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ORIGINAL ARTICLE

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Magnesium deficiency in sugar beets alters sugar partitioning and phloem loading in young mature leaves

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Abstract Magnesium deficiency has been reported to affect plant growth and biomass partitioning between root and shoot. The present work aims to identify how Mg deficiency alters carbon partitioning in sugar beet (*Beta vulgaris* L.) plants. Fresh biomass, Mg and sugar contents were followed in diverse organs over 20 days under Mg-sufficient and Mg-deficient conditions. At the end of the treatment, the aerial biomass, but not the root biomass, of Mg-deficient plants was lower compared to control plants. A clear inverse relationship between Mg and sugar contents in leaves was found. Mg deficiency promoted a marked increase in sucrose and starch accumulation in the uppermost expanded leaves, which also had the lowest content of Mg among all the leaves of the rosette. The oldest leaves maintained a higher Mg content. [^{14}C]Sucrose labelling showed that sucrose export from the uppermost expanded leaves was inhibited. In contrast, sucrose export from the oldest leaves, which are close to, and export mainly to, the roots, was not restricted. In response to Mg deficiency, the *BvSUT1* gene encoding a companion cell sucrose/H^+ symporter was induced in the uppermost expanded leaves, but without further enhancement of sucrose loading into the phloem. The observed increase in *BvSUT1* gene expression supports the idea that sucrose loading into the phloem is defective, resulting in its accumulation in the leaf.

Keywords *Beta* · Magnesium deficiency · Sugar transport · Mineral profile

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Introduction

The allocation of photosynthates within the plant, known as assimilate partitioning, controls the amount of carbohydrates delivered to different sink tissues. Assimilate partitioning is recognized as a major determinant of plant growth, and in the productivity of sink organs having an agronomic interest (Gifford and Evans 1981). Sucrose is the principal form of reduced carbon involved in long-distance transport in most crop plants. Apoplastic phloem loading of sucrose is driven by electrogenic transporters that couple sucrose transport to the proton electrochemical potential difference across the plasma membrane of companion cells (Delrot and Bonnemain 1981; Bush 1989, 1993). The potential difference is energized by the plasma membrane proton-pumping ATPases (for a recent review, see Arango et al. 2003), localized at the phloem plasma membrane of various species (Bouché-Pillon et al. 1994; Dewitt and Sussman 1995).

Magnesium is a crucial divalent cation in plant cells because it is involved in energetic metabolism and acts as an enzyme cofactor (Shaul 2002). Moreover, as a central element of chlorophyll structure, Mg plays a prominent role in the harvesting of solar energy.

Indeed, up to one-fifth of the Mg structural pool is involved in chlorophyll structure (Mengel and Kirkby 1987; Wilkinson et al. 1990). Magnesium deficiency in plants occurs worldwide, affecting productivity and quality in agriculture (Bennett 1997) and forestry (Mitchell et al. 1999). The incidence of Mg deficiency symptoms is increasing, due to amplified rotations per site and intensive harvesting of crop products without recycling or replacing with mineral fertilizers, which raise the demand on soil Mg. Signs of Mg deficiency in most crop plants usually make their appearance first on the uppermost expanded leaves, and systematically progress from them towards the youngest and oldest ones. A characteristic symptom of Mg deficiency is interveinal chlorosis (Bennett 1997).

Alteration of carbon partitioning in Mg-deficient plants has been studied in the last decade. Sucrose accumulation in source leaves was suggested to be the major growth constraint, particularly for roots, under Mg-limiting conditions (Ericsson and Kahr 1995). Fischer and Bremer (1993) concluded that assimilate transport from source to sink leaves might be disrupted, and Cakmak et al. (1994a, 1994b) even proposed that phloem loading of sucrose would probably be the step most sensitive to Mg deficiency in *Phaseolus vulgaris*. Growth of sink organs depends on adequate flow of carbon supply from source leaves and growth can therefore be restricted by inhibition of sucrose loading into the phloem. According to Cakmak et al. (1994a, 1994b), the high susceptibility of sucrose partitioning to Mg delivery may be related to the carrier-mediated uptake of sucrose in the conducting complex of phloem. Mg is possibly involved in phloem loading because it is an allosteric activator of protein complexes (Cowan 2002) and it interacts with ATP fuelling the H^+-ATPases, which creates the proton-motive force energizing the sucrose symporters. Likewise, pyrophosphatases, which play a role in the long-distance transport of sugars (Lerchl et al. 1995), also require Mg for pyrophosphate hydrolysis. All these observations suggest that phloem loading in source leaves may be limited by low Mg availability. Conversely, it has been postulated that a congestion of metabolites in source leaves from *Spinacia oleracea* arises from a limited consumption in sink leaves (Fischer et al. 1998). In that work, phloem loading did not appear to be the most susceptible process, as sucrose content in the phloem sap of mature leaves was identical in Mg-deficient and control plants. Limited use of assimilates in sink organs could result from growth inhibition by low Mg supply. As a result, sucrose would accumulate in source organs. This in turn points to a regulation of sucrose synthesis in the source depending on consumption in the sink tissues.

In the present work, *Beta vulgaris* was chosen as a model species possessing a sucrose storage sink. Low symplasmic connectivity between bundle sheath mesophyll and phloem cells, as well as reported sucrose symport activities in source leaves, argues for apoplastic sucrose phloem loading in sugar beet (Giaquinta 1976; Bush 1989; Lemoine and Delrot 1989). Thus, the analysis of Mg deficiency focuses on sucrose partitioning and the expression of the sucrose/H^+ symporter, which is hypothesized to play a prominent role in phloem loading of sucrose in sugar beet (Vaughn et al. 2002; Ransom-Hodgkins et al. 2003).

Materials and methods

Plant culture and growth conditions

Two weeks after germination in peat-based compost, plantlets of *Beta vulgaris* L. cv. Adonis were transferred to a hydroponic culture system. The macronutrient composition, slightly modified from Cakmak et al. (1994a), was (in mM): 2.00 $Ca(NO_3)_2$, 1.00 $MgSO_4$, 0.88 K_2SO_4, 0.25 KH_2PO_4 and the micronutrient composition (in μM) was 20 FeEDTA, 10 NaCl, 10 H_3BO_3, 1.00 $ZnSO_4$, 1.00 $MnSO_4$, 0.10 $CuSO_4$, 0.01 $(NH_4)_6Mo_7O_{24}$. The pH of the solution was adjusted to 5.8 ± 0.1. At the beginning of the treatment, half of the plant population (physiological stage as indicated in the results) was fed with an Mg-free nutrient solution, which was the same as above, except that 1 mM $MgSO_4$ was replaced with 1 mM Na_2SO_4. The nutrient solution was replaced every 4 days. The photoperiod consisted of 16 h light (300 or 250 μmol photons m^{-2} s^{-1}) and 8 h darkness. Plant fragments were flash-frozen in liquid nitrogen and stored at −80°C until further analysis.

Carbohydrate analysis

The frozen tissues were ground in a mortar and extracted three times with ethanol (80% v/v) at 85°C. Extraction was assumed to be total because a fourth ethanol extraction of the starch pellet did not contain detectable amounts of glucose. Soluble sugars were determined by a spectrophotometric enzymatic assay (Lambda 14 spectrophotometer; Perkin Elmer), through the NAD^+ reduction at 340 nm, following a procedure slightly modified from Sokolov et al. (1998). For starch determination, the pellet was autoclaved and digested with amylase and amyloglucosidase. The glucose released before and after digestion was measured using the same spectrophotometric assay. All enzymes used in this assay were purchased from Roche Diagnostics.

Iodine staining

Starch was visualized by iodine staining. Bleached leaves in 80% (v/v) boiling ethanol were stained with Lugol solution (2 mM I_2, 6 mM KI) and briefly de-stained with distilled water.

RNA gel blot analysis

Total RNAs were isolated as described by Ausubel et al. (1998). Fifteen or 25 µg total RNA was loaded and size-fractioned by electrophoresis in 1% agarose–16% formaldehyde gels and blotted onto Hybond-N^+ nylon membranes (Amersham Pharmacia Biotech). A 0.24- to 9.5-kb RNA Ladder (Invitrogen Life Technologies) was used to determine the size of mRNA transcripts. Uniformity of loading and completeness of transfer to membranes were checked by ethidium bromide staining under UV illumination. RNA was cross-linked to the membrane by heating for 2 h at 80°C. Hybridizations were done with DNA probes labelled with [^{32}P]dCTP, using the Ready-prime II Random Prime Labelling System (Amersham Pharmacia Biotech). Total RNAs were probed with a DNA fragment of *BvSUT1* (AC: X83850) amplified between nucleotides 516 and 1,100, using the forward primer 5′GGAATTCGGGCGTTGTTGGCTGATATG3′ and the reverse primer 5′GCTCTAGACCACCTACTAAACGAGCTAA3′. The PCR fragment obtained was verified by sequencing. Equal loading of membrane was checked by hybridization with an 18S rRNA from *Arabidopsis thaliana*. Hybridizations were carried out for 16 h at 62°C in a phosphate buffer containing 5× SSC (1× SSC is 150 mM NaCl and 15 mM sodium citrate, pH 7.0) and 100 µg ml^{-1} denatured salmon sperm. After hybridization, the blots were washed twice for 20 min at 55°C in 2× SSC/0.1% SDS and once for 20 min in 0.2× SSC/0.1% SDS. Hybridization signals were quantified with a phosphor imager (Storm 860; Molecular Dynamics).

Uptake of ^{14}[C]sucrose

The upper epidermis of the leaf was abraded with Carborundum (0.060 mm) as described in Grusak et al. (1990). [U-^{14}C]Sucrose (37 kBq per leaf; Amersham Pharmacia Biotech) was applied as liquid droplets (25 µl) containing 10 mM sucrose and 20 mM Mes buffer (pH = 5.5 adjusted with KOH).

Radioactivity measurements

Small portions of tissues were bleached and their radioactivity was counted by liquid scintillation spectroscopy using an external standard for correction of quenching (Tri-carB 1900TR Liquid Scintillation Analyzer; Packard Instruments).

Chemical analysis

Powdered dried material was ashed in a muffle furnace at 450°C. Ashes were digested with 7 N nitric acid and the filtrate was assayed for Mg with an atomic flame absorption spectrometer (Perkin Elmer AAS 3110) at 422.6 nm wavelength.

Chlorophyll determination and photosynthesis measurement

Pigments were extracted with 80% (v/v) acetone and chlorophyll content was estimated using the method of Porra et al. (1989). The behaviour of photosystem II (PSII) was assessed through the fast fluorescence kinetics (direct induction with 660 nm exposure), recorded with the a Plant Efficiency Analyser fluorometer (Hansatech Instruments).

Results

Plant growth, mineral status and photosynthesis

Magnesium deficiency was induced in sugar beet plants with seven fully expanded leaves, after 5 weeks growth, by transferring them into Mg-free medium. Interveinal chlorosis appeared in leaves 8, 9 and 10 (leaves numbered from the bottom of the plant) after 17 days, and necrotic spots appeared after 20 days. Physiological measurements were started after 12 days because sugar accumulation in leaves started to be apparent at that time. The biomass of organs (Fig. 1a) and the Mg content (Fig. 1b) were measured at days 12, 16 and 20. No significant ($P < 0.05$) difference could be found in biomass measurement between control and Mg-deficient

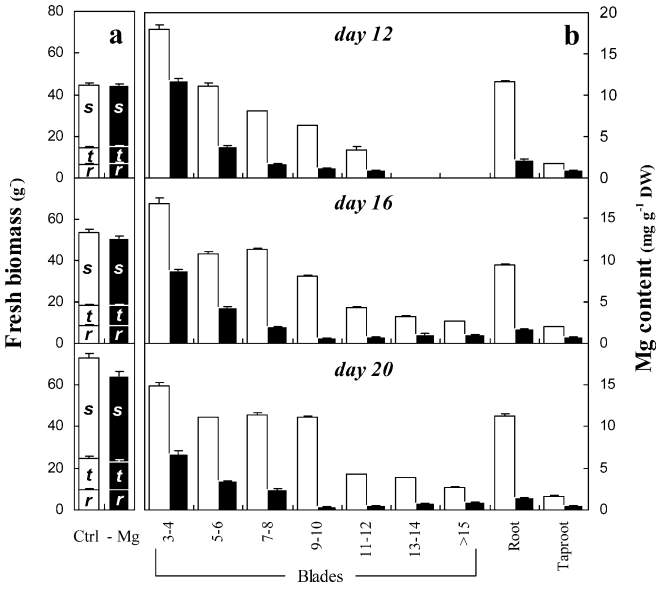

Fig. 1a,b Effect of Mg deficiency on fresh biomass and Mg content of sugar beet (*Beta vulgaris*). **a** Fresh biomass allocation between root (*r*), taproot (*t*) and shoot (*s*) in plants at days 12, 16 and 20 of treatment. Mean ± SE of 14–18 values. **b** Mg content in opposite leaf blade pairs numbered from the oldest to the most recent (pair 1–2 was senescent), root and taproot of control and Mg-deficient beets at days 12, 16 and 20 of treatment. Mean ± SE of 3 mineralizations of a pooled powder of 5–10 plants. Mg was removed from the nutrient solution at day 0, when plants had seven expanded leaves. Photoperiod was 16 h light (300 µmol photons m^{-2} s^{-1})/8 h darkness. *White bars* control; *black bars* Mg deficiency

plants at day 12. At day 16, only the fresh weight (FW) of the shoot was lower (9%) under Mg deficiency, compared to control treatment. At day 20, the biomasses of the shoot, taproot and root were reduced by 15, 8 and 3%, respectively (Fig. 1a). When the Mg-deficiency treatment was prolonged over 20 days, the root biomass did not markedly decrease (data not shown).

In control plants, a gradient of decreasing Mg content was observed from the oldest leaves to those that had emerged after the beginning of the treatment. The profile of Mg content in Mg-deficient plants showed a decrease from the oldest leaves to leaves 9–10, and an increase to the youngest leaves (Fig. 1b). The leaves at position 9–10 exhibited the most dramatic decrease in Mg content, reaching 1.2 ± 0.02 mg g^{-1} dry weight (DW) after 12 days versus 6.3 ± 0.1 mg g^{-1} DW in the control pair. The Mg content of these leaves progressively decreased to 9 and 4% of the control values, after 16 and 20 days of treatment. The Mg contents in root and taproot represented 12.5 and 31% of the control values at day 12, and they remained relatively constant until the end of the treatment.

Chlorophyll content was measured to assess the degradation of the photosynthetic apparatus. The total chlorophyll contents in the deficient leaves represented successively 94, 73 and 57% of the control values at days 12, 16 and 20 (Table 1). The maximum quantum yield for primary photochemistry of PSII (ϕ_{Po} or F_V/F_M) related to the dark-adapted state, decreased by less than 4% in Mg-deficient plants compared to control plants by the end of the treatment (Table 1).

Carbohydrate status

The carbohydrate content in beet leaves was measured at the onset of light, following an 8-h dark period, at days 12, 16 and 20 of the Mg-deficiency treatment (Fig. 2). Two-fold increases in sucrose content relative to the control were observed after 12 days in leaves 7–8 and 9–10 (Fig. 2). Comparable increases were observed in the upper leaves that emerged after Mg removal from the nutrient solution. A more pronounced starch increase was apparent in the same leaves, with a maximum of 5-fold the control values. Iodine staining of the entire plants after an 8-h dark period confirmed this quantitative analysis (Fig. 3). Neither glucose nor fructose content was affected by Mg deficiency at day 12, but at day 20 glucose increased by a factor of 2 in leaves 9–10 and 11–12 (data not shown). Sugar content in the root was little changed by Mg deficiency, although sucrose content in the taproot was reduced by 18% at day 20 only, compared to the control.

Table 1 Chlorophyll content and primary photochemistry of photosystem II (PSII) in sugar beet (*Beta vulgaris*) upon Mg deficiency. Total chlorophyll (*Chl*) content and maximum quantum yield for primary photochemistry of PSII ($\phi_{Po} = F_V/F_M$) were determined in the uppermost expanded leaves (9 and 10) of control and Mg-deficient plants at days 12, 14 and 20 of treatment. *Asterisks* (*) indicate a significant difference between treatments at $P = 0.05$ or less

Days of treatment	Treatment	Chl (nmol g^{-1} FW) n = 5	φ_{Po} n≥25
12	Control	139.6 ± 3.7	0.812 ± 0.009
	Mg deficiency	122.0 ± 3.9*	0.810 ± 0.007
16	Control	133.6 ± 6.8	0.804 ± 0.007
	Mg deficiency	92.8 ± 2.6*	0.798 ± 0.008*
20	Control	129.5 ± 6.2	0.798 ± 0.011
	Mg deficiency	72.5 ± 2.7*	0.767 ± 0.023*

Sucrose partitioning

Radioactive sucrose was fed to intact leaves (Grusak et al. 1990) to monitor sucrose partitioning between sink organs (Fig. 4a,b) in beet plants that had four expanded leaves when the treatment started, and five to six expanded leaves 11 days after Mg withdrawal. [^{14}C]Sucrose was supplied as droplets either on the oldest (numbered 1) or most recently developed (numbered 5) leaves. Whole-leaf autoradiographs confirmed that most of label was found in the organs analysed (data not shown). When [^{14}C]sucrose was supplied to leaf 1, 63.5 and 71.5%, respectively, of the labelled molecules remained in the control and Mg-deficient blades (Fig. 4a). Overall, export was not significantly affected even when there was a strong reduction in the radioactivity contained in the petiole of Mg-deficient plants (Fig. 4a). When [^{14}C]sucrose was supplied to leaf 5, a significantly higher proportion ($P > 0.05$) of label stayed in the treated blade compared to the untreated blade, and there was a drastic decrease of radioactivity in the petiole of the treated leaf as well as in the taproot, root and young leaves (Fig. 4b).

BvSUT transcript profiles

The impact of Mg deficiency on the steady-state transcript levels of *BvSUT1*, which encodes a sucrose/H^+ symporter expressed in companion cells (Vaughn et al. 2002), was determined during the photoperiod when deficiency symptoms were well established (Fig. 5), as well as during the establishment of the Mg deficiency (Fig. 6). Measurements were made in the uppermost expanded source leaves, which emerged after Mg withdrawal, and in which Mg reduction (Fig. 1b) and sucrose export (Fig. 4b) were most affected. The variations in *BvSUT1* transcript abundance and sucrose content in leaves 9–10 were investigated during the photoperiod at day 18 (Fig. 5a–c). Mg deficiency markedly enhanced the amount of *BvSUT1* transcripts, with the highest differences compared to the control at 12 and 16 h into the photoperiod (Fig. 5b). The sucrose content in Mg-deficient leaves was twice as high as in the control leaves at the onset of the light. This difference between Mg-deficient and control leaves was maintained throughout the photoperiod and superimposed on the diurnal variation (Fig. 5c). Since the difference in *BvSUT1*

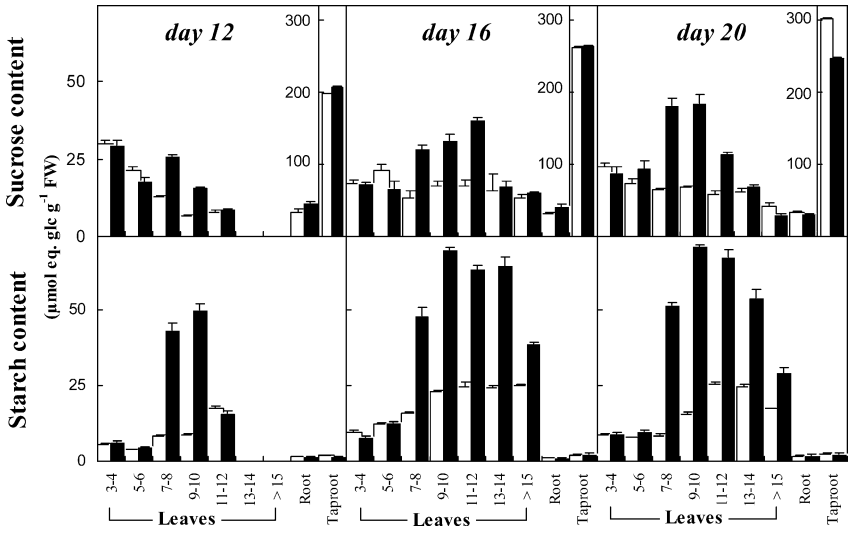

Fig. 2 Effect of Mg deficiency on sugar content in sugar beets. Soluble sugars and starch content (µmol glucose equivalents g^{-1} FW) in blade pairs, roots and taproots were measured at the onset of light in the photoperiod after 12, 16 and 20 days of treatment. *White bars* control, *black bars* Mg deficiency. Each bar is the mean ± SE of 3 extractions of a pooled powder of 5 plants

transcript levels between the treatments was maximal at the end of the photoperiod, sucrose symporter expression was further analysed during the establishment of Mg deficiency in plants, together with sucrose contents in source leaves after 12 h of light (Fig. 6). The transcripts levels of *BvSUT1* increased in the most recently expanded blades of Mg-deficient plants after 6 days of treatment, and after 9 days they were 77% more abundant than in the control (Fig. 6a). Concomitantly, sucrose contents started to increase in Mg-deficient leaves at day 6 of treatment and were 2.5-fold higher than in control blades after 12 days (Fig. 6b).

Discussion

Plant growth and biomass allocation under Mg deficiency

This work analyses physiological responses of *Beta vulgaris* to Mg starvation. Mg deficiency resulted in a lower fresh weight of the plants, by comparison to the control treatment. In particular, the shoot growth was affected (Fig. 1a), resulting in decreased shoot/root biomass ratio. After prolongation of the Mg-deficiency treatment, the root biomass was not markedly lower than in the control (data not shown). These observations differ from other studies showing a severe decrease in root biomass, as in bean plantlets (Cakmak et al. 1994a) or spinach plants (Fisher et al. 1998) after 12 days of complete Mg starvation. Direct comparison is difficult to make because studies were performed with different plant species at different growth stages. However, the bean plants studied by Cakmak et al. (1994a, 1994b) had a much-reduced number of leaves when deficiency was induced, compared to our experiments. Therefore, Mg limitation might have affected leaf area increment of all leaves and shoot biomass, which could have readily restricted assimilate flux to the root as well. Root architecture adaptation to low nutrient availability (N, Fe, P and S) consists of an overall increase in the total absorptive surface of the root system (López-Buico et al. 2003). Here also, unaffected root growth in Mg-deficient beet plants can be regarded as an adaptive mechanism to enhance the capacity for mineral uptake and to explore the nutrient medium in the absence of Mg.

Altered sucrose partitioning between source and sink organs upon Mg deficiency

Mg deficiency has previously been reported to induce sucrose accumulation in leaves (Cakmak et al. 1994b, 1994b; Fisher et al. 1998). The present work aimed at investigating whether all leaves were equally affected by Mg deficiency and whether a decrease in sucrose phloem loading or a decline in sucrose consumption in sink organs was the cause of sucrose accumulation. Sucrose and starch accumulation was an early response to Mg deficiency (Fig. 2) in the uppermost expanded leaves prior to obvious chlorophyll degradation or changes in photosynthetic activity (Table 1). A long-term effect of Mg deficiency could be to engage a carbon-metabolite feedback control of photosynthesis (Paul and Pellny 2003). Actually, a negative correlation is largely reported between sugar levels, chlorophyll content, photosynthetic activities and expression of photosynthetic genes (Foyer 1988; Oswald et al. 2001).

The source leaves that had developed after the removal of Mg from the nutrient solution were the first to accumulate sucrose and starch (Figs. 2, 3). Sucrose accumulated up to 3-fold and starch up to 5-fold without any major increase in fructose or glucose content except at the end of the treatment (data not shown). Starch accumulation was more pronounced between the veins of the distal part of the leaves, and preceded interveinal chlorosis (Fig. 3). The uppermost expanded source leaves had the lowest Mg contents by comparison to older ones (Fig. 1b), reaching 1.2 mg g^{-1} DW after 12 days, which is below the general threshold value in leaves for the

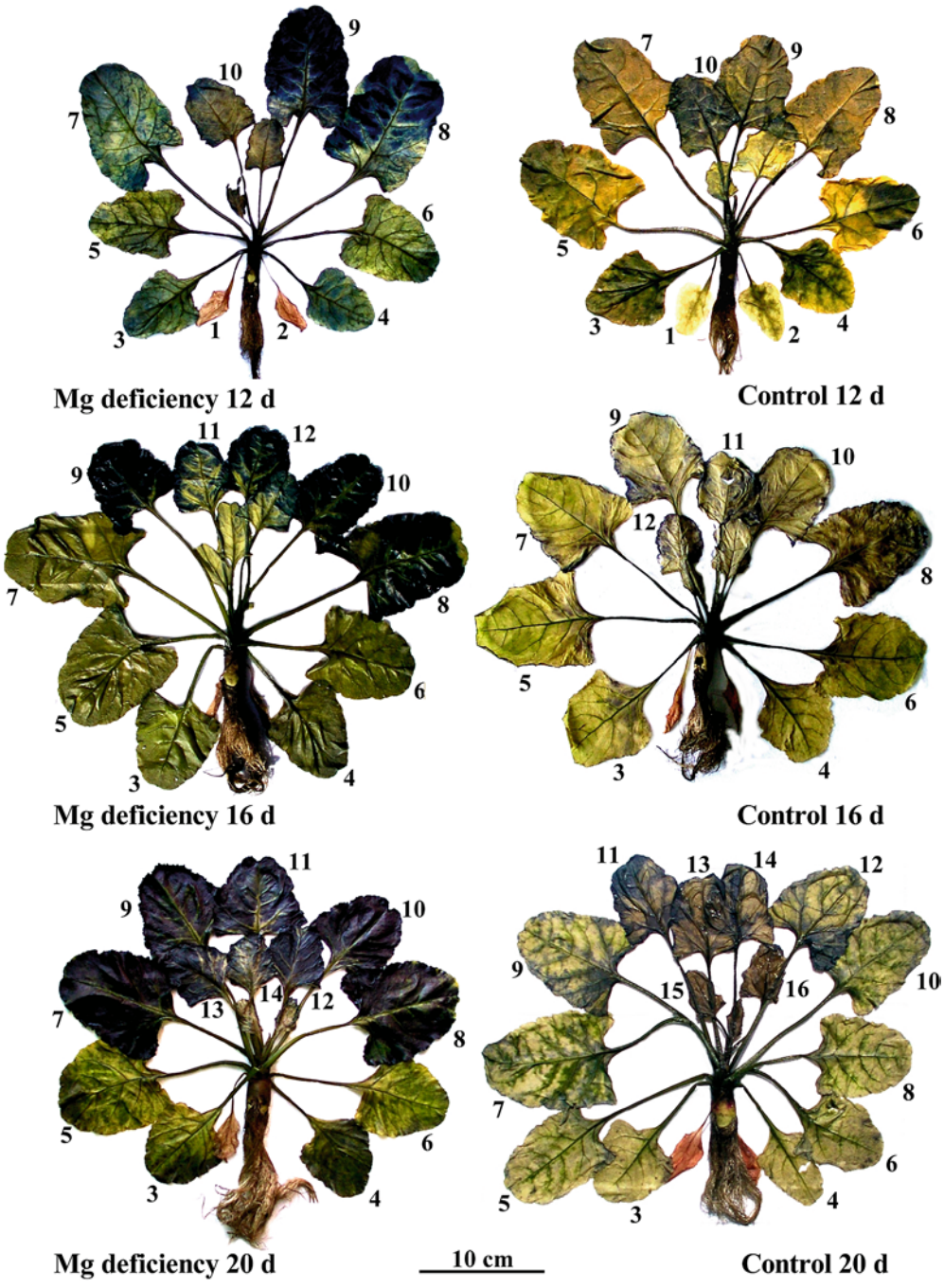

Fig. 3 Effect of Mg deficiency on starch content in sugar beet leaves, as detected by lugol staining. Mg-deficient and control beet plants were stained with lugol solution to reveal the presence of starch at the end of the dark period (8 h) at days 12, 16 and 20 of treatment. Mg was removed from the nutrient solution at day 0, when plants had seven expanded leaves

occurrence of deficiency symptoms, reported to be about 2 mg g^{-1} DW (Mengel and Kirkby 1987). These leaves were indeed the first to show symptoms. Accumulation of starch was at the origin of an early change in the leaf structure, which turned rubber-like, well before the development of chlorosis and necrosis. Transitory starch accumulation can be considered as an intermediate sink in photosynthetic leaves, when the balance between sucrose production and sink utilization is inadequate. Such a situation has been generated either by antisense expression of sucrose symporters in predominantly apoplasmic loader species, such as in *Arabidopsis* (Rook et al. 2001), tobacco (Bürkle et al. 1998) and potato (Kühn et al. 1996; Lemoine et al. 1996), by co-suppression of the plasma membrane H^+-ATPase (Zhao et al. 2000), or alternatively by reduced sink consumption (Ntsika and Delrot 1986; Grusak et al. 1990; Paul and Foyer 2001). Several lines of evidence suggest that the accumulation of starch in Mg-deficient sugar beet leaves is due to ongoing sucrose production without proper export from the sources rather than to decreased consumption of sugars in sinks.

To study sucrose partitioning upon Mg deficiency, [^{14}C]sucrose distribution within the whole plant was analyzed before the development of marked chlorosis. The supply of exogenous [^{14}C]sucrose avoided any

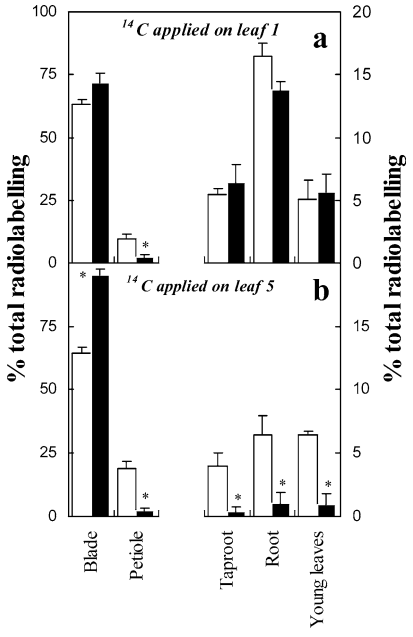

Fig. 4a,b Effect of Mg deficiency on ^{14}[C]sucrose transport in sugar beets. Plants were labeled apoplasmically after deposition of labeled ^{14}[C]sucrose molecules on leaf 1, corresponding to the lowest leaf of the rosette (**a**), or on leaf 5, corresponding to the most recently expanded leaf of the rosette (**b**). Droplets (25 µl) containing ^{14}[C]sucrose (37 kBq) were deposited on abraded zones of leaves after 11 days of Mg-deficiency treatment. Re-partitioning of labeled sucrose molecules between donor blade, petiole and sink organs (taproot, root and youngest immature leaves) is reported relative to the total radioactivity measured in the whole plant. Data are means ± SE of 3 (**a**) or 5–6 (**b**) replicates. *Asterisks* (*) indicate a significant difference between treatments at $P = 0.05$ or less

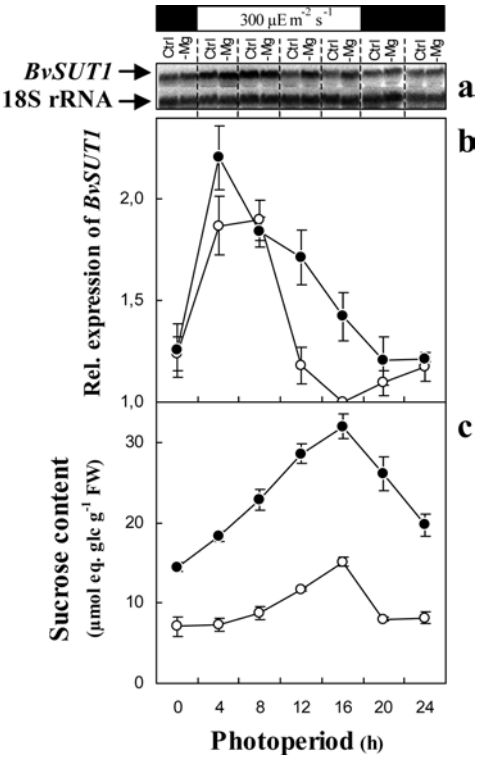

Fig. 5a–c Effect of Mg deficiency on diurnal variations of sucrose concentration and *BvSUT1* expression in sugar beet leaves. **a** Northern analysis of *BvSUT1* in uppermost expanded blades of control (*Ctrl*) and deficient (*−Mg*) leaves at day 18 of treatment. A 25-µg sample of total RNA was loaded and 18S rRNA was used as a loading control. **b** Quantified signals of *BvSUT1* transcripts relative to the 18S rRNA loading control. The relative hybridizing signal of the control at 16 h was defined as 1.0. Values are means ± SE of 3–4 replicates of 2 RNA extractions. **c** Sucrose content in blades. Mean ± SE of 3 extractions from a pooled powder of 5 plants. The Mg-deficiency treatment started on plants with seven expanded leaves (day 0). Tissues were sampled at 12 h into the photoperiod (16 h at 250 µmol photons m^{-2} s^{-1}/8 h darkness) throughout the days of treatment. *Open circles* control, *closed circles* Mg deficiency

indirect effects of Mg deficiency on $^{14}CO_2$ consumption. The proximity between source and sink organs is a significant factor in the distribution of photosynthates among organs (Bonnemain 1964). In sugar beet, the uppermost expanded mature leaves provided photosynthates to the growing shoot tip and young immature leaves, while the lower leaves predominantly supplied the root system (Fig. 4a,b). In Mg-deficient beet plants, the young source leaves provided less carbon resources to the growing shoot than in control conditions (Fig. 4b). Although the sucrose pool size in the Mg-deficient leaves affected the specific activity of the transport of sucrose, the 2-fold sucrose accumulation in those leaves cannot account for the 8-fold reduction in ^{14}C-carbon exported to sink organs. On the contrary, the export of sucrose from old source leaves was not affected or only slightly affected by Mg deficiency and those leaves kept on fuelling the root system with photosynthates (Fig. 4a). Overall carbon allocation to the root was proportionally less affected than to the youngest leaves. These results corroborate the observed decrease in shoot growth and the ongoing root growth (Fig. 1a). Root growth was reduced and the lower leaves began to accumulate high levels of sucrose and starch only after prolonged treatment (data not shown). Sink metabolism was apparently not restricted because labelled molecules were detected in roots and young immature leaves when [^{14}C]sucrose was supplied on the lower leaves of Mg-deficient plants (Fig. 4a). That observation argues against the hypothesis of sugar accumulation due to reduced sink consumption (Fisher et al. 1998).

Mg deficiency, sucrose accumulation and expression of sucrose transporters

In the present context of altered sucrose partitioning, it was important to study the expression of *BvSUT1*, which encodes a sucrose symporter in the companion cells in sugar beet leaves. Light-dependent expression of *BvSUT* was observed in control and Mg-deficient beets (Fig. 5a,b). Similarly, diurnal regulation of the expression of sucrose symporters genes has been previously reported in tobacco (Kühn et al. 1997) and in carrot plants (Shakya and Sturm 1998). In control plants, the relative level of *BvSUT* transcripts increased during the

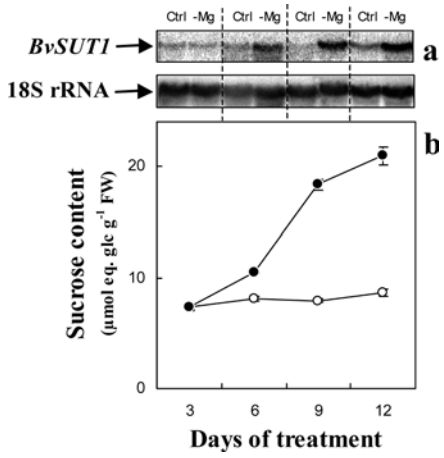

Fig. 6a,b Effect of Mg deficiency on *BvSUT1* steady-state transcript levels and sucrose contents in sugar beets. **a** Northern analysis of *BvSUT1* in control (*Ctrl*) and Mg-deficient (*−Mg*) mature blades at 12 h into the photoperiod. A 15-µg sample of total RNA was loaded. Percentages indicate variation in the *BvSUT1* transcript level (standardized with 18S rRNA signal) of Mg-deficient plants compared to control plants. **b** Sucrose content in mature blades. Mean ± SE of 3 extractions of a pooled powder of 5 plants. Mg-deficiency treatment started on plantlets with four leaves (day 0). Tissues were sampled at 12 h into the photoperiod (16 h at 300 µmol photons m^{-2} s^{-1}/8 h darkness) throughout the treatment. *Open circles* control, *closed circles* Mg deficiency

first 8 h of the light period and decreased after 12 h, as the soluble sugars accumulated in blades (Fig. 5a–c). A circadian cycle cannot be excluded, as mRNA abundance was already high at the onset of light. However, since light regulation of several photosynthetic genes can be overridden by sucrose and glucose (Sheen 1990), a role for endogenous sucrose levels in the transcriptional regulation of *BvSUT1* can be hypothesized. Reported effects of high sugar concentrations on the expression of sucrose transporters differ, which suggests that not all transporters are regulated in the same way (Harms et al. 1994; Truernit and Sauer 1995; Weber et al. 1997; Aoki et al. 1999). Feeding high sucrose via the xylem transpiration stream in detached leaves of sugar beet reduced the *BvSUT1* transcript level and transport activity of the protein (Chiou and Bush 1998; Vaughn et al. 2002). The variation in sucrose symporter responses to sucrose accumulation may be a function of which cells accumulate excess sucrose. Here, *BvSUT* transcript was more abundant in Mg-deficient beets compared to control plants during the photoperiod, but the down-regulation after 12 h of light was still effective in the deficient plants (Fig. 5a,b). The fact that *BvSUT1* transcript accumulation occurs together with sucrose accumulation seems to contradict the data published by Chiou and Bush (1998). However, Bush and co-workers hypothesized that excess cell wall sucrose delivered via the xylem stream overloads the phloem. Inhibition of phloem loading upon Mg deficiency is expected to reduce the sucrose concentration in the sieve element–companion cell complex, and to result in sucrose accumulation in the leaf. However, as Mg deficiency induces *BvSUT1* expression (Figs. 5, 6) whereas sucrose feeding of the xylem represses it (Chiou and Bush 1998), these different effects induced by sucrose accumulation in the leaf may be due to the fact that sucrose infiltrated via the xylem may accumulate preferentially in the apoplast (cell walls), whereas sucrose accumulating upon Mg deficiency may be localized preferentially inside the mesophyll cells (i.e. in the vacuoles).

BvSUT1 is the only known sugar beet gene coding for a sucrose/H^+ symport. However, high levels of sequence identity have been reported between members of the multigenic sucrose transporter (*SUT* or *SUC*) family in other species (Shakya and Sturm 1998; Noiraud et al. 2000; Aoki et al. 2003). Therefore, although stringent conditions were used for the RNA blot analysis (see Materials and methods), a cross-hybridization of the probe with other not yet identified sucrose transporter transcripts cannot be excluded.

Conclusion and perspectives

We propose that phloem loading of sucrose in the uppermost expanded source leaves is a primary target of Mg deficiency in sugar beet. A major challenge in the future would be to localize the site of sucrose accumulation and to understand the relation between Mg level and sucrose phloem loading. The observed effect of Mg deficiency on carbon export may emerge from a direct effect on the sucrose symporter activity, or an indirect effect through a disruption of the proton-motive force in the sieve element—companion cell complex.

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