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REVIEW

Role of magnesium in carbon partitioning and alleviating photooxidative damage

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Magnesium (Mg) deficiency exerts a major influence on the partitioning of dry matter and carbohydrates between shoots and roots. One of the very early reactions of plants to Mg deficiency stress is the marked increase in the shoot-to-root dry weight ratio, which is associated with a massive accumulation of carbohydrates in source leaves, especially of sucrose and starch. These higher concentrations of carbohydrates in Mg-deficient leaves together with the accompanying increase in shoot-to-root dry weight ratio are indicative of a severe impairment in phloem export of photoassimilates from source leaves. Studies with common bean and sugar beet plants have shown that Mg plays a fundamental role in phloem loading of sucrose. At a very early stage of Mg deficiency, phloem export of sucrose is severely impaired, an effect that occurs before any noticeable changes in shoot growth, Chl concentration or photosynthetic activity. These findings suggest that accumulation of carbohydrates in Mg-deficient leaves is caused directly by Mg deficiency stress and not as a consequence of reduced sink activity. The role of Mg in the phloem-loading process seems to be specific; resupplying Mg for 12 or 24 h to Mg-deficient plants resulted in a very rapid recovery of sucrose export. It appears that the massive accumulation of carbohydrates and related impairment in photosynthetic CO_2 fixation in Mg-deficient leaves cause an over-reduction in the photosynthetic electron transport chain that potentiates the generation of highly reactive O_2 species (ROS). Plants respond to Mg deficiency stress by marked increases in antioxidative capacity of leaves, especially under high light intensity, suggesting that ROS generation is stimulated by Mg deficiency in chloroplasts. Accordingly, it has been found that Mg-deficient plants are very susceptible to high light intensity. Exposure of Mg-deficient plants to high light intensity rapidly induced leaf chlorosis and necrosis, an outcome that was effectively delayed by partial shading of the leaf blade, although the Mg concentrations in different parts of the leaf blade were unaffected by shading. The results indicate that photooxidative damage contributes to development of leaf chlorosis under Mg deficiency, suggesting that plants under high-light conditions have a higher physiological requirement for Mg. Maintenance of a high Mg nutritional status of plants is, thus, essential in the avoidance of ROS generation, which occurs at the expense of inhibited phloem export of sugars and impairment of CO_2 fixation, particularly under high-light conditions.

Abbreviations – PEP, phosphoenolpyruvate; ROS, reactive O_2 species; RuBP, ribulose-1,5-bisphosphate.

Introduction

Magnesium (Mg) is exceptional in activating more enzymes than any other mineral nutrient (Epstein and Bloom 2004). Magnesium is, therefore, involved in numerous physiological and biochemical processes in plants affecting growth and development. Some examples of Mg-activated enzymes include ATPases, ribulose-1,5-bisphosphate (RuBP) carboxylase, RNA polymerase and protein kinases (Marschner 1995, Shaul 2002). The role of Mg as the central atom of the Chl molecule is perhaps the best-known function of Mg in plants in that it is associated with the development of leaf chlorosis, typically interveinal, under Mg deficiency stress. Depending on the Mg status of the plant, between 6 and 35% of the total Mg may be bound in the chloroplasts, a higher proportion being associated with a lower Mg status (Scott and Robson 1990). With the onset of Mg deficiency, there is first a decline in the concentration of Chl *b*, which is followed by that of Chl *a* (Hermans et al. 2004). An initial decrease in Chl concentration in Mg-deficient plants has been ascribed to early accumulation of sugars in leaves, rather than to low levels of Mg (Hermans et al. 2004). According to Hermans et al. (2004), high accumulation of sugars may repress the expression of the *cab2* gene that is responsible for encoding Chl *a* and *b* protein.

Magnesium deficiency symptoms vary between plant species, but there are some similarities. As Mg is a relatively mobile nutrient in plants, deficiency always appears first in the older leaves (Bergmann 1992). As discussed below in detail, development of Mg deficiency symptoms is highly affected by light intensity. By increasing light intensity, plants under low Mg supply rapidly develop interveinal chlorosis and reddish spots on the leaves (Marschner and Cakmak 1989). Moreover, for leaves with the same low concentration of Mg, development of leaf chlorosis and necrosis is significantly accentuated by increasing light intensity (Marschner and Cakmak 1989). Critical Mg deficiency concentrations vary depending on plant species and other factors (e.g. light intensity), but values less than 2 g kg^{-1} Mg in the leaf dry matter may be suspect. In crops, Mg deficiency is especially associated with strongly leached sandy acid soils with a low cation exchange capacity. Lateritic soils are also usually poor in Mg. Magnesium deficiency can be induced, however, not only by a direct lack of Mg but also by the presence of competing cations that prevent Mg uptake, such as Ca^{2+} in calcareous soils; H^+, NH_4^+ and Al^{3+} in acid soils and Na^+ in saline soils (Mengel and Kirkby 2001, Shaul 2002).

One of the early reactions of Mg deficiency stress in plants is its impact on partitioning of dry matter between roots and shoots. An increasing body of evidence indicates that Mg plays a fundamental role in phloem export of photosynthates from the source to the sink organs, and its deficiency results in dramatic increases in accumulation of carbohydrates in the source leaves (Cakmak et al. 1994a, 1994b, Hermans et al. 2004, Marschner et al. 1996). Reduced transport and hence accumulation of carbohydrates in Mg-deficient leaves cause alterations in photosynthetic carbon metabolism and restrict CO_2 fixation. Impairment of the photosynthetic electron transport to CO_2 through photosynthetic membranes may cause an accumulation of non-utilized electrons and absorbed energy. Under such conditions, the electrons and excitation energy not used in photosynthetic CO_2 fixation are channelled to molecular O_2, leading to the generation of highly reactive O_2 species (ROS) and consequently to damage of chloroplast constituents such as Chl and membrane lipids (Asada 2006, Marschner and Cakmak 1989, Mittler 2002).

The main emphasis of this review is on the roles of Mg in partitioning of photosynthates between shoots and roots, maintenance of photosynthetic electron transport to CO_2 and occurrence of photooxidative damage resulting from the generation of ROS at the expense of impaired electron transport to CO_2. Readers interested in general physiological functions of Mg are referred to the publications of Marschner (1995), Mengel and Kirkby (2001) and Shaul (2002). There are also comprehensive review papers in relation to the role of mineral nutrition (including Mg nutrition) in dry matter allocation between shoots and roots (Cakmak and Engels 1999, Hermans et al. 2006, Marschner et al. 1996).

Functions of Mg in photosynthesis

Magnesium plays a key role in photosynthesis. Activity of many key enzymes involved in photosynthetic carbon metabolism is greatly affected by small variations in Mg concentration. A number of reports have shown that Mg deficiency in leaves results in a markedly reduced photosynthetic rate (Fischer and Bremer 1993, Hermans et al. 2004, Laing et al. 2000, Peaslee and Moss 1966, Sun and Payn 1999). Besides low concentrations, very high concentration of free Mg may also impair photosynthesis by inhibiting K transport from the cytosol to the stroma and possibly interfering with Mg homeostasis within the chloroplast (Shaul 2002). A decrease in photosynthesis capacity of Mg-deficient leaves is mostly associated with reduced stomatal conductance or increased mesophyll resistance to CO_2, decreased activity of enzymes involved in CO_2 fixation and increased carbohydrate accumulation in leaves (Fischer and Bremer 1993, Hariadi and Shabala 2004, Laing et al. 2000, Lin and Nobel 1971, Peaslee and Moss 1966). Magnesium also

affects photosynthetic CO_2 fixation by its effect on the activity of RuBP carboxylase (Lin and Nobel 1971). The carboxylation process of RuBP-driving CO_2 fixation is highly influenced by Mg (Portis 1992). In C4 and CAM plants, incorporation of CO_2 into phosphoenolpyruvate (PEP) catalysed by PEP carboxylase is also activated by Mg^{2+} (Wedding and Black 1988).

In sugar beet (Hermans et al. 2004) and Arabidopsis plants (Hermans and Verbruggen 2005), accumulation of sugars in leaves precedes a decrease in photosynthesis, suggesting that accumulation of sugars in leaves triggers a decline in photosynthetic CO_2 fixation by causing negative feedback inhibition of photosynthesis. As illustrated in Fig. 1, before any noticeable change occurs in photosynthetic electron transport and Chl concentrations, there is a substantial increase in sugar accumulation in Mg-deficient leaves (Hermans et al. 2004). The effects of Mg deficiency in decreasing photosynthetic electron transport in the PSII and PSI reaction centres occur at advanced stages of Mg deficiency as found in pine seedlings (Laing et al. 2000) and sugar beet plants (Hermans et al., 2004). Laing et al. (2000) also showed that photochemical yield (as a good indicator of the efficiency of PSII) is greatly reduced under Mg deficiency stress in pine seedlings with less than 0.25 g Mg kg^{-1} in the needles.

Magnesium is both a structural component of Chl and needed for its biosynthesis. Magnesium chelatase is an important enzyme catalysing the first step of Chl biosynthesis by inserting Mg^{2+} into protoporphyrin IX (Walker and Weinstein 1991). ATP is essential for the functioning of this enzyme, playing a role both in the activation of the enzyme and in the insertion of Mg into protoporphyrin IX (Walker and Willows 1997). Magnesium-bound ATP is an important form of Mg needed in the activation of the enzyme (Jensen et al. 1998). Protoporphyrin IX is known to be highly toxic and results in leaf chlorosis when accumulated in tissues, especially under high light intensity (Reinhold et al. 2007, Witkowski and Halling 1988). This toxic effect is most probably related to the capacity of protoporphyrin IX to induce light-dependent generation of ROS (Lermontova and Grimm 2006, Mock et al. 1998). Thus, any decrease or alteration in activity of Mg chelatase (e.g. as a result of low ATP availability or reduced Mg concentrations) may result in accumulation of protoporphyrin IX in leaf tissue that may induce development of leaf chlorosis. Recently, Reinhold et al. (2007) showed that a lowering in Mg

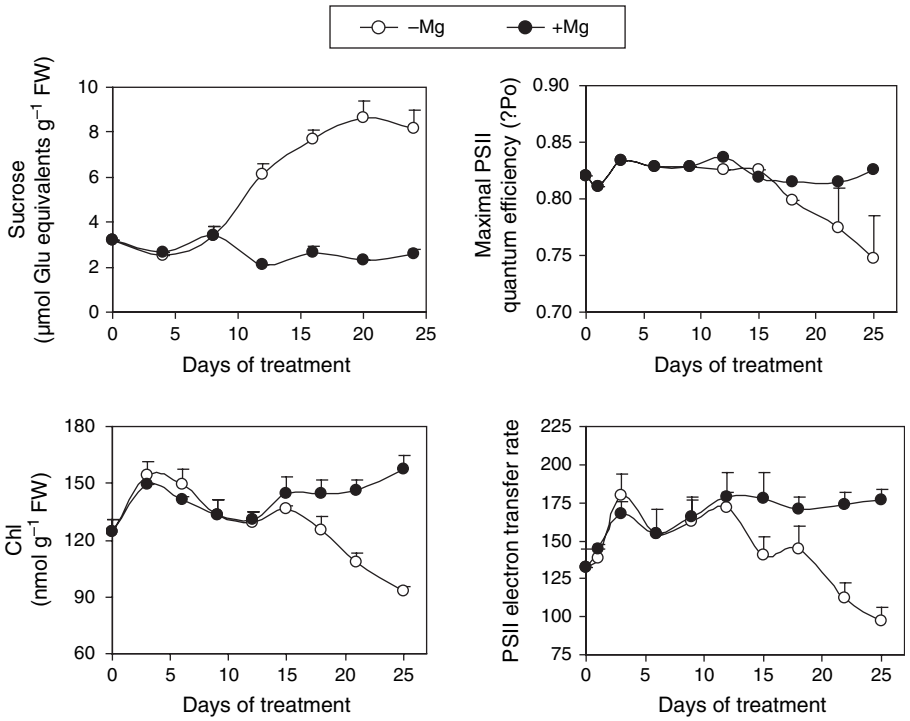

Fig. 1. Concentrations of sucrose and Chl (*a*+*b*) and levels of maximal quantum efficiency of PSII (ΦPo) and electron transport rate of PSII at 1700 μmol photons m^{-2} s^{-1} in uppermost fully expanded leaves of sugar beet plants over 25 days of treatments with deficient (−Mg) and adequate (+Mg) Mg supply. For the Mg-deficient treatment (−Mg), Mg was omitted from the nutrient solution at day 0, when plants had seven to eight expanded leaves (see Hermans et al. 2004 for the further details). FW, fresh weight.

chelatase activity by reduced availability of ATP is associated with higher concentrations of protoporphyrin IX. Further work is needed to establish the role of low Mg supply in accumulation of protoporphyrin IX and its contribution to the development of Mg deficiency leaf chlorosis.

Magnesium also plays a major role in photosynthetic electron transport in PSI and PSII reaction centres that absorb photons and initiate electron flow. During photosynthetic electron transport, protons are pumped from the stroma into the intrathylakoid space (thylakoid lumen), which become more acid, thereby inducing a proton gradient across the thylakoid membrane. It is well documented that this light-induced proton transport into thylakoid spaces is counterbalanced by enhanced transport of Mg from thylakoid lumen into stroma (Marschner 1995, Shaul 2002). The established electrochemical potential gradient across the thylakoid membrane is needed for synthesis of ATP (photophosphorylation). Therefore, increases in Mg supply are associated with enhanced ATP formation (Lin and Nobel 1971, Shabala and Hariadi 2005). The light-driven transport of magnesium into the stroma is associated with alkalization of stromal pH needed for optimal activities of the various stromal enzymes involved in photosynthetic CO_2 fixation (Shaul 2002). Recent measurements of light-dependent changes in Mg concentration showed that stromal Mg concentration in dark-kept intact spinach chloroplasts was 0.5 m*M*, which increased to 2 m*M* on illumination (Ishijima et al. 2003). Similar results were also reported previously by Portis and Heldt (1976). Increases in stromal Mg concentrations are associated with significant increases in RuBP carboxylase activity (Raghavendra et al. 1981).

Partitioning of carbohydrates

Plant Mg status has a pronounced impact on the transport and utilization of photosynthates, thereby markedly influencing carbohydrate partitioning between source and sink organs. In most cases, with the onset of Mg deficiency stress, partitioning of dry matter to roots is reduced, leading to a distinct increase in shoot-to-root dry weight ratio. As shown in Fig. 2, before any significant change in shoot growth occurred, Mg deficiency severely decreased root dry weight. A decrease in root growth and an increase in shoot-to-root dry weight ratio can be considered one of the earliest plant reactions to Mg deficiency. Similar observations have been reported for a wide range of cultivated species, such as subterranean clover (Bouma et al. 1979), birch (Ericsson and Kahr 1995), spinach (Fischer et al. 1998), pepper (Riga and Anza 2003) and *Pinus* (Sun and Payn 1999). However, in

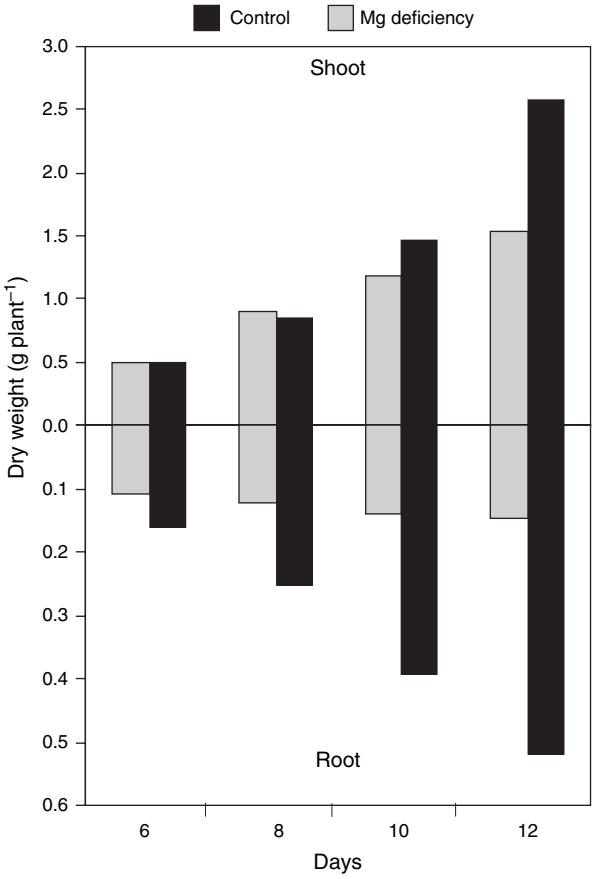

Fig. 2. Shoot and root dry weight of bean plants over 12 days of growth in nutrient solution with adequate (control) and deficient supply of Mg (redrawn from Cakmak et al. 1994a).

contrast to these findings, in studies with *Arabidopsis thaliana* (Hermans and Verbruggen 2005) and sugar beet (Hermans et al. 2005), controversial results have been obtained, showing that Mg deficiency had a greater effect in depressing shoot growth than root growth and thus lowering the shoot-to-root dry weight ratio. The reasons for this difference in expression of Mg deficiency in terms of shoot-to-root dry weight ratio among different plant species is not known but might be related to plant species used, growth stage studied and severity of Mg deficiency stress applied. As discussed by Hermans et al. (2005), proximity between source and sink organs may affect distribution of sugar transport within plants, and therefore, dry matter partitioning between shoot and root organs and thus shoot-to-root dry weight ratio may vary between plant species depending upon the proximity between source and sink organs.

The majority of results in the literature indicate that root growth is very sensitive to Mg deficiency stress. One important reason for such an early and distinct inhibition

of root growth under Mg deficiency may relate to restricted photosynthate supply. This suggestion is in agreement with the published results on carbohydrate partitioning between shoot and root organs under Mg deficiency. Most reports show that carbohydrate transport towards the roots is depressed in Mg-deficient plants. In sugar beet, Mg deficiency caused a massive accumulation of sucrose and starch in the leaves of rosette, which were the lowest in Mg concentration, and this accumulation of sucrose was in accordance with severe inhibition of sucrose export from these leaves into the roots (Hermans et al. 2005, see below). Accumulation of carbohydrates in Mg-deficient source leaves occurs before any adverse effect of Mg deficiency on shoot growth, Chl level, photosynthesis or leaf morphology is observed (Cakmak et al. 1994a, 1994b, Hermans and Verbruggen 2005, Fig. 1). Depending on the severity of Mg deficiency stress, plants under low Mg supply accumulate from 3- up to 12-fold greater concentrations of sucrose in source leaves than are present in adequately supplied plants (Cakmak et al. 1994a).

This increase in accumulation of sugars in Mg-deficient shoots appears to be a direct consequence of Mg deficiency stress and not the result of a decrease in sink activity. In comparing Mg with the effects of other nutrient deficiencies in common bean, in plants at the same stage of growth and grown under the same environmental conditions, accumulation of photosynthates in leaves was also found under K and Zn deficiencies but not under P deficiency (Marschner and Cakmak 1989, Cakmak et al. 1994a, 1994b). Magnesium- and K-deficient plants behaved similarly in their effects in increasing the dry matter shoot-to-root ratio as well as accumulation of sugars in the leaves in comparison with the plants adequately supplied with nutrients. By contrast, under N and P deficiencies, translocation of carbohydrates into the roots increased as a consequence of the relatively much higher sink activity of the roots compared with the shoots as evident from the lower shoot-to-root dry matter ratios and the lower amounts of carbohydrates in the leaves (Cakmak et al. 1994a, Freeden et al. 1990, Rufty et al. 1988). In comprehensive review papers by Marschner et al. (1996) and Hermans et al. (2006), several examples have been presented in relation to the effects of mineral nutrient deficiencies on the distribution of carbohydrates between roots and shoots. These authors conclude that N- or P-deficient plants differ greatly from those subject to K or Mg deficiency in relation to dry matter allocation between roots and shoots. When the main physiological functions of these nutrients are compared, one of the distinguishing features is the role of Mg and K in phloem loading of photoassimilates (Fig. 3 and below), resulting in smaller inhibitory effects of P or N deficiency on root growth compared with Mg or K deficiency (Cakmak 2005, Hermans et al. 2006, Marschner 1995, Marschner et al. 1996).

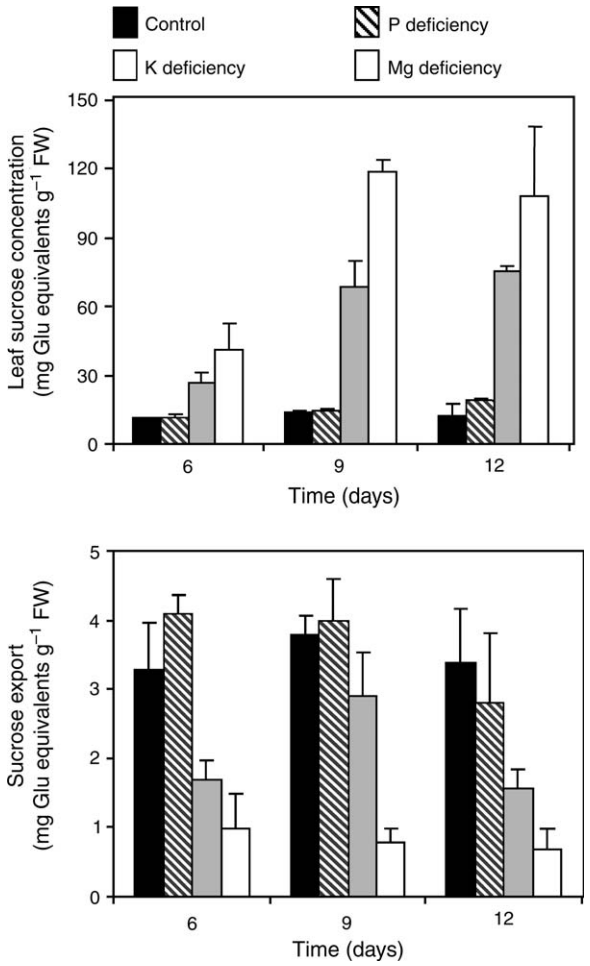

Fig. 3. Effect of adequate (control) and deficient supply of P, K and Mg on sucrose concentrations of source (primary) leaves and on phloem sucrose export from primary leaves of bean plants over 12 days of growth in nutrient solution (redrawn from Cakmak et al. 1994b).

Phloem export of sucrose

Substantial increases in carbohydrate accumulation in Mg-deficient source leaves (Figs 1 and 3) together with the enhanced shoot-to-root dry weight ratios (Fig. 2) indicate that photosynthate export from source into sink organs (e.g. seeds) is impaired by Mg deficiency. Only a few research articles have been published on the role of Mg in the phloem-loading process. In Mg-deficient sugar beet plants, high accumulation of starch and sucrose in the rosette leaves, which have the lowest Mg concentration, was associated with up to an eight-fold decrease in transport of ^{14}C-labelled sucrose (Hermans et al. 2005).

Interestingly, in sugar beet plants, sucrose export from the older leaves was not affected by Mg deficiency. This finding presumably relates to the higher concentrations of Mg in these older leaves compared with the leaves showing reduced sucrose export. In bean plants, Mg deficiency severely depressed sucrose transport from primary (source leaves) into roots, as measured from detached leaves using an EDTA-promoted exudation technique (King and Zeevaart 1974). There is a clear inverse relationship between export rate of sucrose and accumulation of sucrose in Mg-deficient source leaves (Fig. 3). As shown in Fig. 3, the effects of Mg and K deficiencies on phloem export of sucrose were similar, whereas in P-deficient plants, sucrose transport in phloem was not affected (Fig. 3). The effect of Mg on phloem loading of sucrose appears to be very specific and not related to any secondary effect. Resupplying Mg to Mg-deficient plants clearly restored phloem export of sucrose within 12 h (Cakmak et al. 1994b). Following resupply of Mg for 24 h, the export rate of sucrose was similar to the rate obtained with the Mg-adequate plants. Such recovery of the sucrose export in Mg-deficient plants upon Mg resupply was found both under dark and light conditions, indicating that enhancement in sucrose export after Mg resupply is not related to photosynthesis.

Based on the results discussed above, it can be suggested that phloem loading of sucrose is sensitive to low concentrations of Mg at the phloem-loading sites. This may have significant consequences for yield formation, especially during the intensive carbohydrate transport from source organs into generative organs (see Conclusions). One possible explanation for the impairments of the phloem export of sucrose could be related to reduced photosynthesis in Mg-deficient leaves. This possibility appears unlikely, however, because decreases in photosynthetic activity occur at later stages of Mg deficiency stress (Fig. 1). Previously, Peaslee and Moss (1966) reported that a decrease in photosynthesis is not a sensitive indicator of Mg deficiency. A more likely explanation of Mg-deficiency-induced impairment in phloem export of sucrose might be related to a direct functional and/or structural effect(s) of Mg on the phloem-loading process of sucrose.

It is well documented that phloem loading of sucrose is an active process catalysed by H^+/sucrose co-transport, which involves a proton gradient across the plasma membranes of phloem cells. The proton gradient required for the co-transport of H^+ and sucrose is established by an H^+-ATPase located in the plasma membranes of sieve tube cells (Bouché-Pillon et al. 1994, Ward et al. 1998). Growing evidence is available, suggesting that Mg–ATP is a major complex of ATP in biological systems (Igamberdiev and Kleczkowski 2003) and essential for the proper functioning of H^+-ATPase (Bush 1989, Getz and Klein 1995). Any decrease or alteration in activity of H^+-ATPase of phloem cells is associated with reduced export of sucrose into sink organs (Bürkle et al. 1998, Zhao et al. 2000). It seems most probable, therefore, that a fall in concentration of Mg–ATP at the phloem-loading sites is the major reason for inhibited sucrose transport from the Mg-deficient source leaves. In Mg-deficient sugar beet leaves with high accumulation of sugars, an induced expression of *BvSUT1* has been observed that is a sugar beet phloem-specific proton-sucrose symporter located in companion cells of the vascular system (Vaughn et al. 2002). According to Hermans et al. (2005), enhanced expression of the *BvSUT1* gene under Mg deficiency is a reflection of a defect in the phloem loading of sucrose. It would be interesting in future studies to investigate in more detail the expression level of this gene at different Mg concentrations.

Structural damage and destabilization in phloem tissue under Mg deficiency might be an additional factor involved in reduced phloem loading of sucrose under Mg deficiency (Hannick et al. 1993). Recently, Boxler-Baldoma et al. (2006) showed that Mg deficiency in Norway spruce needles induces structural damage in the vascular tissues under high-light conditions that results in loss of functional integrity of phloem cells. Because the inhibition of phloem export of sucrose by Mg deficiency occurs in the early stages of Mg deficiency stress (e.g. before development of leaf chlorosis or reductions in growth) in annual plants, degeneration of phloem tissue and related inhibition of sucrose transport might be important in later stages of Mg deficiency stress.

Photooxidation and leaf chlorosis

Obviously, impairments in photosynthetic CO_2 fixation and massive accumulation of photoassimilates in Mg-deficient leaves may decrease the use of absorbed light energy in photosynthesis, which may induce over-reduction of the photosynthetic electron transport chain. Additionally, under given conditions, chloroplasts of the Mg-deficient leaves might be exposed to an excessive light energy that is not used in CO_2 fixation. A similar situation also occurs in plants grown under different environmental stress conditions, such as chilling (Wise 1995, Zhou et al. 2007), drought (Biehler and Fock 1996) and excess light (Foyer et al. 1994, Marschner and Cakmak 1989). Under such conditions, flow of photosynthetic reducing equivalents to molecular oxygen (instead of CO_2) is expected to be intensified with a concomitant generation of ROS such as superoxide radicals ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\cdot}$) (Fig. 4). Additionally, as a result of limited

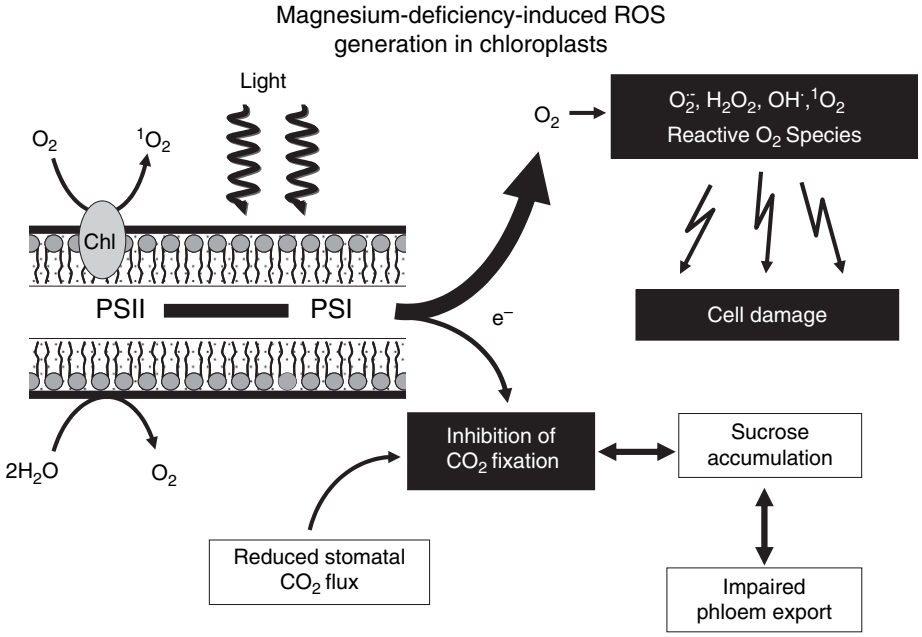

Fig. 4. Schematic representation of Mg-deficiency-induced generation of ROS in chloroplasts.

conversion of absorbed light energy into chemical energy (ATP), the excitation energy may be transferred to O_2, resulting in the generation of highly toxic singlet oxygen ($^1O_2$) in the PSII reaction centre (Asada 2006). When not removed effectively, ROS are extremely toxic to chloroplast constituents and photosynthetic enzymes, leading to inhibition of photosynthesis and induction of leaf chlorosis and necrosis (Asada 1999, Foyer and Noctor 2000). Chloroplasts are well equipped with different antioxidative defence systems to remove ROS and minimize their peroxidative attack [e.g. ascorbic acid, ascorbate-dependent H_2O_2-scavenging enzymes (e.g. ascorbate peroxidase and glutathione reductase) and superoxide dismutase] (Asada 1999, Foyer et al. 1994).

As illustrated in Fig. 4, also in Mg-deficient leaves, inhibition in utilization of absorbed light energy in CO_2 fixation may intensify the flow of light energy and reducing equivalents to molecular O_2 to form ROS. Production of ROS under Mg deficiency is expected to occur at much higher rates when plants are exposed to high light intensity, leading to photooxidative damage. In accordance with this suggestion, it was shown that Mg-deficient plants are extremely sensitive to high light intensity: exposure of Mg-deficient plants to high light intensity (480 μmol photons m^{-2} s^{-1}) rapidly induced leaf chlorosis, whereas Mg-deficient plants grown under low light intensity (80 μmol photons m^{-2} s^{-1}) or Mg-adequate plants grown at high light intensity did not develop leaf chlorosis (Fig. 5A, Marschner and Cakmak 1989). Shading only one of the primary leaves (light intensity 120 μmol photons m^{-2} s^{-1}) of Mg-deficient plants during the growth period prevented development of chlorosis, whereas the non-shaded primary leaf (light intensity exposed 480 μmol photons m^{-2} s^{-1}) developed very severe chlorosis (Fig. 5B).

The enhancing effects of high light on the chlorosis in Mg-deficient plants were not caused by lower Mg concentrations in those leaves exposed to high light (Marschner and Cakmak 1989). A most convincing demonstration for the high light sensitivity of Mg-deficient leaves was shown by partial shading individual leaf blades (Fig. 5C). In the Mg-deficient plants exposed to high light intensity, partial shading of the leaf blade either prevented or effectively delayed development of leaf chlorosis and necrosis, although Mg concentrations in different parts of the leaf blade were not significantly affected by shading (Marschner and Cakmak 1989). These observations clearly indicate a direct involvement of photooxidative destruction of Chl and membrane lipids in development of Mg deficiency symptoms on leaves. Oxidative damage in chloroplasts caused by Mg deficiency may also involve a programmed cell damage (Foyer and Noctor 2005) that needs further investigation. Over-reduction of the photosynthetic electron transport chain and associated decreases in consumption of electrons in CO_2 fixation were also shown in a nutrient-deficient transgenic tobacco plant overexpressing gene delaying leaf senescence (Wingler et al. 2005). According to Wingler et al. (2005), a full nutrient deficiency induces over-reduction of the electron transport chain because of

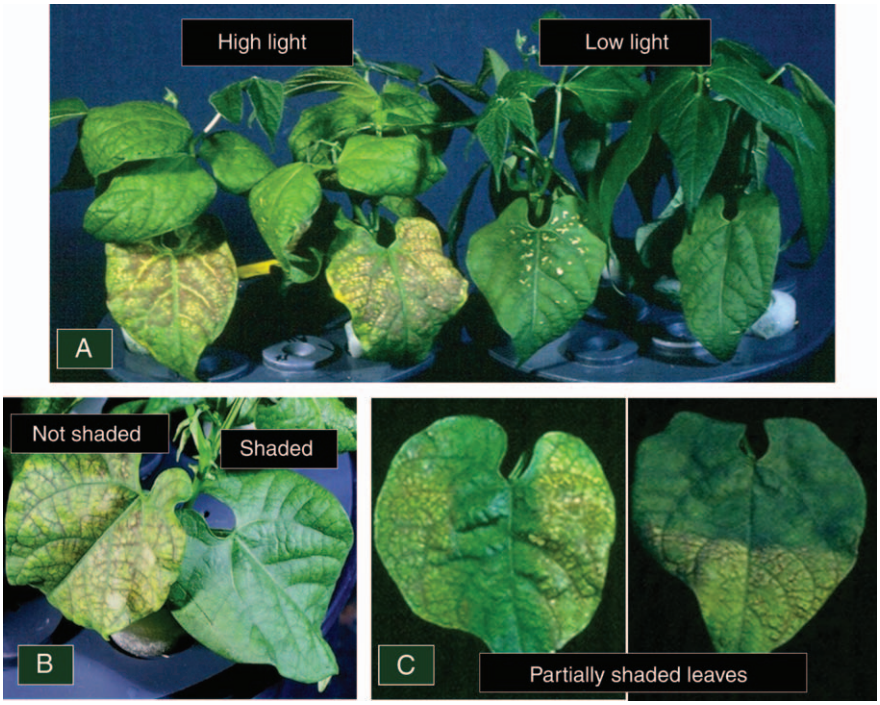

Fig. 5. Development of chlorosis and necrosis on leaves of Mg-deficient bean plants grown at high (480 μmol photons m^{-2} s^{-1}) and low (80 μmol photons m^{-2} s^{-1}) light intensities in nutrient solution (A), differential expression of leaf chlorosis and necrosis on primary leaves not shaded (480 μmol photons m^{-2} s^{-1}) and shaded (l20 μmol photons m^{-2} s^{-1}) completely by filter paper (B) and effect of partial shading of primary leaves on development of leaf chlorosis and necrosis (C). Light intensity was nearly 120 μmol photons m^{-2} s^{-1} on the shaded parts and 480 μmol photons m^{-2} s^{-1} on the non-shaded parts of leaf blades (for further details, see Marschner and Cakmak 1989).

impaired consumption of the reducing equivalents in CO_2 fixation that potentiates generation of ROS and related photooxidative damage to chloroplasts. Increase in severity of leaf chlorosis as a consequence of carbohydrate accumulation (especially starch) has also been shown in Arabidopsis plants infected with mosaic virus (Handford and Carr 2007). Virus-infected plants accumulated abnormal amounts of starch and developed severe leaf chlorosis, especially under continuous light. Recently, a recessive maize mutant has been characterized, the so-called tie-dyed, which develops variegated yellow and green sectors on leaves (Braun et al. 2006). As found in Mg-deficient source leaves, the yellow sectors accumulated excessive amount of sugars (up to 16-fold higher than the green parts) and were not able to export sugars. Following accumulation of sugars, leaf chlorosis appeared, and all these changes were highly light dependent. Chlorotic parts on leaves developed only under high-light conditions (Braun et al. 2006). In this context, it would be of interest to study the distribution of minerals elements between green and yellow sectors on maize leaves (especially Mg and K).

Potassium-, boron- and Zn-deficient plants are also highly light sensitive (Cakmak 2005, Cakmak and Römheld 1997, Marschner and Cakmak 1989), but high light intensity does not induce leaf chlorosis in P-deficient plants (Cakmak 1994). Generally, in contrast to Zn-, Mg- or K-deficient plants, development of leaf chlorosis is not a characteristic of P-deficient plants, indicating that there may be a linkage between sugar accumulation in leaves and induction of leaf chlorosis, especially under high light intensity as shown in K-, Mg- or Zn-deficient plants (Cakmak 1994, Marschner and Cakmak 1989).

Antioxidative defence systems

Besides increases in accumulation of carbohydrates, plants respond to Mg deficiency by substantial increases in levels of antioxidants and activities of antioxidative defence enzymes. A particularly high increase was found in the case of reduced ascorbic acid under Mg deficiency stress (Table 1). Ascorbic acid together with glutathione is used extensively in ascorbate-dependent H_2O_2 scavenging systems and is required in non-enzymatic detoxification of ROS (Foyer and Noctor 2005). Magnesium-deficiency-induced increases in ascorbic acid concentration were already evident in plants with slight decreases in Chl concentrations (Table 1). The

Table 1. Effect of increasing supply of Mg on concentrations of Chl, reduced ascorbic acid (AsA) and SH-containing compounds in primary leaves of 12-day-old bean plants grown at 580 μmol m^{-2} s^{-1} in nutrient solution (Cakmak and Marschner 1992a). DW, dry weight.

Mg supply (μ*M*)	Chl (mg g^{-1} DW)	AsA (μmol g^{-1} FW)	SH compounds (μmol g^{-1} FW)
10	3.8 ± 0.3	6.0 ± 0.2	2.4 ± 0.2
20	5.3 ± 0.8	6.2 ± 0.9	2.3 ± 0.4
50	7.4 ± 1.0	4.8 ± 0.5	1.4 ± 0.0
100	10.8 ± 0.2	1.8 ± 0.5	0.7 ± 0.1
1000	11.3 ± 0.5	0.9 ± 0.5	0.6 ± 0.1

amount of non-protein SH compounds (predominantly glutathione) was also significantly affected by Mg deficiency and showed progressive increases with decreasing Mg supply (Table 1). These increases in the antioxidative capacity by Mg deficiency were particularly pronounced under high light intensity (Cakmak and Marschner 1992a). Similar to the behaviour of antioxidants, the activities of antioxidative enzymes, such as ascorbate peroxidase and glutathione reductase, rise with increasing Mg deficiency stress, especially under high light intensity (Cakmak and Marschner 1992a). Increases in antioxidative defence systems by Mg deficiency were also reported by Candan and Tarhan (2003) in *Mentha*, Riga et al. (2005) in pepper and Tewari et al. (2006) in mulberry plants. Changes in the level of some antioxidative systems have been considered suitable biochemical markers for determination of critical Mg concentrations in leaves (Riga et al. 2005). Cakmak and Marschner (1992a) indicated that increases in antioxidative capacity in Mg-deficient leaves begin at an early stage of Mg deficiency and therefore can be considered one of the first physiological responses of plants to Mg deficiency.

As found for carbohydrate accumulation, the rise in the activities of antioxidative defence enzymes in Mg-deficient leaves occurs before any decrease in plant growth and Chl concentrations could be detected. Such an early increase in antioxidative capacity in Mg-deficient leaves might be interpreted as a rapid response of plants to repress (at least partially) ROS-mediated photooxidative damage and inactivation of photosynthetic enzymes. For example, the enzyme ribulose-1,5-biphosphate-carboxylase (RUBISCO) is very sensitive to ROS formation. Under low temperature stress and high-light conditions, enhanced production of ROS resulted in degradation of RUBISCO in the intact leaves (Nakano et al. 2006, Zhou et al. 2006). Early increases in activity of defence enzymes against ROS under Mg deficiency may also, at least in part, explain why photosynthesis is affected by Mg deficiency much later than is the accumulation of sugars in Mg-deficient leaves (Hermans et al. 2004, Peaslee and Moss 1966). In accordance with these suggestions, Mg-deficient leaves with elevated levels of antioxidants showed a higher tolerance to herbicide paraquat (Cakmak and Marschner 1992b), which induces light-driven generation of ROS and results in damage to chloroplasts (Halliwell 2006). In agreement with early increases in antioxidative capacity, it has been found that tolerance to paraquat toxicity begins at early stages of Mg deficiency stress (Cakmak and Marschner 1992b).

Conclusions

This study shows that Mg plays key roles in (1) partitioning of carbohydrates and dry matter production between roots and shoots; (2) photosynthetic CO_2 fixation and (3) ROS formation and related photooxidative damage (Cakmak 1994, Cakmak et al. 1994a, 1994b, Hermans et al. 2004, 2005, 2006). As illustrated in Fig. 6, these effects of Mg have several ecological and physiological consequences for growth and yield formation. Impairments in maintenance of phloem transport of sugars into the sink organs (e.g. roots and seed) under Mg deficiency may affect the size and number of sink organs. Previously, it has been reported that Mg deficiency decreases carbohydrate concentration in the sink organs such as in pods and tubers (Werner 1959; Fischer and Bussler 1988). Similarly, single grain weight and number of grains per ear were also found to be reduced by Mg deficiency (Beringer and Forster 1981). Impairments in root growth and related decline in root surface may have a very serious impact on the acquisition of mineral nutrients and uptake of water by roots (Fig. 6), especially under water-limited and nutrient-deficient soil conditions. Use efficiency of mineral fertilizers applied to soils can also be reduced by Mg deficiency stress. Because of its fundamental role in phloem export of carbohydrates, nutritional status of plants with Mg is of critical importance during the reproductive growth stage of plants. Sufficiently high amounts of Mg are needed during the reproductive growth stage to maintain and maximize transport of source organs. Late foliar applications of Mg to crop plants could be, therefore, highly beneficial for maintaining and improving yield formation, especially under water deficiency conditions with reduced root uptake of Mg. The marked susceptibility of Mg-deficient plants to high light intensity (Fig. 5) indicates that plants grown under long-term exposure to high-light conditions would need more Mg to avoid the risk of photooxidative damage catalysed by ROS (Figs 4–6). In conclusion, improving Mg nutritional status of plants under abiotic stress conditions (e.g. water stress, heat and high light), especially during the reproductive growth stage, seems

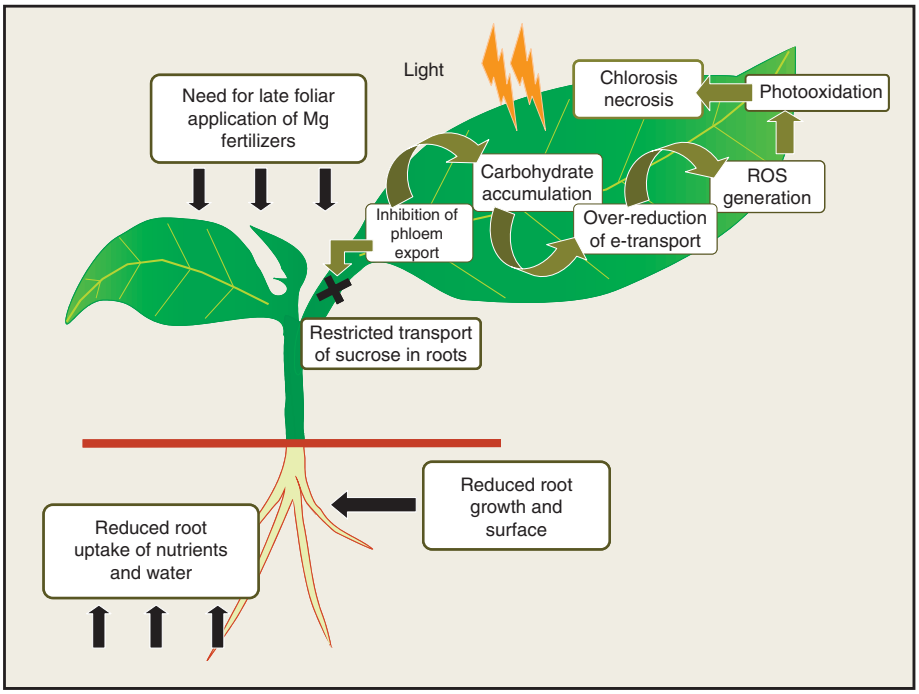

Fig. 6. Schematic presentation of changes in transport and accumulation of carbohydrates, photosynthetic electron transport, ROS generation and development of photooxidative damage in Mg-deficient leaves and their influence on root growth and root uptake of nutrients and water.

to be indispensable to alleviate detrimental effects of abiotic stress factors and to maintain high yield capacity of plants.

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