Plant Soil (2013) 368:87–99
DOI 10.1007/s11104-013-1589-0

REGULAR ARTICLE

Physiological and molecular responses to magnesium nutritional imbalance in plants

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Received: 5 July 2012 / Accepted: 7 January 2013 / Published online: 22 January 2013

Abstract
Background Magnesium (Mg) is pivotal for many biochemical and physiological processes in plants. Mg biological functions include a key role in photosynthesis, in protein synthesis, as well as in nucleotide metabolism. However, Mg nutrition of plants remains little examined compared with other essential elements.
Scope The review summarizes the current knowledge on physiological targets of Mg imbalances. Recently generated transcriptome profiles in response to Mg shortage and excess are also presented.
Conclusions Sugar accumulation in source leaves is a major consequence of Mg shortage that can limit plant growth most probably by down regulation of photosynthesis activity. Newly identified molecular targets of Mg imbalance are appraised in relation to their potential contribution to Mg deficiency phenotypic emergence. In particular a potential role of the circadian clock and change in phytohormones concentration and/or signalling in the orchestration of the Mg deficiency response is possible. The development of markers for incipient Mg deficiency appears to be a challenging task.

Keywords Magnesium nutrition · Sugar transport · Biomass allocation · Global transcriptomics

Responsible Editor: Ismail Cakmak.

Electronic supplementary material The online version of this article (doi:10.1007/s11104-013-1589-0) contains supplementary material, which is available to authorized users.

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Introduction

Magnesium (Mg) is one of the nine essential macronutrients used in large amount by plants for their growth and reproductive success (Williams and Salt 2009). The best known physiological roles for Mg are in harvesting solar energy by occupying the central position in the chlorophyll structure, as a cofactor and allosteric modulator for >300 enzymes (including carboxylases, phosphatases, kinases, RNA polymerases, and ATPases), and in chelation to nucleotidyl phosphate forms (Cowan 2002; Shaul 2002; Hawkesford et al. 2012). About three-quarters of leaf Mg appears to be associated with protein synthesis, and up to one-fifth with chlorophyll pigments, and the remaining fraction stored in the vacuole (reviewed in Karley and White 2009). Magnesium has a large hydrated radius and sorbs weakly to soil colloids, predisposing it to leaching, particularly in acidic soil with low cation exchange capacity (Aitken et al. 1999; Grzebisz 2009). On alkaline soils, $MgCO_3$ formation and excess calcium (Ca), potassium (K) and sodium (Na) reduce Mg availability to crops (reviewed in Broadley and White 2010). Mg scarcity in agricultural soils can increase with intensive harvesting of crop products without concomitant Mg fertilization

(Grzebisz 2009). Mg deficiency in plants is a common nutritional disorder that affects productivity and quality in agriculture (Aitken et al. 1999; Graeft et al. 2001), horticulture (Troyanos et al. 2000; Ruan et al. 2012; Yang et al. 2012) and forestry (Mehne-Jakobs 1995; Mitchell et al. 1999; Laing et al. 2000; Sun et al. 2001; Boxler-Baldoma et al. 2006; Vacek et al. 2006). Mg toxicity can appear in certain locations, mostly in extreme serpentine (high magnesium:calcium) soils (Brady et al. 2005) and in semi-arid regions, where water stress conditions can increase Mg metabolic pool in plants (Hawkesford et al. 2012).

This review will describe the physiological and transcriptional targets of Mg nutritional imbalance in plants. In many species, the earliest indications of Mg deficiency consist of an impairment in sugar partitioning leading to starch accumulation and the enhancement of antioxidative mechanisms, prior to noticeable effects on photosynthetic activity (Cakmak and Kirkby 2008; Hermans and Verbruggen 2008). In recent years, the global transcriptional response to Mg imbalance has been studied by microarray technologies. The availability of the complete Arabidopsis genome sequence (www.arabidopsis.org) has dramatically accelerated research on plant nutrition. Genome scale studies of the model species under Mg depletion (Hermans et al. 2010a, b) or excess (Visscher et al. 2010) have allowed unbiased views of the responses to Mg imbalance.

Symptoms and threshold levels for Mg toxicity and deficiency occurrence in plants

Numerous studies have examined the relationship between Mg nutrition and plant growth in various species. There is much more research interest in Mg deficiency than its toxicity, which we will first briefly address. (i) Toxicity symptoms are hardly visible in plants, even upon culture with concentrations as high as 60 mM Mg^{2+} (Shaul et al. 1999). One possible reason for the scarcity of Mg toxicity reports could be the large vacuolar storage capacity for Mg within the plant (Hawkesford et al. 2012). Nonetheless, some authors associate Mg toxicity with copper coloured patches along the marginal veins (Venkatesan and Jayaganesh 2010) or necrotic spots (Messenger and Hruby 1992) on leaves. (ii) In most plant species, Mg deficiency symptoms arise first in the oldest and recently expanded leaves, and systematically progress from them towards the youngest ones. Indeed, due to the fairly mobility of the element, plants will remobilize Mg from older leaves to younger ones (Taiz and Zeiger 2010). A characteristic deficiency symptom is leaf interveinal chlorosis (Bennett 1997), a phenotype worsened by high light conditions which promote the spreading of chlorotic and necrotic lesions (Marschner and Cakmak 1989; Cakmak and Marschner 1992; Cakmak and Kirkby 2008). Mg concentrations required for optimal growth vary and are usually between 1.5 and 3.5 mg g^{-1} dry weight in vegetative tissues (Grzebisz 2009; Römheld 2012). Mg concentrations below 1–2 mg g^{-1} leaf dry weight are frequently associated with the onset of chloroses (Hermans et al. 2004; Hermans and Verbruggen 2005; Ding et al. 2006). Below that threshold, a reduction of plant growth and a modification in biomass allocation between organs are commonly reported.

Plant growth and biomass allocation upon Mg limitation

Although Mg deficiency symptoms are well depicted in aerial parts of the plant, impact on root growth and biomass allocation between root and shoot in response to Mg limitation is more variable. For the illustration, Fig. 1 presents morphological adaptations of pea (*Pisum sativum* Norli) and Chinese cabbage (*Brassica rapa* Michihili) plants to Mg shortage using experimental hydroponics system, where plants were replete before being transferred to Mg-free nutrient solution. After 2 weeks treatment, the occurrence of chlorotic symptoms in Mg-deficient pea plants (Fig. 1a, g) is accompanied with a significant reduction in total fresh biomass, mainly due to a decrease in petioles and stem fresh weight (Fig. 1c). Lower dry weights of roots (slight decrease) and stems are measured, while dry matter accumulates in trifoliate limbs (Fig. 1d). The specific leaf weight, calculated as the dry weight per unit leaf area, is increased in Mg deficient pea plants (Fig. 1f). Considering Chinese cabbage, none of those parameters are significantly different between Mg-deficient and Mg-fully supplied plants at that time of treatment. However a tendency for lower fresh and dry shoot biomass can be drawn (Fig. 1h, i).

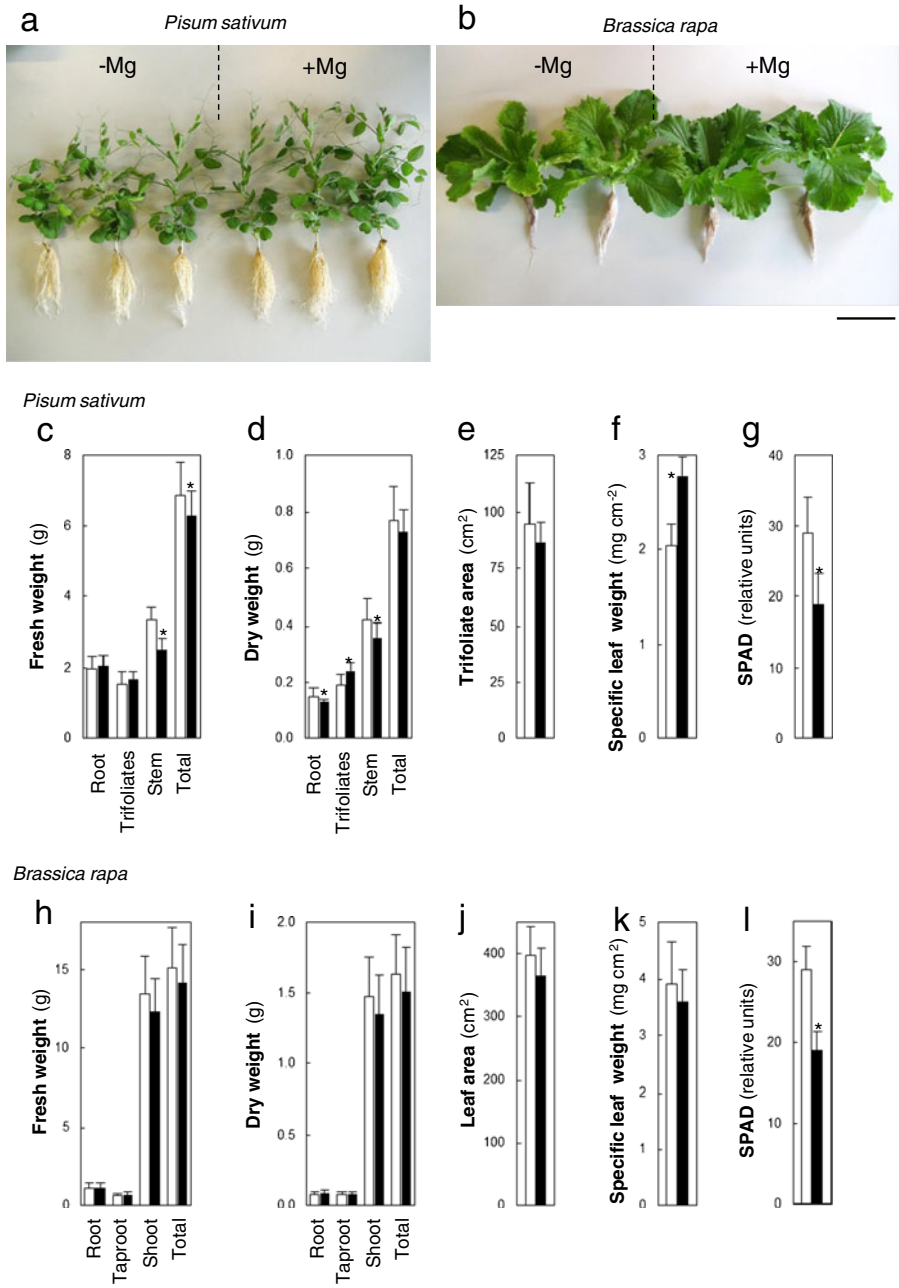

Fig. 1 Magnesium deficiency symptoms in plants grown hydroponically. Plants of *Pisum sativum* (Norli) and *Brassica rapa* (Michihili) were initially grown at a photoperiod of 16 h (100 µmol photons m^{-2} s^{-1}) light/ 8 h darkness for 9 day and 16 day after germination, respectively. After that time, plants were fed with Mg-supplied (1 mM Mg^{2+}) solution or Mg-depleted (without Mg added) solution for 2 weeks. For details on nutrient solution composition, see Hermans et al. (2010a). Color pictures of *P. sativum* (**a**) and *B. rapa* (**b**) plants were taken at the end of the treatment. Scale bar: 10 cm. Fresh (**c**, **h**) and dry (**d**, **i**) biomasses in various plant organs, total leaf area per plant (**e**, **j**), specific leaf dry weight (**f**, **k**) and SPAD-chlorophyll meter values (numerical outputs of leaf transmittance readings in the red and infrared wavelengths) indicating leaf greenness (**g**, **l**) were measured, n=8–15±std. *Stars* indicate significant differences between treatments (P<0.05)

Previous reports showed a more severe impact on the root or on the shoot growth, depending on the plant species and the system used to induce Mg depletion. In particular, applying Mg shortage at germination or at a very young growth stage is usually accompanied by a severe reduction of root growth. Under those conditions, reduction in dry matter production is observed earlier in the root than in the shoot of bean (Cakmak et al. 1994a, b), birch (Ericsson and Kahr 1995), pine (Sun and Payn 1999), pepper (Riga and Anza 2003), clover (Bouma et al. 1979). On the contrary, when plants have developed in Mg replete conditions before being transferred to Mg deficient solution (as described in Fig. 1), mature leaves keep on feeding roots with photoassimilates (see further explanations). Interestingly, a stimulation of rice root growth can even be observed upon continuous culture at low Mg (Ding et al. 2006). Almost no effect on root

fresh biomass production is documented in sugar beet (Hermans et al. 2004, 2005) upon transfer of plants first grown without Mg supply constraints to a solution fully-depleted of Mg. Mg starvation affects the shoot more than the root in Arabidopsis grown with the same experimental procedure (Fig. S1, Hermans and Verbruggen 2005; Hermans et al. 2006, 2010a, b) and that visual observation is confirmed by the alteration of 33 % of the gene transcripts in leaves compared to less than 2 % of the transcripts in roots after 1 week Mg starvation treatment (Hermans et al. 2010b). Indeed Mg concentration in roots decreases more rapidly than in leaves but then plateaus at a low concentration during prolonged Mg starvation (Hermans and Verbruggen 2005; Hermans et al. 2010a, 2011), indicating an efficient Mg recycling mechanism to support root growth. Finally, replenishment of Mg to the nutrient solution restores initial patterns of gene expression for one-fifth of the transcripts in the leaves and half in the roots within 24 h (Hermans et al. 2010b).

Survey of molecular targets reveals potential signaling mechanisms of Mg imbalance

Physiological targets of fluctuation in Mg availability have been well described in the literature in general terms over several decades (see following sections). However, knowledge of upstream molecular targets was limited until recently. Interesting outcomes of recent global transcriptomics studies are here briefly reviewed, those may help to maximize plant fitness at low Mg input (Fig. 2). The roles played by the circadian clock and hormones in the orchestration of responses to mineral availability are interesting research leads.

Impact of Mg nutrition on the circadian clock oscillator and possible physiological output pathways

The circadian system is built on three interconnected parts: the receptors that perceive the environmental signals (such as light, temperature or nutritional status) to provide input to the clock, the core oscillator that generates the rhythms, and the output pathways that produce rhythmic physiology (Haydon et al. 2011; McWatters and Devlin 2011). Briefly, the central oscillator of Arabidopsis is based on interlocked regulatory feedback loops of transcription factors, namely *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), *LATE ELONGATED HYPOCOTYL* (*LHY*) and *TIMING OF CAB EXPRESSION 1* (*TOC1*) (Harmer 2009; Nakamichi 2011; Huang et al. 2012). Interestingly, Mg status was recently documented as one of the sensory inputs in Arabidopsis (Hermans et al. 2010a, b). The perturbation of the oscillator may occur within hours of Mg shortage in roots and within days in leaves. The expression levels of early morning-induced *CCA1* and *LHY* genes have a slower decay in -Mg organs than in control ones but with larger amplitude variations in long-standing Mg-deficient leaves than in roots (Hermans et al. 2010a, b). The extent to which the circadian clock regulates the timing of plant growth and metabolism has only recently begun to be explored (McWatters and Devlin 2011; Farré 2012). Upon Mg depletion, alteration of expression of those clock-related transcription factors occurs before the build-up in sugar concentration in source leaves (see following section), and it could therefore, in part, orchestrate the decline in the photosynthetic apparatus beforehand (Fig. 2). In support of that view, the clock controls the rate of starch degradation (Graf and Smith 2011) and the synthesis of light-harvesting complex proteins and chlorophyll pigments (Prombona and Argyroudi-Akoyunoglou 2004; Dodd et al. 2005). It is to be noted that chloroses can occur under very long photoperiods, because the duration of light-induced degradation of light-harvesting complex proteins extends beyond their period of clock-enhanced transcription (Dodd et al. 2005). Previous work showed that the expression of *CHLOROPHYLL A/ B-BINDING PROTEIN 2* (*CAB2 / LHCB1.1*) lost rhythmicity in Mg-deficient Arabidopsis plants, well before leaves displayed lower chlorophyll content (Hermans and Verbruggen 2005). Further work should be dedicated to decipher the impact of Mg nutrition on the circadian rhythmicity of plants and how it translates into physiological output pathways.

Hormonal signaling of Mg imbalance

A key role for plant growth regulators in the Mg imbalance response is documented mainly through the expression modulation of several genes in hormonal biosynthetic and signaling pathways upon the element depletion in roots and leaves (Hermans et al. 2010a, b) or excess in roots (Visscher et al. 2010). The hormone measurement is a tantalizing prospect

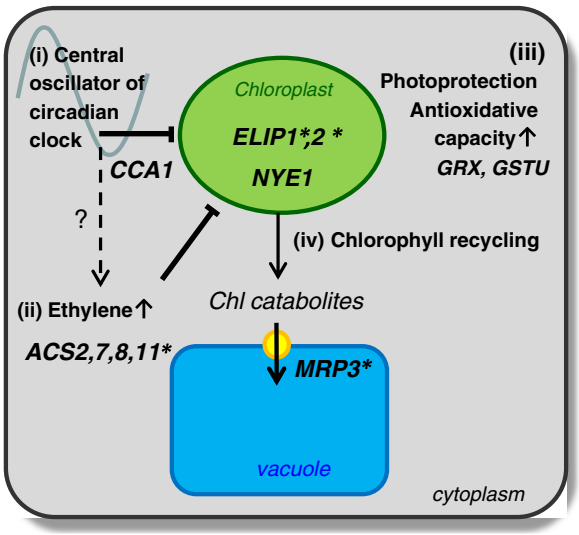

Fig. 2 Transcriptomic responses of Mg deficiency in the leaf cell. (*i*) The alteration of the expression of transcription factors in the central oscillator of the circadian clock, namely the master controller *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*), can impact on the turn-over of photosystems components and ethylene production; (*ii*) induction of *1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE* (*ACS*) genes and measured higher ethylene production could induce early senescence of the leaves, or alternatively have a potential role as nutrient starvation stress-protecting hormone; (*iii*) enhanced protection of the pigment-protein complexes and anti-oxidative stress mechanisms are supported by higher expression of *EARLY LIGHT-INDUCED PROTEIN 1 and 2* (*ELIP1* and *ELIP2*); higher expression of genes in the ROS detoxification like *GLUTAREDOXIN* (*GRX*) and *GLUTATHIONE S-TRANSFERASE TAU* (*GSTU*) and higher ratio of oxidized/reduced ascorbate and gluthatione forms (*iv*) increased chlorophyll catabolism is supported by higher expression of *NON-YELLOWING 1* (*NYE1*) and chlorophyll catabolites transport to the vacuole by *MULTIDRUG RESISTANCE PROTEIN 3* (*MRP3*). *Stars* indicate gene whose expression is restored after 24 h Mg re-supply following one week Mg starvation (Hermans et al. 2010b)

for investigating the drivers for those early transcriptomic responses seen in plant tissues. Short-term Mg shortage triggers the abscisic acid (ABA) signalosome in leaves, without detectable change in ABA content (Hermans et al. 2010a). That discrepancy can possibly be explained by a lower activity of certain Mg^{2+}-dependent phosphatase 2C proteins which are negative regulators of ABA-induced responses (Raghavendra et al. 2010). After 1 week of -Mg treatment, several genes encoding isoforms of the 1-aminocyclopropane-1-carboxylic acid synthase (ACS) family, catalyzing the conversion of S-AdoMet to ACC in the ethylene biosynthesis pathway (Harpaz-Saad et al. 2012), are strongly induced by Mg starvation (Fig. 2). That observation correlates with double ethylene emanation in Mg-starved plants compared with controls (Hermans et al. 2010b). Although C_2H_4 is generally depicted as a leaf senescence inducer (Jing et al. 2005), ethylene-regulated stress responses also seem to be essential for mineral deficiencies tolerance (Jung et al. 2009; Iqbal et al. 2011). We previously discussed the potential impact of the circadian clock on the ruining of the photosynthetic apparatus; we can also highlight a possible link between the clock and ABA and ethylene signaling upon Mg deficiency. The expression of a large number of ABA-responsive genes oscillates in a manner dependent on the circadian clock (Mizuno and Yamashino 2008). Ethylene production also follows a circadian rhythmicity and in particular the expression of *ACS8* is controlled by *CCA1* and *TOC1* (Thain et al. 2004; Convington et al. 2008).

Survey of physiological targets upon Mg deficiency

Impact on photosynthates and allocation to sink organs

As previously mentioned, Mg deficiency has been characterized in several plant species through hydroponics culture by lowering or omitting Mg in nutrient solutions. A hierarchy of physiological perturbations is largely documented upon Mg depletion: the sugar and amino acid accumulation in source organs usually precedes the growth inhibition of sink organs, the decrease in photosynthetic rate and appearance of chloroses. The early impairment of sugar and amino acid partitioning is reported in Arabidopsis (Hermans and Verbruggen 2005; Hermans et al. 2006), bean (Fischer and Bremer 1993; Fischer 1997; Cakmak et al. 1994a, b), citrus (Yang et al. 2012), spruce (Mehne-Jakobs 1995), spinach (Fischer et al. 1998), rice (Ding et al. 2006; Cai et al. 2012) and sugar beet (Hermans et al. 2004, 2005; Hermans and Verbruggen 2008). Furthermore, the activity of key enzymes in nitrogen assimilation and carbon partitioning such as nitrate reductase and sucrose-phosphate synthase, are decreased upon Mg depletion in maize (Zhao et al. 2012). Figure 3 shows the difference in starch accumulation patterns between Mg-deficient beet leaves and those suffering from deficiencies of other major elements such as nitrogen (N), phosphorus (P) and potassium (K). Upon Mg starvation, starch

accumulates preferentially in the most recently expanded leaves, which have the lowest Mg concentration (Hermans et al. 2005). Increase in amino acids concentrations of source leaves is also frequently reported in various species like spinach and tea plants (Fischer et al. 1998; Ruan et al. 2012). Causes of photosynthate accumulation in source leaves might be due to (i) structural damage and destabilization in phloem tissues (Boxler-Baldoma et al. 2006 and references therein), (ii) a decrease in the metabolic activity of sink organs (Fischer et al. 1998) or (iii) impaired phloem loading (Cakmak et al. 1994b; Hermans et al. 2005, 2006; Hermans and Verbruggen 2005). Figure 4 illustrates the third possibility and points to the susceptibility of sucrose phloem loading to low Mg availability in sugar beet, which uses a Type II strategy (apoplasmic phloem loading through H^+/sucrose symporter). Mg is essential for sucrose phloem loading because it interacts with nucleotidyl tri-phosphate (Mg-ATP) fuelling the H^+-ATPases, which creates the proton-motive force energizing the sucrose symporter BvSUT1 (Chiou and Bush 1998; Vaughn et al. 2002; Ayre 2011). ^{14}C-sucrose labeling studies show that sucrose export from young mature leaves, which preferentially provide carbon resources to growing shoot, is inhibited upon -Mg. Meanwhile, sucrose export from the oldest leaves, which maintain relatively high Mg concentrations and which supply more to the roots, does not seem to be restricted. The differential impact on leaves that developed before Mg depletion and on those that developed after the onset of the treatment could explain why sucrose export to the root is proportionally less affected than transport to the youngest leaves (Fig. 4). With the increasing severity of the deficiency, lower leaves also accumulate higher starch amounts; and finally the root and taproot growth starts to be restricted when treatment is prolonged. In response to sucrose accumulation, possibly in the apoplasm of the mesophyll, the *BvSUT1* gene is induced in Mg-deficient source leaves of sugar beet, but without further enhancement of sucrose loading into the phloem (Hermans et al. 2005). It was shown that feeding sucrose via the xylem transpiration stream in detached leaves reduced the *BvSUT1* transcript levels and transport activity (Chiou and Bush 1998; Vaughn et al. 2002). Hermans et al. (2005) suggest that sucrose accumulation in the apoplasm and not into the companion cell- sieve element (CCSE) complex may account for the observed difference in *BvSUT1* transcript levels. Indeed, upon Mg depletion, higher *BvSUT1* gene expression supports the idea that sucrose loading into CCSE complex is defective. It is to be noted that phloem loading of sucrose can be quickly restored upon resupply of Mg to starved bean plants (Cakmak et al. 1994b).

Impact on chlorophyll synthesis, photosynthetic apparatus and carbon assimilation

Chlorophyll synthesis, photochemical reactions, carbon fixation and stomata functioning can all be affected by Mg shortage. Magnesium occupies the central position of the chlorophyll structure and between 10 and 20% of the total Mg can be bound to that pigment (Wilkinson et al. 1990), with an even higher proportion upon Mg depletion (Hawkesford et al. 2012). Magnesium is required for grana stacking (Hall et al. 1972; Kaftan et al. 2002) and its depletion results in disorganized thylakoid membranes (Lu et al. 1995). Unsurprisingly, the impact of Mg deficiency on total chlorophyll concentration is heavily reported in various plant species (Fischer 1997; Lavon et al. 1999; Balakrishnan et al. 2001; Hermans et al. 2004; Hermans and Verbruggen 2005; Ayala-Silva and Beyl 2005; Werner et al. 2010; Ceppi et al. 2012; Yang et al. 2012). Chlorophyll catabolism (Hörtensteiner 2009; Hörtensteiner and Kräutler 2011) can be seen as a strategy of Mg-deficient plants to dechelate Mg from pigment molecules and to recycle it in favor of young tissues growth, considering that Mg is a fairly phloem-mobile element (White et al. 2009). In support of a higher chlorophyll catabolism, global transcriptomic studies have shown that *NON-YELLOWING 1* (*NYE1*) and *MULTIDRUG RESISTANCE PROTEIN 3* (*MRP3*) are up-regulated upon Mg deficiency (Fig. 2, Hermans et al. 2010b). Respectively, *NYE1* encodes a chloroplastic protein that can initiate chlorophyll degradation during senescence in Arabidopsis and other species (Ren et al. 2007; Wei et al. 2011) and *MRP3* encodes a vacuolar ABC transporter that has chlorophyll catabolites and glutathione conjugates transport activity (Tommasini et al. 1998), in addition to a possible role in toxic metals detoxification (Zientara et al. 2009).

Upon Mg deficiency, a higher Chlorophyll *a*/*b* ratio is often observed (Lavon et al. 1999; Balakrishnan et al. 2001; Hermans et al. 2004; Hermans and Verbruggen 2005), but the ratio can also be decreased (Ayala-Silva and Beyl 2005) or unaffected (Ceppi et al. 2012). Given

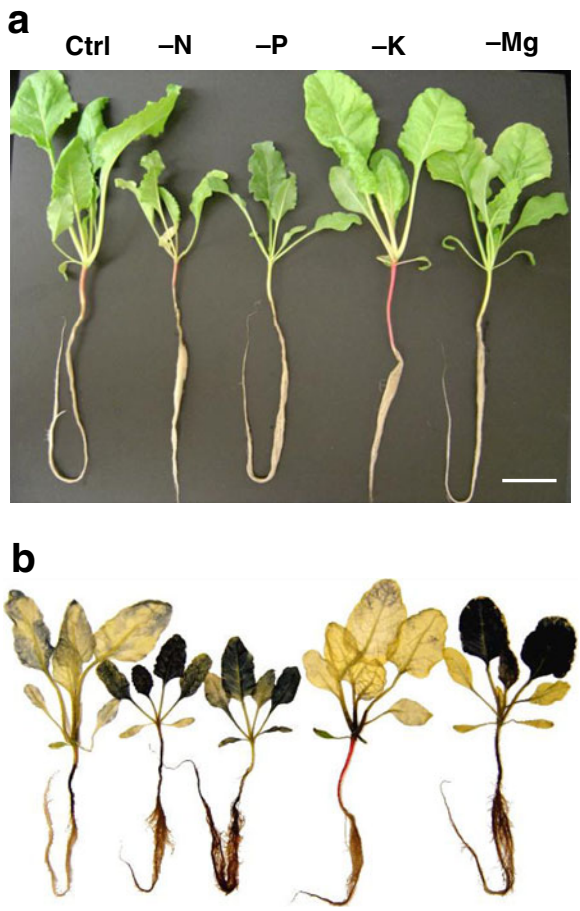

Fig. 3 Influence of mineral supply on the morphology of and starch accumulation pattern in sugar beet. Plants were grown for 3 weeks in hydroponic systems with a fully-supplied nutrient solution, then for 10 day with the same solution (control: Ctrl) or one lacking the mineral element as indicated (nitrogen deficiency: –N, phosphorus deficiency: –P, potassium deficiency: –K and magnesium deficiency: –Mg). For growth conditions and nutrient solution, see Hermans et al. (2004). Salt substitutions from the complete nutrient solution were as follows, –N: 0.2 mM $CaNO_3$, 1.8 mM $CaCl_2$; –P: 0.25 mM KCl; –K: 0.88 mM Na_2SO_4, 0.25 NaH_2PO_4; –Mg: 1.00 mM Na_2SO_4. (**a**) Pictures of sugar beet plants. Scale bar=5 cm. (**b**) Iodine staining of the plants shown in panel **a**. The staining of whole plants was performed as a qualitative approach to visualize the differences in the distribution of starch (*dark blue*) following a dark (8 h) period. Plants were grown under long day conditions (16h light/ 8h darkness)

that Chl *b* is mostly associated with the light harversting complex connected to photosystem II (PSII), the increase of Chl *a*/*b* ratio is probably indicative of a relative loss of PSII peripheral antenna, or, alternatively, of a change in photosystem stoichiometry in favour of photosystem I (PSI) (Hermans et al. 2004). Chlorophyll *a* fluorescence emitted by PSII turned out to be an easy tool for screening the photosynthetic vitality of plants submitted to Mg deficiency. Information on the efficiency of photochemical and de-excitation processes involved in light conversion by the plant were gathered by monitoring the fluorescence fast rise (one sec recording) upon dark-to-light transition of leaf samples in Arabidopsis (Hermans and Verbruggen 2005; Hermans et al. 2011), citrus (Yang et al. 2012), *Pinus radiata* (Sun et al. 2001) and sugar beet (Hermans et al. 2004; Ceppi et al. 2012). Fluorescence levels F_O (50μs), F_J (2ms), F_I (30ms) and $F_{MAX} = F_P$ were integrated into the JIP-test procedure (Strasser et al. 1995) in order to provide an extended range of parameters to assess the effects of Mg deficiency on PSII (Hermans et al. 2004; Yang et al. 2012). In sugar beet, the electron transfer beyond the primary quinone electron acceptor of PSII (Q_A) was affected earlier and to a greater extent than the 'light' reactions upon Mg deficiency induction (Hermans et al. 2004). That result was supported by other observations of a more reduced plastoquinone (PQ) pool in spinach submitted to combined Mg and S deficiencies (Godde and Dannehl 1994; Dannehl et al. 1996). The late fluorescence rise was further investigated in Mg-deprived sugar beet leaves (Ceppi et al. 2012). The amplitude of the relative variable fluorescence of the I-to-P-rise, shown to be correlated with PSI activity, decreased as a function of the Mg depletion treatment time. Other studies of spectroscopic observations in the near-infrared of Mg-deficient leaves point to a loss of total PSI centres occurring per leaf area and a limitation in the pool of PSI available to take part in electron transport (Hermans et al. 2004).

Magnesium is also involved in the modulation of the activity of key photosynthetic enzymes, with the best illustrated example of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) that catalyzes the first major step of carbon fixation (Andersson 2008; Engels et al. 2012). Low Mg levels affect the net rate of photosynthesis across a wide variety of plant species (Fischer and Bremer 1993; Laing et al. 2000; Ridolfi and Garrec 2000; Hariadi and Shabala 2004; Ding et al. 2006; Ling et al. 2009). The decrease in CO_2 assimilation may not be caused by stomatal limitation (e.g. stomatal conductance) because it is accompanied by higher intracellular CO_2 concentration upon Mg depletion (Li et al. 2001; Ling et al. 2009; Yang et al. 2012). As sugar transport efficiency is reported to influence photosynthetic productivity by relieving product (sucrose) inhibition (Ayre 2011), it is conceivable that sugars accumulion in source

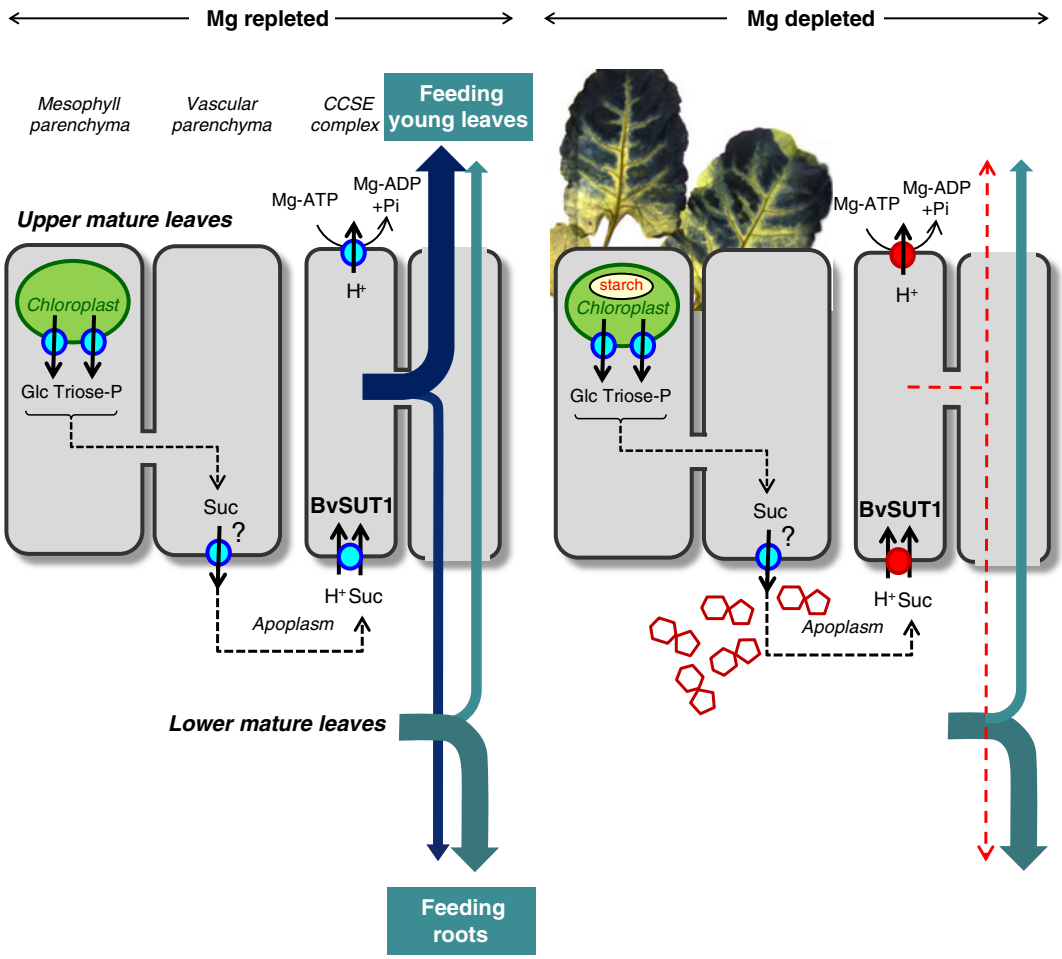

Fig. 4 Allocation of sugars to sink organs during Mg deficiency in sugar beet. Model of sucrose (Suc) phloem loading in strategy II (apoplastic loading) species. After synthesis in the cytoplasm of mesophyll cells, sucrose is exported (through uncharacterized transport systems) to the apoplasm for uptake into companion cell- sieve element (CCSE) complex. Sucrose transport across the plasma membrane of companion cell is operated through symport with protons (SUT1). That step is energized by the H^+-motive force generated by proton-pumping ATPase in the plasma membrane of the CCSE complex, which requires Mg-ATP for their activity. Impact is here illustrated in an experimental setting inducing Mg deficiency with mature plants (Hermans et al. 2005). Lower Mg metabolic pool, particularly in most upper Mg-deficient leaves, is thought to impact on ATPase activity in CCSE complex. Upon Mg deficiency, sucrose export to the roots is proportionally less affected than that to the youngest leaves. The upper mature leaves, where the deficiency symptoms first manifest, have the highest starch (revealed here by iodine staining) and sucrose contents and the lowest Mg concentration among all plant organs. Those leaves provide less carbon resources to young leaves. Consequently, the overall aerial biomass is decreased compared to control plants. At a later stage of Mg deficiency induction, intermediate and lower leaves also accumulate higher starch amount; and finally the root and taproot growth is affected when treatment is prolonged. Pathways and carriers highlighted in red are potential prime targets of Mg depletion. The width of the arrows is proportional to the flow of assimilates delivered to the sink (immature leaves or roots) organs

leaves upon Mg depletion can trigger a decline in photosynthesis (Ding et al. 2006; Hermans et al. 2006). In particular sucrose is not just a passive carrier molecule for long-distance transport of assimilated carbon and it is generally reported that high sugar levels negatively affect the expression of photosynthetic genes and ultimately photosynthetic activities and chlorophyll content (Pego et al. 2000; Oswald et al. 2001). Some authors further contend that upon Mg deficiency, a decline in chlorophyll concentration could be caused in part by a sugar–mediated down-regulation of photosynthetic gene expression (e.g. chlorophyll *a*/*b* binding protein), rather than just by a lack of Mg ions for chlorophyll synthesis (Hermans and Verbruggen 2005).

Enhancement of defence capacities against oxidative stress

Magnesium depletion induces conditions in which sugar export away from the leaf is restricted and photosynthetic end-products accumulate. A consequence of the decline in CO_2 fixation in Mg-deficient leaves is that less of the absorbed light energy captured by the chlorophyll antenna is used in photosynthetic electron transport chain, which becomes over-reduced (Cakmak and Kirkby 2008; Yang et al. 2012). Mg deficient leaves are therefore highly photosensitive and necroses commensurate with light intensity. As a result, reactive oxygen species (ROS) are expected to be increasingly produced and to cause damage to the photosynthetic apparatus. Some distinct adaptations of the photosystiems (PSII s probably down-regulated largely through a loss of antenna, and PSI through a loss of reaction centres) were depicted to lower the overall rate of linear electron transport in order to prevent an excess of reductant being produced upon Mg depletion (Hermans et al. 2004). Also, the photoprotection of the photosynthetic apparatus is indicated by the up-regulation of *EARLY LIGHT-INDUCED PROTEIN 1* and *2* (*ELIP1*, *ELIP2*) upon Mg deficiency and their restoration upon Mg re-supply (Fig. 2). ELIPs seem to be involved in developmental processes under abiotic stress (Rizza et al. 2011) but also to fulfill a photoprotective function (Hutin et al. 2003). They could bind pigments released during turnover of chlorophyll-binding proteins or stabilize the proper assembly of those proteins. The *elip1*/*elip2* double mutant only show a slight reduction in the chlorophyll content in mature leaves upon high light conditions (Rossini et al. 2006), while constitutive expression of *ELIP2* results in a marked reduction of chlorophyll content due to a down-regulation of the activity of some biosynthetic enzymes (Tzvetkova-Chevolleau et al. 2007). It is suggested that ELIPs could work as sensors that modulate chlorophyll synthesis to prevent accumulation of free chlorophyll, and hence to prevent photo-oxidative stress.

Higher synthesis of antioxidant metabolites, higher regeneration rates of reduced ascorbate and glutathione, higher expression of genes that potentially detoxify or alleviate the function of ROS like *GLUTAREDOXIN* (*GRX*) and *GLUTATHIONE S-TRANSFERASE TAU* (*GSTU*) and higher activities of antioxidant enzymes (superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase) have been reported in Mg-deficient leaves of several species (Cakmak and Marschner 1992; Candan and Tarhan 2003; Tewari et al. 2004, 2006; Anza et al. 2005; Cakmak and Marschner 2006; Hermans et al. 2010b; Yang et al. 2012). For further review, the effect of Mg deficiency on ROS scavenging enzymes and antioxidants molecules was extensively considered by Cakmak and Kirkby (2008).

The development of markers for incipient Mg deficiency

The identification of molecular markers responding to the availability of a given element could report the physiological limitation for that element before any growth restriction. The possibility to develop such markers for incipient Mg deficiency has already been explored (Hermans et al. 2010a; Kamiya et al. 2012). It is to be noted that Mg depletion, contrary to other mineral deficiencies does not trigger a higher expression of annotated genes in Mg transport at the organ level (Hermans et al. 2010a, b), like *MAGNESIUM/PROTON EXCHANGER 1* (*MHX1*) (Shaul et al. 1999; Berezin et al. 2008) or the members of the *MITOCHONDRIAL RNA SPLICING 2* (*MRS2*) family (Li et al. 2001; Gardner 2003; Waters 2011). Those genes are therefore not suitable for developing markers. However, the specificity of the response of some other Mg-regulated genes was challenged by studying their expression after other mineral deficiencies and environmental stresses (Hermans et al. 2010a). Nonetheless, no -Mg specifically responsive biomarker could successfully be discriminated. Kamiya et al. (2012) have repeated the micro-array analysis of Mg depleted Arabidopsis plants in order to find other markers. Five genes were selected for further analysis but only *CAX3*, member of the *CATION/*H^+ *EXCHANGERS* family (Chanroj et al. 2011), was demonstrated to be responsive *in planta* to Mg availability. *CAX3* was shown to be induced by Mg shortage (Hermans et al. 2010b) and repressed by Mg excess (Conn et al. 2011, Visscher et al. 2010) but that gene cannot be considered as specific Mg marker because it is also induced by Ca excess (Chan et al. 2008). Nonetheless, transgenic lines that express luciferase under the activity of the *CAX3* promoter can be used to evaluate plant Mg status, as well as to develop new research tool to isolate mutants with altered response to Mg fluctuations (Kamiya et al. 2012).

Conclusion

While global transcriptomics studies have highlighted novel aspects of plant response to Mg (Fig. 2), a plant is not just the sum of its genes. A better understanding of the plant response to Mg level fluctuations will require further transcriptomic, proteomic and metabolomic studies to be integrated and combined at finer resolutions. Deciphering the dynamical bases between the mineral nutrition and the circadian clock is an original research theme and could contribute to efforts to improve plant productivity and resistance to nutrient intake limitation.

Acknowledgments C.H. is research associate of the Fonds pour la Recherche Scientifique-FNRS. Research at LPGMP is partly supported by Action de Recherche Concertée (ARC) 'Nutrition and circadian rhythms in plants'. We thank Simon Conn for critical reading of the manuscript.

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