CSIRO PUBLISHING
www.publish.csiro.au/journals/fpb *Functional Plant Biology*, 2004, **31**, 529–537

Screening broad beans (*Vicia faba*) for magnesium deficiency. I. Growth characteristics, visual deficiency symptoms and plant nutritional status

Yuda Hariadi[A,B] *and Sergey Shabala*[A,C]

[A]School of Agricultural Science, University of Tasmania, Private Bag 54, Hobart, Tas. 7001, Australia.
[B]Department of Physics, University of Jember, Indonesia.
[C]Corresponding author; email: Sergey.Shabala@utas.edu.au

Abstract. We used broad beans (*Vicia faba* L.) as a case study to characterise the development of magnesium (Mg) deficiency symptoms in plants and make a comparative evaluation of the suitability of various physiological characteristics as prospective tools for early diagnosis of Mg deficiency. Growth characteristics were measured at monthly intervals from plants grown in soil solution with a wide range of Mg concentrations (from 1 to 200 ppm). The data were then correlated with plant yield responses, pigment composition and nutrient content in leaves, as well as with visual deficiency symptoms. At the age of 4 weeks, no visual symptoms of deficiency were evident even for plants grown at 1 ppm (severe Mg deficiency). Shoot growth characteristics were very similar for a wide range of treatments, although a pronounced difference in plant yield was observed at the end of the experiment. It appears that neither plant biomass nor leaf area are good indicators for use as diagnostic tools for detection of Mg deficiency in broad beans. Although pigment analysis revealed some difference between treatments, at no age was it possible to distinguish between moderately Mg-deficient (10 or 20 ppm) and sufficient (50–80 ppm) treatments. Leaf elemental analysis for Mg content remained the most sensitive and accurate indicator of Mg deficiency in broad beans. However, it is unsuitable for screening purposes as it is both costly and time-consuming. There is a need for less expensive but effective, rapid screening tools of Mg deficiency in crops at early stages of plant ontogeny.

Keywords: chlorophyll, deficiency, growth, magnesium, screening, toxicity, *Vicia faba*.

Introduction

Magnesium is a central part of the chlorophyll molecule and therefore plays an essential role in photosynthesis (Marschner 1995; Fischer 1997; Sun *et al*. 2001). In addition, many metabolic processes are directly affected by Mg availability. These include numerous enzymatic reactions of photosynthesis, cellular pH control, RNA polymerisation and ATP synthesis (Marschner 1995; Laing *et al*. 2000). As a bridging element for the aggregation of ribosome subunits, Mg affects the rate of protein biosynthesis (Marschner 1995). Magnesium also plays a key role in phloem loading and carbohydrate partitioning (Cakmak *et al*. 1994*a*, *b*).

Although Mg content varies between species, it is generally accepted that the Mg requirement for optimal plant growth is in the range of 0.1–0.4% of the dry weight of vegetative tissues (Hailes *et al*. 1997; Reuter and Robinson 1997). Under severe deficiency conditions, photosynthesis is impaired (see above), and plant yield is markedly reduced.

In many soils of temperate regions, the magnesium concentration of soil solution is typically between 5 and 50 ppm (Tisdale *et al*. 1993). However, many crops (including pasture, maize, potato, cotton, tobacco, sugar beet) and tree species can exhibit Mg deficiency symptoms under certain environmental conditions even at these levels of available magnesium but are highly responsive to application of Mg (Tan *et al*. 1991; Tisdale *et al*. 1993; Hailes *et al*. 1997; Aitken *et al*. 1999). Moreover, there are several types of soils where Mg levels are deficient for optimal growth of plants. The most obvious are acid soils. For example, the dieback of forest trees, growing on acid soils in Central Europe, was linked to magnesium deficiency (Marschner 1995; van Praag *et al.* 1997). Magnesium deficiency was recognised as a common nutrient disorder linked to ‘new type forest decline’ or ‘crown thinning’ in north-eastern North America (Landaman *et al*. 1997) and Mg supply has been shown to be a critical factor in several acid soil types in Australia, particularly on the wet tropical coast of north Queensland (Tan *et al*. 1991; Aitken *et al*. 1999). In New Zealand, Mg deficiency is an issue affecting sustainable productivity of the forest estate (Mitchell *et al*. 1999) and

Abbreviations used: CEC, cation exchange capacity; chl, chlorophyll; DW, dry weight; FW, fresh weight; LSA, leaf surface area; ppm, parts per million.

 10.1071/FP03201 1445-4408/04/050529

the most affected are forests grown on pumice, podzol and brown soils. However, Mg deficiency is not restricted to acid soils. Symptoms of Mg deficiency may also be associated with soils of sandy texture (Hailes *et al*. 1997), highly leached soils with low cation exchange capacity (CEC) or calcareous soils with inherently low Mg levels (Tisdale *et al*. 1993). Also, the availability of Mg for plants can be significantly reduced by high rates of NH_4^+ and K^+ fertilisers applied (Tisdale *et al*. 1993; Mitchell *et al*. 1999).

The most obvious symptom of severe Mg deficiency in many species is interveinal chlorosis of fully expanded leaves (Tan *et al*. 1991; Cakmak *et al*. 1994*a*; Cakmak 1994; Marschner 1995; Fischer *et al*. 1998; Hermans *et al*. 2001). The best studied are conifers, where the upper mid-crown yellowing and dieback are very pronounced under conditions of Mg deficiency (Mehne-Jakobs 1995, 1996; Kölling *et al*. 1997; Sun and Payn 1999; Laing *et al*. 2000; Sun *et al*. 2001). However, in many crops only very severe Mg deficiency may be diagnosed by visual assessment (Masoni *et al*. 1996; Fischer *et al*. 1998; Hermans *et al*. 2001). In the case of a moderate deficiency, such diagnostics often become possible only at later stages of plant ontogeny.

Despite this wide recognition of Mg deficiency in several soil types, there has been little documentation of the relationship between Mg concentration in the soil solution, plant growth and yield responses, and kinetics of development of deficiency symptoms in crops. Most of the literature deals with forest tree-species. Only a handful of crops have been characterised and most of these papers address very severe cases of Mg deficiency (Bottrill *et al*. 1970; Cakmak *et al*. 1994*a*, *b*; Fischer *et al*. 1998). Meanwhile, in many crops, visual symptoms of Mg deficiency are either virtually absent, or become obvious only at later stages of plant development. One of these species is broad bean (*Vicia faba*). In this investigation, broad beans were used as a case study to characterise the development of magnesium deficiency symptoms in plants and make a comparative evaluation of the suitability of various physiological characteristics as prospective tools for early diagnostics of Mg deficiency. Growth characteristics were measured at regular intervals from plants grown in a range of Mg concentrations from 1 ppm (severely deficient) to 200 ppm (excessively deficient; Tisdale *et al*. 1993). This data was then correlated with plant yield responses, pigment composition and nutrient content in leaves as well as with visual deficiency symptoms.

Materials and methods

Plant material and growth conditions

Broad beans (*Vicia faba* L cv. Coles Dwarf, Cresswell's Seeds, New Norfolk, Tas.) were grown from seeds in approximately 4.5 L pots in a 70:30% (v/v) sterilised sand:perlite mix in a glasshouse at 22–25°C under natural irradiance. The daylength was extended to 16 h by incandescent lamps.

An automatic hydroponic flood system was used in this experiment for water and nutrient supply. Each pot was placed in an open-top cylindrical container connected to reservoir tanks by tubing. The reservoir tanks were placed on a computer-driven movable platform. During the experiment, the growth container was periodically flooded (from the base) with nutrient solution from the reservoir tank for 15 min and then drained back into the reservoir tank. Throughout the experiment, pots were sub-surface irrigated five times per day with a full strength, modified Hoagland solution containing (in mol m^{-3}): 5.0 $Ca(NO_3)_2$ $4H_2O$, 5.0 KNO_3, 1.0 KH_2PO_4, 1.0 K_2SO4 and 1.0 $(NH_4)_2SO_4$ plus micronutrients. Magnesium was added as $MgCl_2$ to give the required concentration. Six different magnesium treatments were used: 1, 10, 20, 50 (control), 80 and 200 mg L^{-1} (or ppm), which equals 0.04, 0.4, 0.8, 2.0, 3.3 and 8.2 mol m^{-3}, respectively. Treatments were labelled Mg1, Mg10, Mg20, Mg50, Mg80 and Mg200. Our preference for ppm rather than SI units is explained by an attempt to make our results comparable with the bulk of data in the literature (Tisdale *et al*. 1993). Reservoir tanks were refilled with fresh solutions every few days, at which time solution pH was also checked and adjusted when required. Once per month, solutions were completely renewed. The glasshouse trial was in a completely randomised block design with seven replications per treatment, two plants per replication. The experiment was repeated three times (spring 2000, autumn 2001 and spring 2002).

Sampling

Plant fresh (FW) and dry (DW) weight and leaf surface area were measured fortnightly. At weeks 4, 8 and 12 of the experiment, leaf samples were taken from the fifth fully expanded mature leaf selected from the main stem and used for chlorophyll and nutrient content analysis. The total plant seed yield, average bean weight and number of pods and bean seeds per plant were measured at the end of the experiment for each treatment.

Nutrient content analysis

Leaf elemental content (K, Mg and Ca) was analysed essentially as described by Walinga *et al*. (1995). Leaf samples were placed in paper bags and dried in an oven at 60°C for 48 h. About 100 mg of dried leaves from each treatment were ground and placed in a digestion tube. After adding 5 mL of digestion acid [5:1 HNO_3 (16 N):70% $HClO_4$], the tubes were placed in an aluminium block and left at room temperature overnight. The digestion was completed by heating tubes on a hot plate (180–200°C) for approximately 3.5 h, until the liquid residue became clear. Nitric acid (1–2 mL) was added if incomplete digestion occurred. The tube was made up to 10 mL with distilled water, and 5 mL of the top of the liquid were then transferred and diluted to 12.5 mL with 0.25 N HCl.

For Ca analysis, 0.8 mL of releasing agent [0.4% lanthanum (La) in H_2 SO_4] was added to 6 mL of leaf extract and a final volume of the sample was made up to 8 mL by adding distilled water. For Mg analysis, a final volume was made by mixing 1 mL of leaf extract with 1 mL of releasing agent followed by dilution to 10 mL with distilled water. The Ca and Mg content of leaf tissue were determined by double beam atomic absorption spectrometer (GBC902, GBC Instruments, Dandenong, Vic.). Potassium content was measured by flame photometer (Evans Electroselenium Ltd, Halstead, UK). Measurements were based on six to eight replicates.

Chlorophyll content

Twelve discs were collected from each leaf with a cylindrical 4-mm sample punch; areas with major veins were avoided in the sampling. The sample (approximately 130 mg in total) was placed into a 15 mL tube containing 10 mL of 96% methanol and a few crystals of $MgCO_3$. The sealed tubes were stored in the dark at 4°C for 2 d for extraction. For each treatment, six to eight replicates were taken. Optical densities (OD) at the wavelengths 649 and 665 nm were measured using a

Lambda 20 UV/Vis Spectrophotometer (Perkin Elmer, Melbourne, Vic.), 96% of methanol was used as a blank control. The amount of chlorophyll *a* and *b* (mg L^{-1}) in the extract was calculated using the following equations (Smethurst and Shabala 2003):

$$\text{chl } a \text{ (mg l}^{-1}) = 13.7\ OD_{665} - 5.76\ OD_{649} \quad (1)$$

$$\text{chl } b \text{ (mg l}^{-1}) = 25.8\ OD_{649} - 7.6\ OD_{665} \quad (2)$$

Statistics

One-way analysis of variance was used to establish a significant difference at $P \leq 0.05$ level between treatment means.

Results

Bean yield responses to magnesium treatment showed a classical dose–response curve, with several clearly defined regions (Fig. 1): (i) severe Mg deficiency (Mg1 treatment), with a seed yield only 5% of maximal (Mg50), (ii) moderate Mg deficiency (Mg10 and Mg20), with a seed yield < 80% of maximal, (iii) adequate Mg supply (optimal treatments Mg50 and Mg80) and (iv) excessive Mg supply (toxicity range; Mg200).

The average bean weight was not significantly different between treatments except for Mg1 (approximately 30% of the control, Mg50). Therefore, for most treatments higher plant yield was achieved simply by a larger number of pods per plant.

Despite the pronounced differences in plant yield, shoot growth was very similar in the range 10–200 ppm of Mg treatment. No significant difference in plant fresh weight was found at any time between those treatments (Fig. 2*A*). Mg1 treatment resulted in 50% reduction in plant fresh weight (FW). Statistically significant (P=0.05) differences between Mg1 and other treatments were found from week 8.

Leaf surface area followed essentially the same trend as plant biomass (Fig. 2*B*). There was a progressive increase in the total leaf surface area (LSA) for all 'optimal treatments' (above Mg20). The most severe magnesium deficiency (Mg1) resulted in early plateauing of LSA between weeks 4 and 8. Then, after week 8, Mg1 plants resumed leaf growth at the same rate as Mg-sufficient plants.

At the age of 4 weeks, no visual symptoms of deficiency were evident even for Mg1 leaves other than smaller leaf sizes (Fig. 3). At later stages (8 or 12 weeks) Mg1 treatment resulted in pronounced interveinal chlorosis of mature leaves (Fig. 4). However, no visual symptoms of Mg deficiency were found in leaves of any other treatment in the range of Mg concentrations from 10 to 200 ppm. Even by the end of experiments, when plants were 12 weeks old, the only obvious difference between Mg10 and Mg50 plants was the leaf size (Fig. 4).

Pigment analysis revealed a greater difference between treatments. A statistically significant (P=0.05) decrease in chl *a* content was found in Mg1 leaves at week 4 (Table 1). This difference was further enhanced during the second month of growth, when most plants (except Mg1, which showed a marked reduction) gained more chl *a*. During the third month, chl *a* content in most leaves decreased, probably due to plant ageing. By the end of the experiment, Mg1 plants had only half of the chl *a* of Mg50 and Mg80

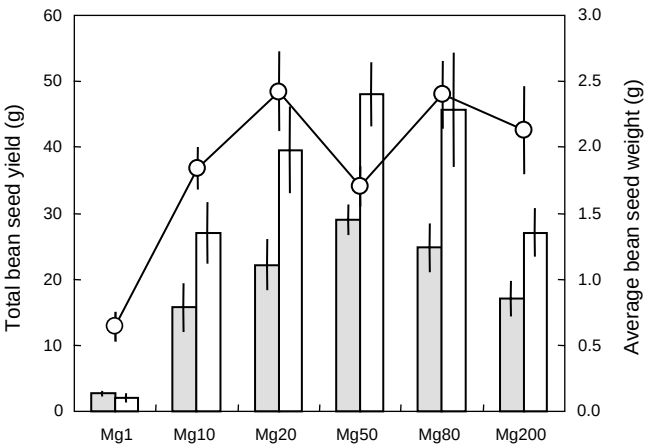

Fig. 1. Effect of Mg concentration on plant yield. Total bean seed yield (open bars), number of beans per plant (closed bars) and average bean weight (○) are plotted against Mg concentration in nutrient solution (in ppm). Error bars indicate SE (n = 14).

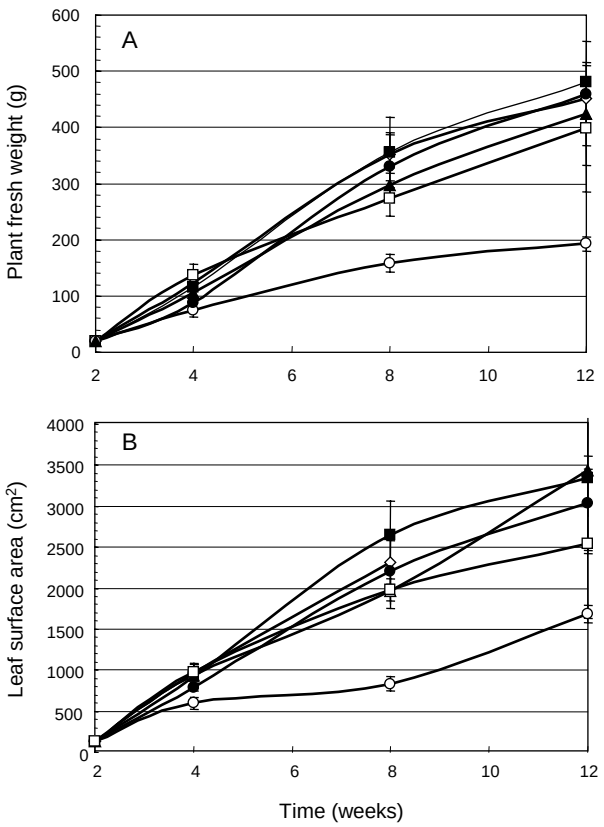

Fig. 2. Effect of Mg concentration (○, Mg1; ◇, Mg10; □, Mg20; ■, Mg50; ●, Mg80; ▲, Mg200) on plant growth. Fresh weight of plants (*A*) and leaf surface area (*B*) are plotted against time (in weeks). Error bars indicate SE (n = 4–6).

treatments (Table 1). However, at no age was it possible to distinguish between Mg10 or Mg20 (both deficient, according to Fig. 1) and Mg-sufficient (Mg50 and Mg80) treatments. Plants in the excessive Mg treatment (Mg200) had the second lowest chl *a* content.

By the end of the experiment chl *b* content in leaves declined in the following progression:

Mg-sufficient > Mg-deficient =
Mg-excessive > severely deficient,

with an almost 4-fold difference between extreme treatments (Table 1). However, for the first 2 months, only Mg1 and Mg200 plants showed significant (P=0.05) reduction in chl *b* levels. The differences between other treatments were not statistically significant (Table 1). Similar trends were obvious for total chlorophyll content (Table 1). The chlorophyll *a* to *b* ratio was highest for extreme cases of Mg1 and Mg200 after 8 weeks of treatment. On average, for most plants chl *a*/*b* ratio was within between 3 and 4 (data not shown).

There was a significant (P=0.05) difference in Mg content and kinetics of Mg accumulation in leaves of different treatments (Table 2). In Mg50 and Mg80 treatments, Mg content had increased from approximately 0.19% in week 4 to 0.4–0.55% in week 12 (per leaf DW basis). Even more dramatic was the Mg increase in leaves of the Mg200 treatment (from 0.27 to 0.77%; Table 2), whereas Mg-deficient plants had lower and much more stable levels of magnesium in leaves (between 0.07 and 0.17%).

There was also an interesting trend in kinetics of potassium accumulation in leaves. In young plants (age 4 weeks), Mg-deficient plants had much higher K content than sufficient treatments (Table 2). At 8 weeks, this difference was not observed, and by the end of the experiment Mg1 leaves had three to four times less K than all other treatments (Table 2). No apparent trends were revealed for changes in leaf Ca content except for a statistically significant (P=0.01) reduction in Mg1 treatment by the end of the experiment (Table 2).

Discussion

Mg availability and plant yield responses

Plant yield responses to Mg supply showed a classical dose-response curve, with three distinct ranges: deficiency (1–20 ppm), optimal supply (50–80 ppm), and excessive supply (200 ppm; Fig. 1). Even Mg1 plants were able to produce a few beans, although the total yield was less than 5% of control (sufficient Mg50 plants). At the same time, the overall reduction in both shoot biomass and leaf surface area was only 50% (Fig. 2). This suggests that Mg deficiency

Fig. 3. Leaves of 1-month-old broad bean plants grown in nutrient solution containing different concentrations of Mg. Fully developed mature leaf (number five) is shown. Note the absence of visual deficiency symptoms between all treatments.

becomes crucial at the reproductive stage of plant development. This is further supported by an analysis of Mg content in leaves (Table 2). At the vegetative stage of development (age 4 weeks), there was no difference between Mg20 and Mg80 treatments. However, by the end of the experiment the difference between these treatments is 3-fold (0.19 and 0.58%, respectively; Table 2).

Our data is consistent with other reports. Grundon (1987) showed that Mg-deficient soybean plants are only slightly shorter, but set much fewer pods, with fewer seeds than Mg-sufficient plants. Sunflower plants, grown at Mg0, showed a 40–50% reduction in both root and shoot biomass and leaf area compared with sufficient plants (Lasa *et al*. 2000). After 5 months of growth in Mg-deficient conditions, the height of pine trees was only 20% smaller than that of control plants (Laing *et al*. 2000). A 50% reduction in plant dry weight for 30-week-old Mg-deficient pine trees was reported by Sun *et al*. (2001). Similar values (2.5-fold decrease) were reported for Mg-deficient spinach plants (Fischer *et al*. 1998). Some reports even suggested that leaf area in Mg-deficient plants is not significantly different from the control (Troyanos *et al*. 1997).

From these data, it appears that neither plant biomass nor leaf area are good indicators for early diagnosis of Mg deficiency, especially in annual crops. Only severely deficient (Mg1) plants showed a statistically significant (P=0.05) decrease in biomass (Fig. 2*A*) or leaf area (Fig. 2*B*). Plant yield analysis appears to be the most accurate test in this study (Fig. 1). However, it appears that this can only be used for monitoring purposes, when the nutritional status of crops in successive years is used to adjust fertiliser use according to revealed trends (Reuter and Robinson 1997). Obviously, this might be applicable to a range of perennial plants, but not broad beans or other annual crops with a similar type of response to Mg.

Applicability of visual assessment to screen broad beans for Mg deficiency

The early diagnostics of mineral deficiencies is an important issue, as it helps to prevent any irreversible loss of crop yield and decrease of crop quality (Hermans *et al*. 2001). Undoubtedly, among many other methods, visual assessment of nutrient deficiency in plants is the most popular among plant growers (Grundon 1987; Marschner 1995; Reuter and Robinson 1997). It is a quick, labour- and money-saving method. Moreover, it is completely independent of analytical bases.

The most obvious visual symptom of Mg deficiency is interveinal chlorosis of fully developed leaves (Tan *et al*. 1991; Cakmak 1994; Cakmak *et al*. 1994*a*, *b*; Broschat 1997; Fischer *et al*. 1998; Lavon and Goldschmidt 1999; Papenbrock *et al*. 2000). In addition, under very severe Mg deficiency, brown and purple lesions and red suffusions at the tips and edges of old leaves become obvious

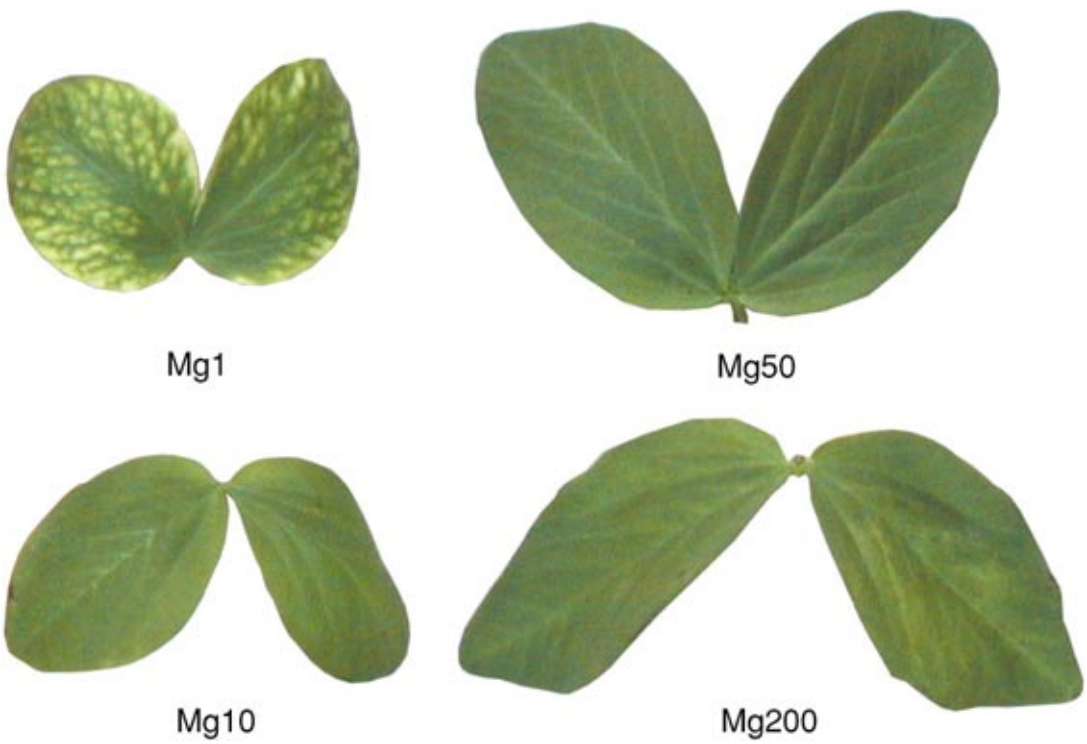

Fig. 4. Visual symptoms of Mg deficiency in 3-month-old broad bean plants. Note the difference in leaf size between different treatments.

(Grundon 1987; Tan *et al*. 1991; Bennett 1993). Magnesium deficiency symptoms are especially pronounced in conifers, where upper mid-crown yellowing occurs (Mehne-Jakobs 1995; Kölling *et al*. 1997; Sun and Payn 1999; Mitchell *et al*. 1999; Laing *et al*. 2000; Sun *et al*. 2001). At the same time, current-year needles usually remain green (Mehne-Jakobs 1995), which could be explained by the high mobility of Mg in the phloem (Marschner 1995).

In broad beans, in addition to obvious interveinal chlorosis, there was a significant reduction in leaf size (Mg1 and Mg10 *v.* Mg50; Fig. 4). However, these symptoms became evident only at the later stages of plant development (at the age of 3 months). One month after the start of the experiment, only severely deficient leaves (Mg1) showed some signs of chlorosis (Fig. 3) while deficient Mg10 and Mg20 treatments were not distinguishable from control (Mg50; Fig. 3). By the time the visual diagnosis of Mg deficiency in broad beans became possible, most of the pods were already set, and the yield was pre-determined. Therefore, visual assessment of deficiency symptoms is not an efficient tool for diagnosis of Mg deficiency in broad beans.

Changes in leaf pigment composition

It is believed that chlorotic appearance of Mg-deficient leaves is associated with chlorophyll destruction due to photo-oxidation (Cakmak 1994). From our data, changes in leaf pigment composition were more accurate indicators of plant Mg status than growth characteristics (Table 1). There was a statistically significant difference in the level of chl *a* between Mg1 and Mg50 leaves as early as 4 weeks after the experiment commenced (Table 1). It should be noted that no visual symptoms of deficiency were observed at that time (Fig. 3).

Significant decrease in chlorophyll level in Mg-deficient leaves has been widely reported (Bottrill *et al*. 1970; Cakmak 1994; Marschner 1995: Laing *et al*. 2000). Severe cases of Mg deficiency have caused a 40–50% reduction in chlorophyll content of bean leaves (Fischer 1997). Similar results were reported for a range of tropical fruit (Balakrishnan *et al*. 2000) and cereal (Masoni *et al*. 1996) crops. This is consistent with our observations that by the end of the experiment, severely deficient Mg1 plants had half the chl *a* compared with magnesium-sufficient Mg50 and Mg80 treatments (Table 1).

There have been some controversial reports showing that the leaf pigment composition remained unchanged under Mg deficiency conditions (Fischer *et al*. 1998). In our experiments, at no age was it possible to distinguish between deficient (Mg10 or Mg20) and optimal Mg50 treatments (Figs 3, 4). It appears that only extreme cases of Mg deficiency cause significant reduction in leaf pigment composition (and associated leaf chlorosis), while under moderate Mg deficiency these symptoms are less detectable. Therefore, it is unlikely that leaf pigment analysis might be used as a diagnostic tool for early assessment of Mg deficiency in broad beans.

Table 1. Effect of magnesium concentration (in the nutrient solution) on pigment content in bean leaves
Data are average ± SE (n = 4–6). Level of significance compared with control (Mg50) is indicated as follows: *, P=0.05; **, P=0.01; ***, P=0.001

Treatment	Plant age (weeks) 4	8	12
Chlorophyll *a* (mg g^{-1} FW)			
Mg1	0.147 ± 0.023*	0.094 ± 0.008***	0.095 ± 0.010***
Mg10	0.189 ± 0.042	0.256 ± 0.011	0.169 ± 0.026
Mg20	0.178 ± 0.008*	0.288 ± 0.011	0.171 ± 0.032
Mg50	0.209 ± 0.005	0.271 ± 0.008	0.196 ± 0.025
Mg80	0.190 ± 0.008	0.261 ± 0.025	0.228 ± 0.010*
Mg200	0.161 ± 0.012*	0.202 ± 0.017**	0.184 ± 0.019
Chlorophyll *b* (mg g^{-i} FW)			
Mg1	0.045 ± 0.007*	0.024 ± 0.002***	0.034 ± 0.003*
Mg10	0.065 ± 0.017	0.102 ± 0.007	0.072 ± 0.013
Mg20	0.054 ± 0.004*	0.084 ± 0.006	0.095 ± 0.035
Mg50	0.068 ± 0.003	0.079 ± 0.007	0.112 ± 0.027
Mg80	0.059 ± 0.005	0.081 ± 0.010	0.128 ± 0.018
Mg200	0.048 ± 0.005*	0.053 ± 0.006*	0.085 ± 0.015
Total chlorophyll (mg g^{-1} FW)			
Mg1	0.192 ± 0.024*	0.119 ± 0.008***	0.129 ± 0.011**
Mg10	0.255 ± 0.045	0.358 ± 0.013	0.240 ± 0.029
Mg20	0.232 ± 0.009**	0.372 ± 0.013	0.266 ± 0.048
Mg50	0.277 ± 0.006	0.350 ± 0.010	0.308 ± 0.036
Mg80	0.249 ± 0.010	0.342 ± 0.027	0.356 ± 0.021
Mg200	0.209 ± 0.013*	0.256 ± 0.018**	0.269 ± 0.025

Leaf elemental analysis

Magnesium content in most plants is within the range from 0.1 to 0.4% (per shoot DW basis; Hailes *et al*. 1997; Troyanos *et al*. 1997; Sun and Payn 1999; Barta and Tibbitts 2000; Laing *et al*. 2000). Our data for broad beans are consistent with these observations (Table 2). However, it is apparent that plant Mg demands and accumulation of Mg in leaves are highly variable and may change by a factor of two to three depending on plant age. The most obvious difference between sufficient (Mg50 and Mg80) and deficient (Mg1, Mg10 and Mg20) plants was the ability to progressively increase Mg level in leaves in plant ontogeny. It further suggests that Mg might play a crucial role at later (reproductive) stages of plant development. Such a trend might be caused by an increased number of sinks resulting in higher demands for photoassimilates.

We found that leaf elemental analysis for Mg content remains the most sensitive and accurate indicator of Mg deficiency in broad beans. As early as at the age of 4 weeks, when no visual symptoms are obvious, (Fig. 3), Mg content in deficient leaves (Mg1 and Mg10) was significantly (P=0.01) lower than in sufficient plants (Mg 50 and Mg80). At the age of 8 weeks, the difference between Mg20 and Mg50 became statistically significant (P=0.001). Therefore, assessment of leaf Mg content could be used as a tool for early diagnostics of Mg deficiency in broad beans.

There is also an apparent relationship between Mg availability and accumulation of other mineral nutrients in bean leaves (Table 2). The most pronounced was the interaction between Mg and K. Increased availability of Mg at early stages of plant growth (age 4 weeks) caused a significant decline in leaf K content (Table 2). This is a classical example of a phenomenon known as the secondary induced deficiency (Marschner 1995). As plant demand grew (age 8 weeks), there was no significant difference in leaf K content between treatments. In old plants (age 12 weeks), severe Mg deficiency (Mg1) significantly reduced leaf K (Table 2). This is consistent with reports in the literature (Masoni *et al*. 1996; Lavon and Goldschmidt 1999).

Surprisingly, no significant difference was found in leaf Ca content between Mg-deficient and -sufficient plants, except for a reduction in Mg1 treatment by the end of the experiment (Table 2). This might be explained by the different mobilities of Mg (high) and Ca (extremely low) in the phloem (Marschner 1995). As a result, there was progressive accumulation of Ca in mature bean leaves (the ones which were analysed), while leaf Mg content might undergo significant variations as a result of the changing sink demands.

Conclusions

It is obvious that plant responses to Mg deficiency are highly species-specific. In the case of broad beans, our data suggest

Table 2. Elemental content (Mg, K, and Ca) of 5th leaf of broad bean plants grown under different Mg supply.

Data are average ± SE (n = 8–12). Level of significance compared with control (Mg50) is indicated as follows: *, P=0.05; **, P=0.01; ***, P=0.001

Treatment	Plant age (weeks) 4	8	12
Magnesium (% DW)			
Mg1	0.09 ± 0.002***	0.07 ± 0.003***	0.07 ± 0.003**
Mg10	0.09 ± 0.004***	0.11 ± 0.006***	0.12 ± 0.010*
Mg20	0.18 ± 0.017	0.17 ± 0.006***	0.19 ± 0.004
Mg50	0.19 ± 0.009	0.27 ± 0.012	0.39 ± 0.086
Mg80	0.19 ± 0.009	0.32 ± 0.010*	0.58 ± 0.008
Mg200	0.27 ± 0.006***	0.61 ± 0.035***	0.77 ± 0.044**
Potassium (% DW)			
Mg1	2.53 ± 0.43	4.14 ± 0.14*	1.05 ± 0.04***
Mg10	2.76 ± 0.04***	4.01 ± 0.07	3.39 ± 0.20
Mg20	2.19 ± 0.06**	3.51 ± 0.07	2.57 ± 0.09**
Mg50	1.54 ± 0.12	3.53 ± 0.20	3.37 ± 0.09
Mg80	1.02 ± 0.09*	3.51 ± 0.07	3.33 ± 0.18
Mg200	0.67 ± 0.29*	3.86 ± 0.07	4.20 ± 0.15**
Calcium (% DW)			
Mg1	0.61 ± 0.05	0.73 ± 0.03	0.33 ± 0.01**
Mg10	0.64 ± 0.01**	0.79 ± 0.06	1.40 ± 0.01
Mg20	0.75 ± 0.04*	0.74 ± 0.05	1.27 ± 0.11
Mg50	0.59 ± 0.05	0.75 ± 0.05	1.64 ± 0.22
Mg80	0.46 ± 0.07	0.66 ± 0.04	0.55 ± 0.05**
Mg200	0.35 ± 0.02***	0.62 ± 0.04	0.95 ± 0.04*

that neither growth rate characteristics, nor visual assessment are suitable for early diagnostics of magnesium deficiency symptoms in broad beans. Pigment analysis was only marginally better as a screening tool for this species. The most reliable and sensitive of all methods used was the assessment of Mg content in bean leaves. However, this is a time consuming method that requires strong analytical support. In addition, its application as a screening tool would be a costly exercise (the average cost of one sample analysis in Australia is between AUD$40 and $70). Obviously, there is a need to continue searching for rapid screening tools to detect Mg deficiency in annual crops at early stages of plant ontogeny.

Acknowledgments

This work was supported by the DUE Scholarship from the University of Jember, Indonesia, to Yuda Hariadi. The technical assistance of Stas Shabala and Christiane Smethurst is greatly appreciated.

References

Aitken RL, Dickson T, Hailes KJ, Moody PW (1999) Response of field-grown maize to applied magnesium in acidic soils in north-eastern Australia. *Australian Journal of Agricultural Research* **50**, 191–198.

Balakrishnan K, Rajendran C, Kulandaivelu G (2000) Differential responses of iron, magnesium, and zinc deficiency on pigment composition, nutrient content, and photosynthetic activity in tropical fruit crops. *Photosynthetica* **38**, 477–479.

Barta DJ, Tibbitts TW (2000) Calcium localization and tipburn development in lettuce leaves during early enlargement. *Journal of the American Society for Horticultural Science* **125**, 294–298.

Bennett WF (1993) 'Nutrient deficiencies and toxicities in crop plants.' (APS Press: St Paul, MN)

Bottrill DE, Possingham JV, Kriedemann PE (1970) The effect of nutrient deficiencies on photosynthesis and respiration in spinach. *Plant and Soil* **32**, 424–438.

Broschat TK (1997) Nutrient distribution, dynamics, and sampling in coconut and Canary Island date plants. *Journal of the American Society for Horticultural Science* **122**, 884–890.

Cakmak I (1994) Activity of ascorbate-dependent H_2O_2-scavenging enzymes and leaf chlorosis are enhanced in magnesium- and potassium-deficient leaves, but not in phosphorus-deficient leaves. *Journal of Experimental Botany* **278**, 1259–1266.

Cakmak I, Hengeler C, Marschner H (1994*a*) Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. *Journal of Experimental Botany* **278**, 1251–1257.

Cakmak I, Hengeler C, Marschner H (1994*b*) Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phosphorus, potassium and magnesium deficiency. *Journal of Experimental Botany* **278**, 1245–1250.

Fischer ES (1997) Photosynthetic irradiance curves of *Phaseolus vulgaris* under moderate or severe magnesium deficiency. *Photosynthetica* **33**, 385–390.

Fischer ES, Lohaus G, Heineke D, Heldt HW (1998) Magnesium deficiency results in accumulation of carbohydrates and amino acids in source and sink leaves of spinach. *Physiologia Plantarum* **102**, 16–20. doi:10.1034/J.1399-3054.1998.1020103.X

Grundon NJ (1987) 'Hungry crops: a guide to nutrient deficiencies in field crops.' (Department of Primary Industries: Brisbane, Qld)

Hailes KJ, Aitken RL, Menzies NW (1997) Magnesium in tropical and subtropical soils from north-eastern Australia. II. Response by glasshouse-grown maize to applied magnesium. *Australian Journal of Soil Research* **35**, 629–641.

Hermans C, Delhaye JP, Straser RJ (2001) Magnesium deficiency in sugar beets probed by optical *in vivo* measurement. In 'Proceedings of 12th international congress on photosynthesis'. (Brisbane, Qld)

Kölling C, Pauli B, Häberle K-H, Rehfuess KE (1997) Magnesium deficiency in young Norway spruce [*Picea abies* (L.) Karst.] trees induced by NH_4NO_3 application. *Plant and Soil* **195**, 283–291. doi:10.1023/A:1004291200514

Laing W, Greer D, Sun O, Beets P, Lowe A, Payn T (2000) Physiological impacts of Mg deficiency in *Pinus radiata*: growth and photosynthesis. *New Phytologist* **146**, 47–57.

Landaman G, Hunter IR, Hendershot W (1997) Temporal and spatial development of magnesium deficiency in forest stands in Europe, North America and New Zealand. In 'Magnesium deficiency in forest ecosystems'. (Eds RF Huttl and W Schaaf) pp. 23–64. (Kluwer Academic Publisher: Dortrecht, The Netherlands)

Lasa B, Frechilla S, Aleu M, Gonzales-Moro B, Lamsfus C, Aparicio-Tejo PM (2000) Effects of low and high levels of magnesium on the response of sunflower plants grown with ammonium and nitrate. *Plant and Soil* **225**, 167–174. doi:10.1023/A:1026568329860

Lavon R, Goldschmidt EE (1999) Effect of potassium, magnesium, and calcium deficiencies on nitrogen constituents and chloroplast components in *Citrus* leaves. *Journal of the American Society for Horticultural Science* **124**, 158–162.

Marschner H (1995) 'Mineral nutrition of higher plants. 2nd edn.' (Academic Press. San Diego, CA)

Masoni A, Ercoli L, Mariotti M (1996) Spectral properties of leaves deficient in iron, sulfur, magnesium, and manganese. *Agronomy Journal* **88**, 937–943.

Mehne-Jakobs B (1995) The influence of magnesium deficiency on carbohydrate concentrations in Norway spruce (*Picea abies*) needles. *Tree Physiology* **15**, 577–584.

Mehne-Jakobs B (1996) Magnesium deficiency treatment causes reductions in photosynthesis of well-nourished Norway spruce. *Trees* **10**, 293–300. doi:10.1007/S004680050036

Mitchell AD, Loganathan P, Payn TW, Tillman RW (1999) Effect of calcinated magnesite on soil and *Pinus radiata* foliage magnesium in pumice soils of New Zealand. *Australian Journal of Soil Research* **37**, 545–560.

Papenbrock J, Pfundel E, Mock H-P, Grimm B (2000) Decreased and increased expression of the subunit CHL1 diminishes Mg chelatase activity and reduces chlorophyll synthesis in transgenic tobacco plants. *The Plant Journal* **22**, 155–164. doi:10.1046/J.1365-313X.2000.00724.X

Reuter DJ, Robinson JB (1997) 'Plant analysis: an interpretation manual. 2nd edn.' (CSIRO Publishing: Melbourne)

Smethurst CF, Shabala S (2003) Screening methods for waterlogging tolerance in Lucerne: comparative analysis of waterlogging effects on chlorophyll fluorescence, photosynthesis, biomass and chlorophyll content. *Functional Plant Biology* **30**, 335–343. doi:10.1071/FP02192

Sun OJ, Payn TW (1999) Magnesium nutrition and photosynthesis in *Pinus radiata*: clonal variation and influence of potassium. *Tree Physiology* **19**, 535–540.

Sun OJ, Gielen GJHP, Sands R, Smith CT, Thorn AJ (2001) Growth, Mg nutrition and photosynthetic activity in *Pinus radiata*: evidence that NaCl addition counteracts the impact of low Mg supply. *Trees* **15**, 335–340. doi:10.1007/S004680100111

Tan K, Keltjens WG, Findenegg R (1991) Role of magnesium in combination with liming in alleviating acid-soil stress with the aluminium-sensitive sorghum genotype CV323. 1991. *Plant and Soil* **136**, 65–71.

Tisdale SL, Nelson WL, Beaton JD, Havlin JL (1993) 'Soil fertility and fertilisers. 5th edn.' (Prentice-Hall: Upper Saddle River, NJ)

Troyanos YE, Hipps NA, Moorby J, Ridout MS (1997) The effects of external magnesium concentrations on the growth and magnesium inflow rates of micropropagated cherry rootstocks 'F.12/1' (*Prunus avium* L.) and 'Colt' (*Prunus avium* L. × *Prunus pseudocerasus* L.). *Plant and Soil* **197**, 25–33. doi:10.1023/A:1004264806741

van Praag HJ, Weissen F, Dreze P, Cogneau M (1997) Effects of aluminium on calcium and magnesium uptake and translocation by root segments of whole seedlings of Norway spruce (*Picea abies* Karst.). *Plant and Soil* **189**, 267–273. doi:10.1023/A:1004266826855

Walinga I, van der Lee JJ, Houba VJG, Novozamsky I (1995) 'Plant analysis manual.' (Kluwer Academic Publishers: Dordrecht, The Netherlands)

Manuscript received 27 October 2003, received in revised form 21 January 2004, accepted 4 February 2004