentrations reached 24.9 ± 0.9% of the control concentration when supplied with 0.01 mM (Table 1). Leaves of sunflower which were supplied with 1 mM Mg contained 4.9 ± 0.4 mg Mg g^{-1} DM. Low Mg supply of 0.025 and 0.01 mM induced tissue concentrations of 1.4 ± 0.1 and 0.56 ± 0.05 mg Mg g^{-1} DM, respectively, hence below the critical value. Under the latter supply, Mg concentrations decreased to 11.8% of that in control plants (Table 1).

3.2. Leaf area and specific leaf mass

The leaf area (LA) of the single wheat leaf was not affected by Mg supply (Fig. 2). The leaf area averaged over all treatments was 5.27 ± 0.20 cm^2, with control treatment showing the highest LA of 6.02 ± 0.31 cm^2 and a supply of 0.075 mM the lowest LA of 4.15 ± 0.24 cm^2. The LA of the single sunflower leaf was significantly reduced with decreasing Mg concentrations (Fig. 2). Significant reduction of LA compared to control was observed at a Mg supply of 0.05 and 0.025 mM Mg (49.95 ± 5.40 and 49.26 ± 5.50 cm^2, respectively). Lowest LA of 30.75 ± 1.06 cm^2 was obtained at lowest Mg concentrations. The specific leaf mass (SLM) of both wheat and sunflower was not affected by Mg tissue concentrations (Fig. 3). The mean SLM of wheat ranged from 4.35 ± 0.60 to 5.5 ± 0.26 mg cm^{-2} and that of sunflower from 3.53 ± 0.74 to 4.5 ± 0.32 mg cm^{-2}.

3.3. SPAD values and net assimilation rates

SPAD values as an estimate for relative chlorophyll concentrations were not affected by Mg concentrations in wheat leaves (Fig. 4). The mean SPAD value was 50 ± 0.57. In sunflower leaves, a curvilinear relationship was found between SPAD values and Mg concentrations (Fig. 4). High SPAD values of 39.88 ± 1.45 were observed at highest Mg concentrations. Low Mg concentrations of < 1 mg g^{-1} DM reduced SPAD values to 27.95 ± 1.54.

In wheat leaves, net assimilation rates (A_n) were not affected by Mg concentrations in the leaf tissue (Fig. 5). The mean A_n was 29.54 ± 6.69 µmol CO_2 cm^{-2} s^{-1}. In contrast, in sunflower leaves a strong positive curvilinear relationship between A_n and Mg concentrations was found (Fig. 5). The lowest Mg concentrations of 0.46–0.70 significantly reduced A_n. Both Mg-deficient wheat and sunflower leaves showed A_n comparable to control plants, though Mg concentrations fell below the critical threshold of 1.5 mg Mg g^{-1} DM.

3.4. Chlorophyll fluorescence

Measurement chlorophyll fluorescence was used to obtain the maximum quantum yield (F_v/F_m), the electron transport rate (ETR), the effective quantum yield (Φ_{PSII}), the quantum yield of regulated energy dissipation (Φ_{NPQ}) and the quantum yield of non-regulated energy dissipation (Φ_{NO}). Mg deficiency did not affect F_v/F_m in wheat where the mean F_v/F_m was 0.79 ± 0.00 (Fig. 6). In sunflower leaves, only the lowest Mg concentration reduced F_v/F_m by 12% (0.708 ± 0.02) compared to the control (Fig. 6). Pictures obtained during measurement of F_v/F_m of sunflower illustrate the lower F_v/F_m of leaves supplied with 0.01 mM by more greenish colours (i.e. lower range on the false-colour scale) (Fig. 10). The heterogeneity of the leaf regarding F_v/F_m is clearly visible as interveinal areas show lower F_v/F_m than the major veins. In contrast, leaves of the other treatments show a homogenous distribution of F_v/F_m.

The ETR in wheat did not respond to decreasing Mg concentrations and was in average 70 ± 2 µmol m^{-2} s^{-1} (Fig. 7) whereas in sunflower, lowest Mg concentrations significantly reduced ETR by 23 ± 8% compared to control. In order to identify the proportions of light energy that are used to drive photochemistry (Φ_{PSII}), dissipated as heat (Φ_{NPQ}) and dissipated non-regulatory (Φ_{NO}), the quantum yields are displayed as a sum of 1 (Fig. 8 and Fig. 9). In wheat plants, the proportion of each quantum yield did not differ between the treatments. From Fig. 9, it is clearly visible that wheat plants were not affected by reducing Mg concentrations. The proportion of Φ_{PSII} and Φ_{NPQ} were almost equal, thus the lowest proportion constituted Φ_{NO}. Mean Φ_{PSII} was 0.388 ± 0.004, mean Φ_{NPQ} 0.387 ± 0.005 and mean Φ_{NO} 0.225 ± 0.001. In sunflower, lowest Mg concentrations reduced Φ_{PSII} and increased Φ_{NPQ}, whereas Φ_{NO} remained unaffected. The decrease of Φ_{PSII} was by 24 ± 7% and the increase of Φ_{NPQ} by 27 ± 7%. Pictures obtained during measurement of Φ_{NPQ} of sunflower demonstrate higher Φ_{NPQ} of leaves supplied with 0.01 mM by more blueish-purple colours (i.e. higher range on the false-colour scale) (Fig. 10). Similarly to F_v/F_m, the leaves display heterogeneity in Φ_{NPQ} distribution over the leaf area whereas leaves of the other treatments show a homogenous

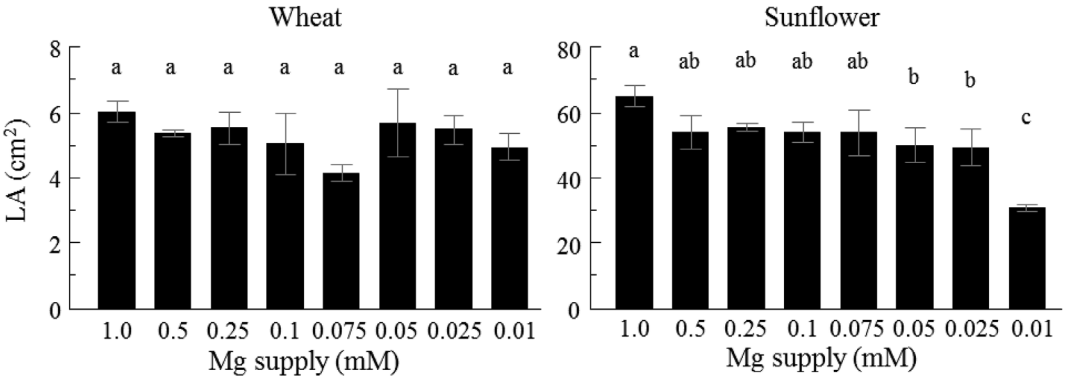

Fig. 2. Leaf area (LA) of latest fully expanded leaves of wheat and sunflower supplied with different magnesium concentrations. Mean values ± SE are shown (n = 4). Different letters indicate significant differences within the plant species ($p \leq 0.05$).

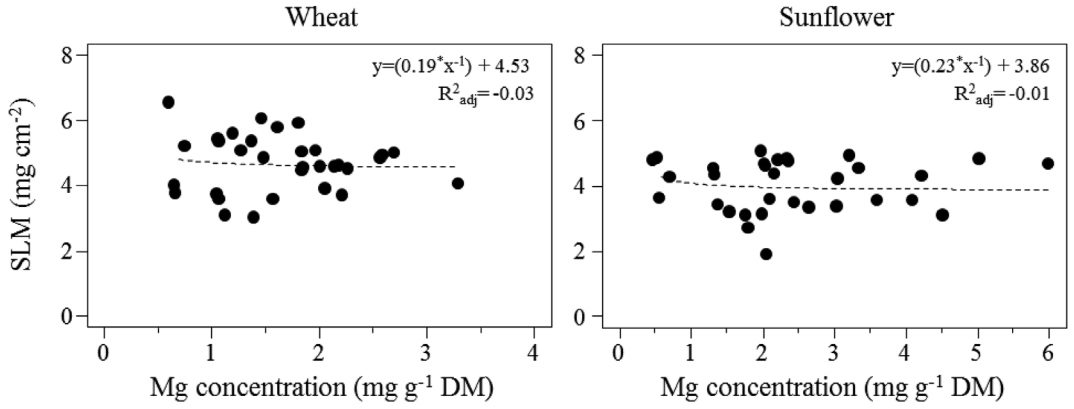

Fig. 3. Specific leaf mass (SLM) of latest fully expanded leaves of wheat and sunflower in dependence on different magnesium concentrations in the leaves.

distribution. Non-photochemical quenching, reflecting heat dissipation of excitation energy in the PSII antenna system was not affected by Mg supply in wheat leaves (Fig. 11 A). The mean NPQ was 1.72 ± 0.04. In sunflower leaves, NPQ was increased by 21 ± 5% under lowest Mg concentrations (2.298 ± 0.071). In both wheat and sunflower leaves, Mg concentrations did not affect the parameter qP that is an estimate for the fraction of PSII centres. The mean qP was 0.663 ± 0.005 and 0.781 ± 0.009 in wheat and sunflower, respectively.

4. Discussion

In the present study, the 8 different decreasing Mg supply levels induced a gradient of Mg concentrations youngest fully expanded leaves. Reducing the Mg supply reduced Mg concentrations of both wheat and sunflower. Assuming a Mg concentration of at least 1.5 mg g^{-1} DM optimal, Mg supply of less than 0.075 mM in wheat and less than 0.025 mM in sunflower induced Mg concentrations which fell below the optimal range and induced deficient concentrations. The magnitude of decrease in Mg concentration was larger in sunflower. Despite having much higher Mg concentrations under 1 mM Mg supply, sunflower leaves had lower concentrations than wheat leaves when supplied with only 0.01 mM Mg. Hence, sunflower reacts more sensitive than wheat to decreasing Mg concentrations.

SLM can be considered a measure to reflect relative carbon accumulation (Witkowski and Lamont, 1991) as it describes the mass per unit leaf area. In tobacco, nitrogen deficiency decreased the specific leaf area (SLA; the inverse of SLM), thus increased SLM (Senbayram et al., 2015). The authors contribute this effect to increases in non-structural carbohydrates such as starch which had higher concentrations in nitrogen deficient plants. An accumulation of photosynthates under Mg deficiency was previously often described in numerous crops such as sugar beet (Hermans et al., 2005), bean (Cakmak et al., 1994), and maize (Mengutay et al., 2013). The proposed mechanism underlying a carbohydrate accumulation is based on limited sucrose loading into phloem cells in source leaves due to reduced plasma membrane H^+-ATPase activity which establishes an H^+-gradient necessary to for the sucrose co-transporters to transport sucrose from the apoplast to the cytosol of phloem cells. In the present study, SLM was unaffected by decreasing Mg concentrations, which does not indicate accumulation of non-structural carbohydrates. As leaf area in sunflower leaves was decreased under deficient Mg concentrations, dry matter must have been decreased proportionally to result in unaltered SLM. This is in line with a study on potato where Mg deficiency decreased leaf area (Cao and Tibbitts, 1992) and a study in birch seedlings where SLA was not affected by decreasing Mg supply (Ericsson and Kähr, 1995). However, Riga and Anza (2003) observed a decrease of SLA under Mg deficiency in pepper plants. This study shows that decreased Mg concentrations affect growth of sunflower (decreased LA), but does not affect the weight of a unit leaf area (SLM) both in wheat and sunflower leaves.

In order to determine the Mg concentration which exerts a limitation on photosynthesis and related processes, SPAD, A_n and chlorophyll fluorescence were measured. One of the first steps in photosynthesis is the harvest of light energy by antenna complexes. The antenna complexes transfer the absorbed light energy to the reaction centres (RC) of the PS where photochemical reactions take place. The majority of the pigments in the antenna complexes are chlorophylls. The relative concentration of chlorophyll can be estimated by SPAD readings using a non-linear relationship as presented by Netto et al. (2005) and Uddling et al. (2007). In the present study, SPAD values and thus relative chlorophyll concentrations were reduced when Mg concentrations were approx. 0.6 mg g^{-1} DM in sunflower leaves. The decrease of chlorophyll concentrations under Mg deficiency are well known and were previously described in numerous reports (Hermans et al., 2004; da Silva et al., 2014; Faust and Schubert, 2016). Similarly to SPAD, the A_n

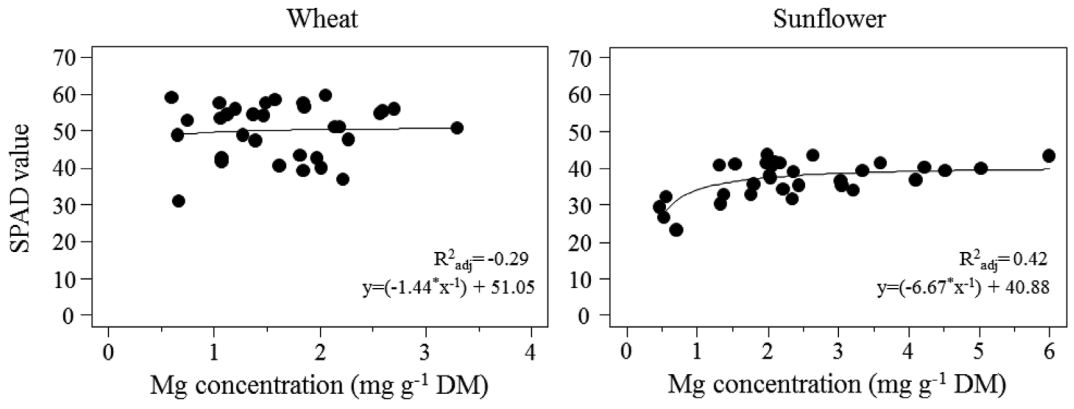

Fig. 4. SPAD values of latest fully expanded leaves of wheat and sunflower in dependence on different magnesium concentrations in the leaves.

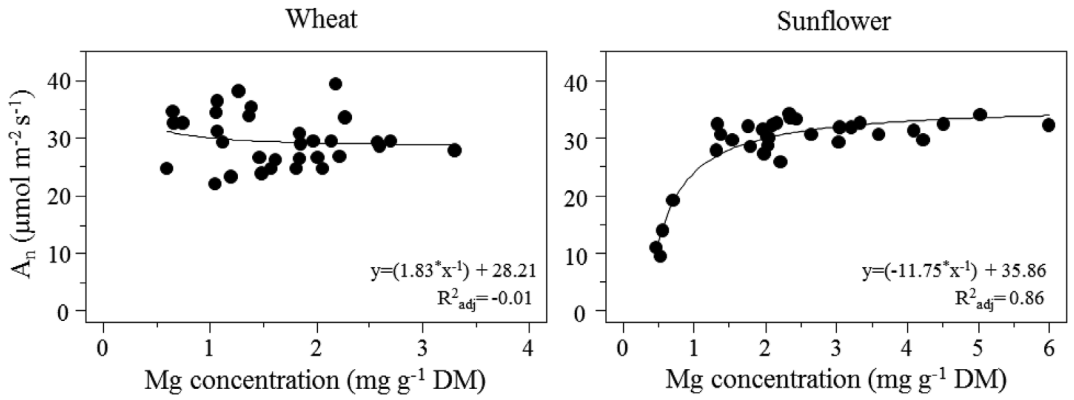

Fig. 5. Net assimilation rates (A_n) of latest fully expanded leaves of wheat and sunflower in dependence on different magnesium concentrations in leaves.

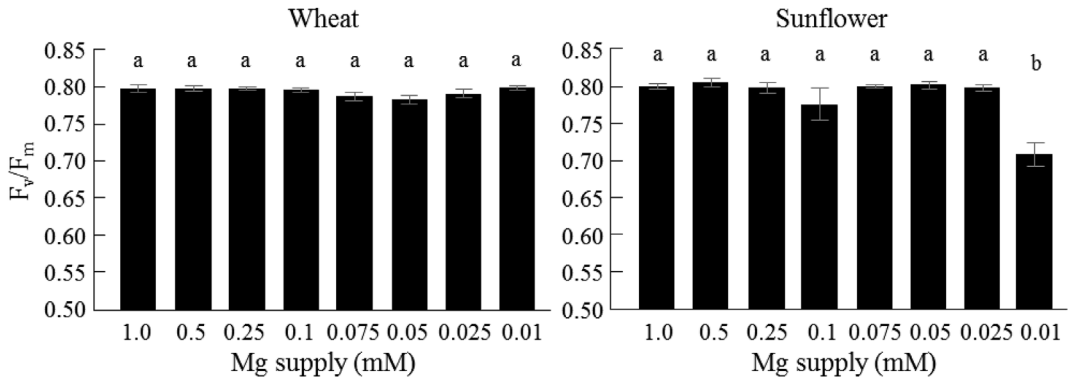

Fig. 6. Maximum PSII quantum yield (F_v/F_m) of wheat and sunflower leaves supplied with different magnesium concentrations. Latest fully expanded leaves were dark adapted for 20 min prior to measurements. Mean values ± SE are shown (n = 4). Different letters indicate significant differences within the plant species ($p \leq 0.05$).

was decreased when Mg concentrations in sunflower leaves were reduced to 0.6 mg g^{-1} DM. Reduced A_n under Mg deficiency is commonly associated with decreased activities of enzymes involved in CO_2 fixation such as Rubisco, and accumulation of carbohydrates triggering a negative feedback on Rubisco, whereas the former and the latter reactions are primary and secondary effects, respectively (Tränkner et al., 2018). A decline in assimilation rates under low Mg supply was observed in numerous species such as sugar beet (Terry and Ulrich, 1974), maize (Jezek et al., 2015), broad bean (Hariadi and Shabala, 2004) and *Sulla carnosa* (Farhat et al., 2015). However, in the present study only the lowest Mg supply level induced decreased SPAD and A_n, though Mg tissue concentrations were reduced already at higher Mg supply levels. Hence, the present study shows that the critical Mg concentration of 1.5 mg g^{-1} DM can be lower in sunflower leaves under these experimental conditions. In wheat plants, neither SPAD nor A_n were affected by Mg concentrations, although Mg tissue concentrations fell below the critical value. Mengutay et al. (2013) induced comparable Mg concentrations in 22-days-old wheat seedlings, but they could observe a decrease in SPAD values and increases in specific dry weights and antioxidant enzyme activities. In another study on wheat, low Mg supply did not affect vegetative biomass formation, but grain yield when Mg concentrations in leaves were approx. only 0.3 mg g^{-1} DW (Ceylan et al., 2016). Mg concentrations were determined after 148 days, hence in fully mature plants and might have been higher in young growth stages when total demand is lower. According to Dow and Roberts (1982), the growth stage of the plant at the time of sampling must

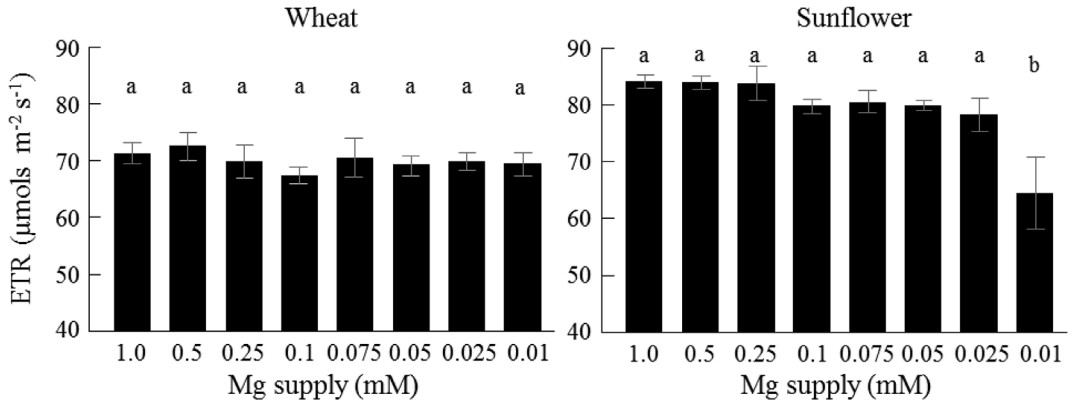

Fig. 7. Electron transport rate (ETR) of wheat and sunflower leaves supplied with different magnesium concentrations. Latest fully expanded leaves were taken for measurements. Mean values ± SE are shown (n = 4). Different letters indicate significant differences within the plant species ($p \leq 0.05$).

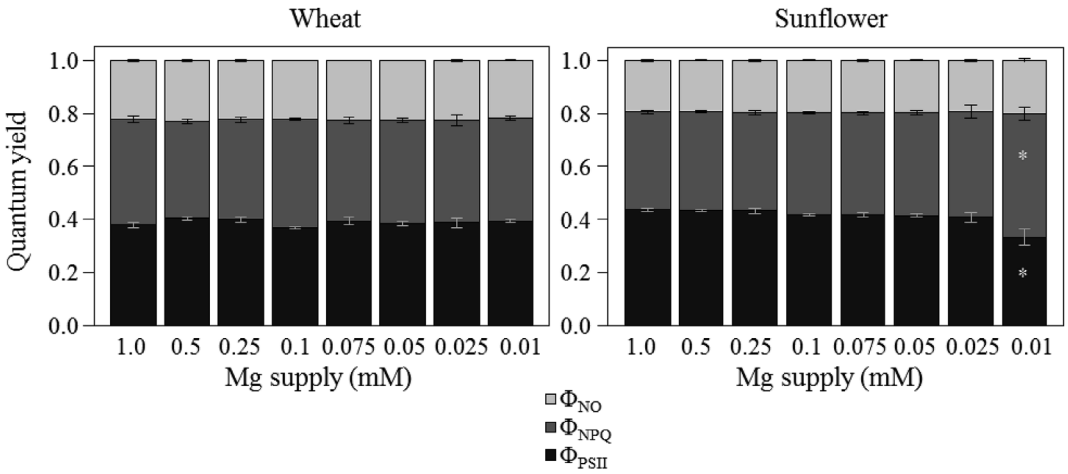

Fig. 8. Effective PS II quantum yield (Φ_{PSII}), non-photochemical quenching (Φ_{NPQ}) and non-regulated energy dissipation (Φ_{NO}) of wheat and sunflower leaves supplied with different magnesium concentrations. Mean values ± SE are shown (n = 4). Asterisks indicate significant differences within the plant species ($p \leq 0.05$).

always be noted when critical nutrient concentrations are considered. They showed that rapid changes in nutrient concentration occur from one growth stage to another in potato petioles. Moreover, photosynthetic performance was shown to be affected by leaf and plant age in a study on Arabidopsis (Bielczynski et al., 2017). However, in the present study all measurements were performed on the identical leaf, thus we can exclude differences due to different development stages.

Measurements of A_n provide insight about photosynthetic processes on a broader spatial scale, whereas chlorophyll fluorescence measurements allow to assess the redox state of PSII, and thus provide detailed understandings on biochemical processes on a narrower scale. The chlorophyll fluorescence parameters Φ_{PSII}, Φ_{NPQ} and Φ_{NO} were assessed to determine changes in the proportion of the fate of absorbed light induced by Mg deficiency. In wheat leaves, the proportion of light used for driving photosynthetic electron transport and the proportion of light which is dissipated as heat are similar irrespective of Mg concentrations. This indicates that the photosynthetic performance is not restricted and is in line with the results of A_n and ETR. In sunflower, the proportion of light which is dissipated as heat (Φ_{NPQ}) is increased under lowest Mg supply, whereas the proportion of light used for driving photosynthetic electron transport is decreased, but the proportion of non-regulated energy dissipation remained unaltered. Φ_{NO} is composed of chlorophyll fluorescence internal changes through the triplet state of chlorophyll, which leads to the formation of singlet oxygen (Moustaka et al., 2015). The constant Φ_{NO} and the increase of Φ_{NPQ} indicates that in sunflower leaves, which had a concentration of 0.56 mg Mg g^{-1} DM, light is excessive and Φ_{NPQ} serves as a photoprotective mechanism to prevent photoinhibition of PSII (Niyogi, 1999). This can be confirmed by increased NPQ. The site for NPQ is the LHCII antenna and the proton gradient (ΔpH) across the thylakoid membrane serves as a trigger for LHCII antenna aggregation required to induce the NPQ state (Ruban, 2016). Over 75% of absorbed photons can be eliminated by NPQ (Niyogi, 1999), hence NPQ can be a substantial regulatory valve for dissipation of excess energy. However, if light energy cannot be sufficiently quenched, loss of PSII activity occurs and photoinhibition takes place (Edreva, 2005). In contrast to NPQ, qP was not affected by low Mg concentrations. The parameter qP can be considered a measure of the fraction of oxidized PSII centres, thus serving as an indicator for the redox state of primary quinone electron acceptor (Q_A). The maintenance of a constant Q_A redox state might be a result of down-regulated ETR and increased energy dissipation as heat (NPQ) (Moustaka and Moustakas, 2014). Thus, in Mg-deficient sunflower leaves photoprotection was efficiently regulated.

In order to assess the PSII functionality under varying Mg supply, F_v/F_m was studied which gives insight about the maximal proportion of absorbed light that can be used to drive photosynthesis. Optimal values of F_v/F_m were reported to be approx. 0.83 (Maxwell and Johnson, 2000), but they might differ between different plant species and

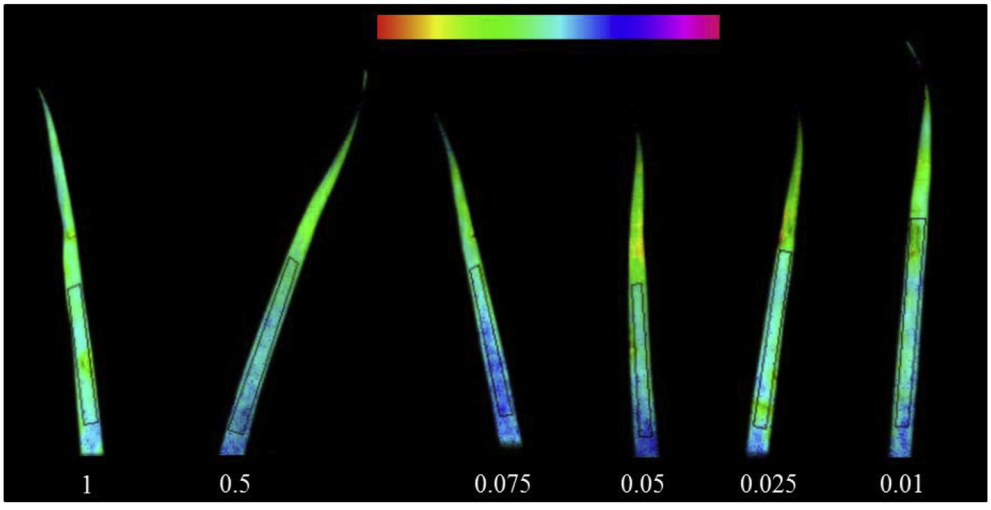

Fig. 9. Representative false colour images of chlorophyll *a* fluorescence showing effective PSII quantum use efficiency (Φ_{PSII}) of wheat leaves. Each image shows leaves of wheat grown under a different Mg supply (mM) as indicated next to the leaves. Imaging was performed 10 days after onset of Mg treatments. Rectangles indicate the area used for calculation of Φ_{PSII}. The colour scale depicted at the top represents a range of 0.133 (red) to 0.647 (pink). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

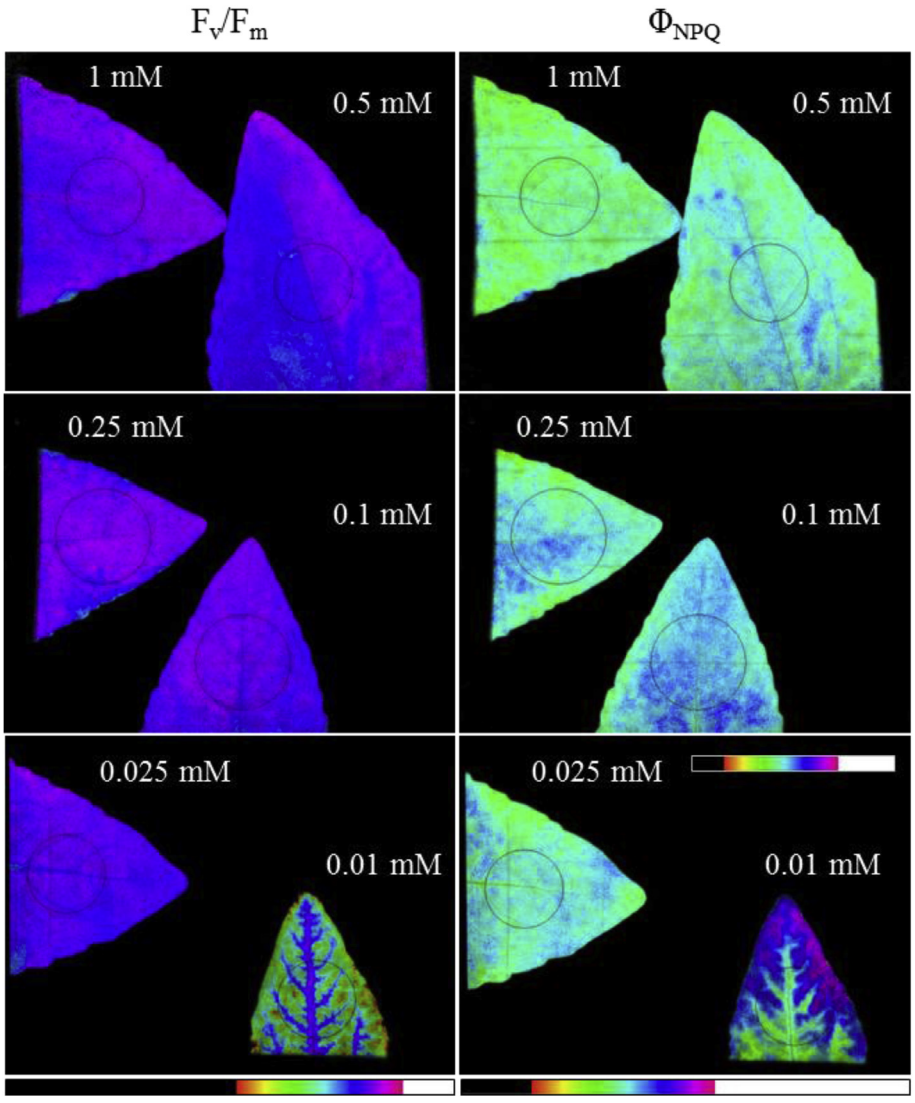

Fig. 10. Representative false colour images of chlorophyll *a* fluorescence showing maximum PSII quantum use efficiency (F_v/F_m, left column) and non-photochemical quenching (Φ_{NPQ}, right column) of sunflower leaves. Each image shows leaves of sunflower grown under a different Mg supply as indicated next to the leaves. Imaging was performed 10 days after onset of Mg treatments. Circles indicate the area used for calculation of F_v/F_m and Φ_{NPQ}. The colour scale depicted at the bottom of images represents a range of 0.514 (red) to 0.878 (pink) for F_v/F_m and 0.157 (red) to 0.561 (pink) for Φ_{NPQ}. Note that the range of the colour scale depicted in the lower right image is broader in order to display higher Φ_{NPQ} in 0.01 mM leaf (0.157 (red) to 0.718 (pink)). It only refers to the 0.01 mM leaf. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

environmental growth conditions. In the present study, the F_v/F_m of wheat and sunflower of control treatment was 0.797 and 0.800, respectively. In a phenotyping study on more than 1000 wheat cultivars, the mean F_v/F_m was 0.802 (Sharma et al., 2012), thus substantially lower than 0.83. Another study on young wheat plants reported F_v/F_m values of control plants of 0.784 (Zlatev, 2009). Hence, there is considerable variation of F_v/F_m among studies. In the present study, F_v/F_m values in Mg-deficient wheat leaves did not differ from those adequately supplied indicating that quinone A, the primary electron acceptor, is in a reduced state and that PSII functionality is not affected under Mg deficiency. However, F_v/F_m of sunflower leaves was decreased under lowest Mg supply, i.e. a Mg concentration of 0.56 mg g^{-1} DM. In two different citrus seedlings, F_v/F_m was decreased under Mg deficiency (Yang et al., 2012). Interestingly, the decrease in F_v/F_m was only observable when Mg leaf concentrations were below 1 mg g^{-1} DM, similarly to our observations in the present study. A decreased F_v/F_m points at a disturbance in or damage at the photosynthetic apparatus (Lichtenthaler et al., 2005). The decrease in F_v/F_m might be due to photoinhibition or have other causes. For determining photoinhibition, measurements of relaxation kinetics to obtain the photoinhibitory quench qI are necessary (Lichtenthaler et al., 2005). Photoinhibition may occur when plants suffer from unfavourable growth conditions like nutrient deficiency or drought (Aro et al., 1993). It is suggested that photoinhibition is induced by an overreduction of the acceptor side of PSII, formation of triplet chlorophyll and production of singlet oxygen (Vass et al., 1992; Telfer et al., 1994; Lindahl et al., 2000). Singlet oxygen is the most important species responsible for degradation of the D1 protein in the reaction centre of PSII (Krieger-Liszkay, 2005). According to Ruban (2016), besides measurement of F_v/F_m, assessment of D1 protein degradation is commonly used to analyse photoinhibition. Hermans et al. (2004) showed that D1 protein (encoded by the gene *PsbA*) content in sugar beets is not affected by Mg deficiency. However, an unchanged Y(NO) indicates no enhanced singlet oxygen production, hence, a decrease in F_v/F_m might have other causes, such as leaf optical properties as indicated by (Murchie and Lawson, 2013). Detailed studies are needed to reveal the reasons for this phenomenon.

In sunflower leaves which were exposed to lowest Mg concentrations, the chlorophyll fluorescence parameters, SPAD values and A_n were affected by Mg deficiency. However, A_n responded with the largest magnitude. The decrease of A_n under lowest Mg supply compared to control was 68%, whereas SPAD values and Φ_{PSII} decreased only by 26% and 24%. Hence, we suggest that from all measured parameters A_n reacts most sensitive to decreasing tissue Mg concentrations. Numerous enzymes involved in CO_2 fixation are activated by chloroplastic free Mg^{2+} concentrations (Wolosiuk et al., 1993). Using a divalent ionophore stromal Mg^{2+} concentration of illuminated spinach chloroplasts were lowered and consequently, sedoheptulose 1,7-bisphosphate and fructose 1,6-bisphosphate levels increased, and this reaction was reversible when Mg^{2+} was added again. The authors suggest that CO_2 fixation is limited due to inhibited activity of the bisphosphatases and

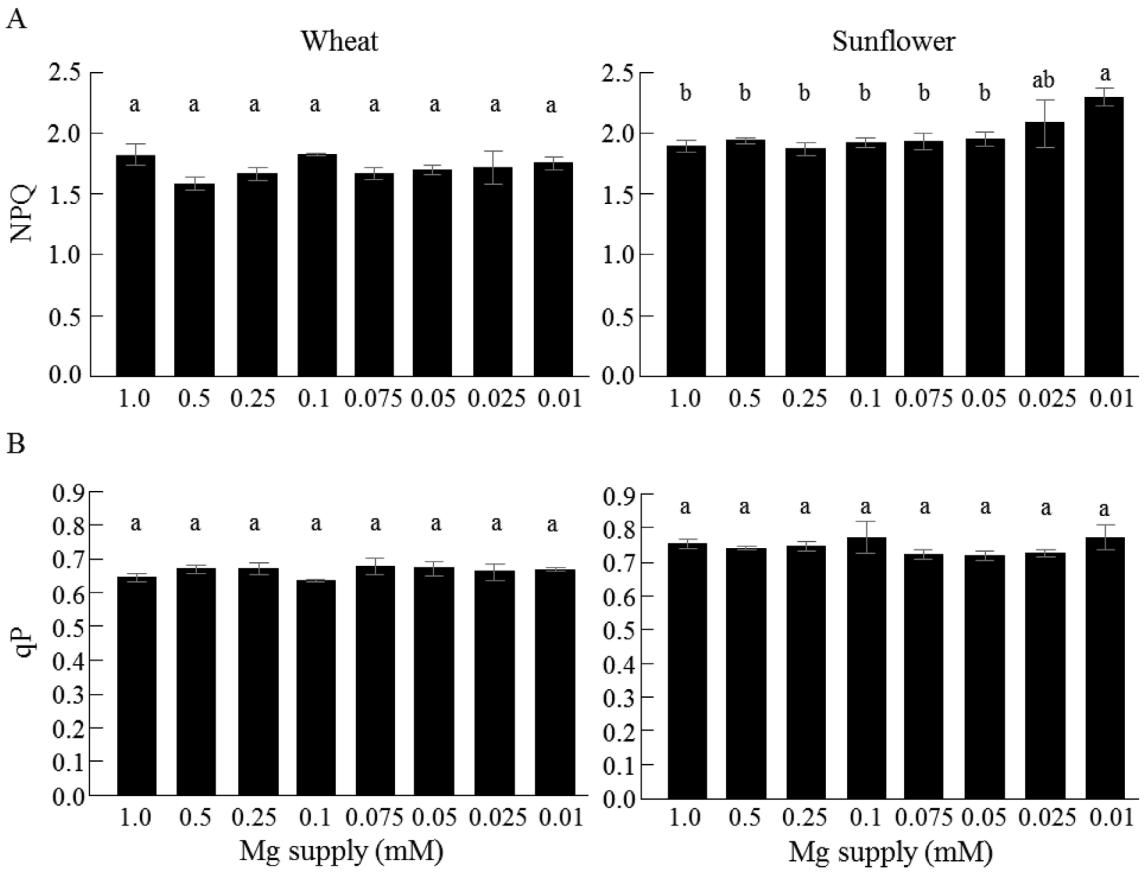

Fig. 11. Non-photochemical quenching (NPQ) (A) and coefficient of photochemical quenching (qP) (B) of wheat and sunflower leaves supplied with different magnesium concentrations. Latest fully expanded leaves were taken for measurements. Mean values ± SE are shown (n = 4). Different letters indicate significant differences within the plant species ($p \leq 0.05$).

this conclusion was confirmed by Ishijima et al. (2003). A higher sensitivity of A_n over Φ_{PSII} was also observed in a study by Farhat et al. (2015) where *Sulla carnosa* plants showed decreased A_n at higher Mg-deficient supply levels, whereas Φ_{PSII} and F_v/F_m where decreased only when Mg was absent. However, how the Mg tissue concentrations observed in the present study, affect stromal Mg concentrations and whether this might affect the activity of bisphosphatases requires further studies.

In summary, in wheat leaves photosynthetic capacity was not decreased though Mg concentrations were considerably reduced. In sunflower leaves, photosynthetic capacity was diminished when leaves reached a Mg concentration of 0.56 mg Mg g^{-1} DM. Hence, critical concentrations were much lower than the reported value of 1.5 mg g^{-1} DM under our experimental conditions. We propose that critical concentrations should be precisely linked to a physiological process, e.g. yield formation, and sampled tissue age as sensitivity to the abundance of Mg might not be equal among several physiological processes.

Contributions

S. Jamali Jaghdani performed the experiment and contributed to data analysis. M. Tränkner analysed the data and wrote the manuscript.

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