Planta (2005) 221: 56–65
DOI 10.1007/s00425-004-1425-0

ORIGINAL ARTICLE

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Effects of magnesium availability on the activity of plasma membrane ion transporters and light-induced responses from broad bean leaf mesophyll

Received: 12 September 2004 / Accepted: 14 October 2004 / Published online: 12 January 2005
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Abstract Considering the physiological significance of Mg homeostasis in plants, surprisingly little is known about the molecular and ionic mechanisms mediating Mg transport across the plasma membrane and the impact of Mg availability on transport processes at the plasmalemma. In this study, a non-invasive ion-selective microelectrode technique (MIFE) was used to characterize the effects of Mg availability on the activity of plasma membrane H^+, K^+, Ca^{2+}, and Mg^{2+} transporters in the mesophyll cells of broad bean (*Vicia faba* L.) plants. Based on the stoichiometry of ion-flux changes and results of pharmacological experiments, we suggest that at least two mechanisms are involved in Mg^{2+} uptake across the plasma membrane of bean mesophyll cells. One of them is a non-selective cation channel, also permeable to K^+ and Ca^{2+}. The other mechanism, operating at concentrations below 30 μM, was speculated to be an H^+/Mg^+ exchanger. Experiments performed on leaves grown at different levels of Mg availability (from deficient to excessive) showed that Mg availability has a significant impact on the activity of plasma-membrane transporters for Ca^{2+}, K^+, and H^+. We discuss the physiological significance of Mg-induced changes in leaf electrophysiological responses to light and the ionic mechanisms underlying this process.

Keywords Light · Magnesium · Ion transport · Plasma membrane · *Vicia faba*

Abbreviations: ROS: Reactive oxygen species · NSCC: Non-selective cation channel

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Introduction

Magnesium is an essential nutrient that plays a key role in plant photosynthesis. In addition to being a central atom of the chlorophyll molecule, Mg is essential for the functioning of many enzymes, including RNA polymerases, ATPases, protein kinases, phosphatases, glutathione synthase, and carboxylases (Marschner 1995; Shaul 2002). Insertion of Mg^{2+} into the porphyrin structure during chlorophyll formation is catalyzed by Mg^{2+}-chelatase. Many key chloroplast enzymes are strongly affected by small variations in Mg levels (Shaul 2002). Thus, Mg plays a fundamental role in both the 'light' and 'dark' reactions of photosynthesis. Both Mg deficiency and Mg oversupply have detrimental effects on plant photosynthesis. There are numerous reports that the rate of photosynthesis is severely reduced in leaves of Mg-deficient plants (Fischer 1997; Sun and Payn 1999; Ridolfi and Garrec 2000). Several factors, including structure and function of chloroplasts (Lavon and Goldsmith 1999), impaired export of carbohydrates from source to sink sites (Cakmak et al. 1994), and production of reactive oxygen species (ROS) (Cakmak 1994), have been implicated in photosynthetic inhibition under Mg-deficiency conditions. Increased concentrations of free Mg may also impair photosynthesis via multiple pathways such as inhibition of K^+ transport from the cytosol to the stroma, possible interference with Mg homeostasis inside the chloroplast, and impaired regulation of transport events across the tonoplast (Shaul 2002).

Magnesium control over membrane-transport processes in plant cells is rather complex. To date, the best studied are effects of Mg on transport activity in tonoplast and chloroplast membranes. Physiological levels of Mg significantly affect activity of both slow (SV) and fast (FV) activating vacuolar ion channels (Allen and Sanders 1997; Tikhonova et al. 1997; Pottosin et al. 1997; Pottosin and Muniz 2002), thus playing an important role in maintaining the cytosolic homeostasis

and in the regulation of cell turgor (Bruggemann et al. 1999; Pei et al. 1999; Shaul 2002). In addition to regulation of vacuolar ion channels, Mg acts as an allosteric activator of vacuolar inorganic pyrophosphatase (with at least two Mg-binding sites known; Fraichard et al. 1996) and thus can modulate the activity of both the V-ATPase and the VPPase (Shaul 2002). This is crucial for cellular pH homeostasis and turgor regulation, in both photosynthetically competent mesophyll cells and stomata guard cells. Also important is the role of Mg^{2+} in membrane-transport processes in chloroplasts, where free Mg contributes to the regulation of photosynthetic enzyme activity (Shaul 2002). Additionally, proton pumping from the stroma into thylakoids lumen results in significant (ΔpH 2–3; Remis et al. 1986) acidification of the thylakoid lumen. This massive light-driven transport of H^+ into the thylakoid lumen is electrically balanced by the counterflow of other ions (Hinnah and Wagner 1998) and, particularly, Mg^{2+} (Pottosin and Schönknecht 1996). No details of specific transporters mediating Mg^{2+} transport across the chloroplast envelope are known to date.

Even more rudimentary is our knowledge of specific molecular and ionic mechanisms mediating Mg transport across the plasma membrane. It is not currently known through which transport proteins Mg enters the root symplasm and is loaded and unloaded from the xylem and phloem (Shaul 2002). The impact of Mg availability on the activity of other plasma membrane transporters also remains to be described. Despite recent advances in molecular biology studies of Mg^{2+} transport in yeast and bacterial cells (Gardner 2003), very little is known about the specific Mg transporters at the plasma membrane in higher plant cells. The aim of this study was to characterize the effects of Mg availability on the activity of major plasma-membrane transporters (those for K^+, H^+, Ca^+) in photosynthesizing leaf tissues, and to relate Mg-induced ion-flux changes in response to light/dark fluctuations with cell photosynthetic performance and leaf expansion growth. Using the non-invasive ion-flux measuring (MIFE) technique, we were able to quantify (to our knowledge, for the first time) net Mg fluxes across the plasma membrane of leaf mesophyll cells and their responses to light fluctuations. Our data suggest that Mg availability significantly modifies transport of all major ions (H^+, Ca^{2+}, and K^+) and provides some clues about mechanisms underlying Mg^{2+} transport across the plasma membrane.

Materials and methods

Plant material

Broad beans (*Vicia faba* L cv. Coles Dwarf, Cresswell's Seeds, New Norfolk, Australia) were grown hydroponically from seeds in a 70% : 30% (v/v) sterilized sand: perlite mix in a glasshouse as described in our previous publication (Hariadi and Shabala 2004). Plants were grown at three different magnesium concentrations: deficient (1 ppm), optimal (50 ppm), and excessive (200 ppm), or 0.04, 2.0, and 8.0 mM, respectively. These are designated as Mg1, Mg50, and Mg200, respectively. For the yield data, see Hariadi and Shabala (2004). Our preference for parts per million rather than SI units is explained by an attempt to make our results comparable with the bulk of literature data (Tisdale et al. 1993). Plants were used for measurements after 4 weeks. Four hours before measurements, the fifth leaf was excised, transferred to the laboratory, and kept at room temperature (22°C) under 0.45 W m^{-2} illumination (Sylvania TLD 58 W cool fluorescence tubes, Australia), with the petiole immersed in distilled water. All measurements were made at the same time of the day, between 2 and 6 p.m.

Non-invasive ion-flux measurements

The leaf epidermis was gently removed, and mesophyll segments of about 5 mm×7 mm in size were cut and left floating in an aerated nutrient solution (0.1 mM $CaCl_2$ + 0.2 mM KCl) essentially for about 1.5–2 h as described by Shabala et al. (2000). One hour prior to the measurements, mesophyll segments were immobilized in a Perspex holder and then placed into a measuring chamber, filled with the appropriate solution (see details below). Dim green microscope light (1 W m^{-2}) was used as background illumination during segment preparation and throughout the experiments.

Net fluxes of H^+, K^+, Ca^{2+}, and Mg^{2+} were measured using the non-invasive ion-selective vibrating microelectrode (MIFE) technique (University of Tasmania, Hobart), generally as described in our previous publications (Shabala et al. 1997; Shabala 2000; Shabala and Lew 2002). Specific details of the liquid ionic exchanges (LIX) and backfilling solutions used are given in Table 1. All LIX were purchased from Fluka. The reference electrode was a glass capillary of about 30 μm diameter, filled with 1 M KCl made up in 2% agar.

Prior to measurements, electrode tips were positioned 50 μm above the leaf surface, with their tips aligned and separated by 1–3 μm. During measurements, electrodes were moved back and forth in a square-wave manner by a computerized stepper motor between two positions, 50 and 80 μm above the leaf surface, with 0.1 Hz

Table 1 Liquid ion exchangers and backfilling solutions used for preparation of ion-selective microelectrodes

Ion	LIX catalog no. (Fluka)	Backfilling solution
Hydrogen	95297	15 mol m^{-3} NaCl + 40 mol m^{-3} KH_2PO_4
Potassium	60031	500 mol m^{-3} KCl
Calcium	21048	500 mol m^{-3} $CaCl_2$
Magnesium	63048	500 mol m^{-3} $MgCl_2$

frequency. Net ion fluxes were calculated from measured differences in electrochemical potential for each ion between these two positions as described earlier (Shabala et al. 1997; Newman 2001).

Nutrient uptake kinetics experiments

Mesophyll segments from severely Mg-deficient leaves (Mg1) were used for these experiments. Leaf samples were pre-incubated in 500 µl of measuring solution (so-called "buffer" solution, 0.1 mM $CaCl_2$ + 0.2 mM KCl, pH 5.6) until steady fluxes were reached (usually 1–1.5 h after removal of epidermis). Steady-state fluxes were measured for about 5 min, and then an appropriate amount of Mg was added to the measuring solution, and transient ion flux kinetics recorded. To minimize the time required to reach the unstirred layer conditions (a prerequisite for MIFE measurements; Newman 2001), the chamber volume was made very small (1 ml), and Mg was added as a double-stock Mg solution made up in 500 µl of the "buffer". With such experimental design, the final solution concentration was reached at 20–25 s after Mg addition. Consequently, only the first 30–40 s were rejected during the data analysis, significantly improving the time resolution of experiments compared with our previous reports (Shabala 2000; Shabala et al. 2000, 2003). Altogether, nine different magnesium concentrations were used (Fig. 1), covering the range of Mg in the measuring solution from 10 µM to 100 mM.

For each segment, transient flux responses were measured for about 15–20 min. Then, a new chamber containing a fresh segment, pre-incubated in a "buffer" (i.e. Mg-free) solution, was put onto the microscope stage. The steady-state fluxes were measured, and the procedure repeated again, this time adding a different amount of Mg. Four to six segments were measured for each Mg treatment; each of these segments was cut from a different leaf.

Light-induced transients

Leaves were excised from bean plants grown in Mg1, Mg50, and Mg200 solutions (Hariadi and Shabala 2004) and prepared and immobilized as described above. A mesophyll segment was transferred to a measurement chamber containing 5 ml measurement solutions (in mM: 0.1 $CaCl_2$ + 0.2 KCl + 0.2 $MgCl_2$) and left for 20–30 min to adapt to light conditions (dim green microscope light of about 1 W m^{-2}). After measuring steady-state (dark) ion fluxes for 5–10 min, the light treatment was applied (white light of 80 µmol m^{-2} s^{-1}; fibreoptics cool light KL 1500 LCD, Schott, Germany). Transient light responses were measured for 20–30 min or until the steady state was reached. This process was repeated for different mesophyll leaf segments, grown at different Mg levels, from deficient (1 ppm; Mg1) to excessive (200 ppm; Mg200) (see Hariadi and Shabala 2004 for more details) in a random sequence. A total of 8–12 segments were measured for each treatment.

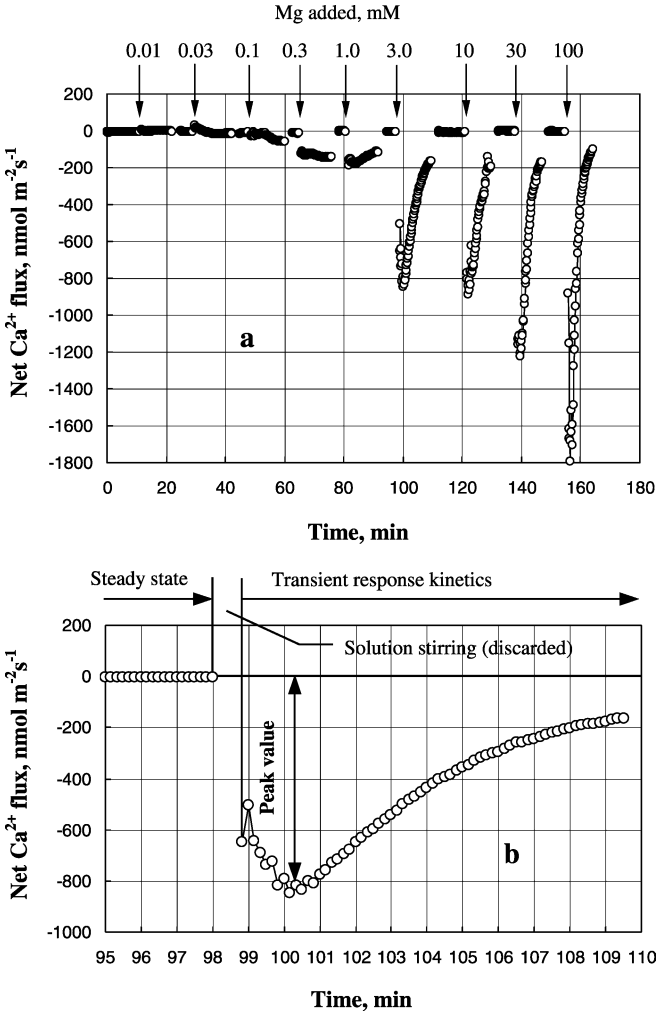

Fig. 1 Methodological aspects of measurements of ion uptake kinetics by the MIFE technique. **a** Typical example of net Ca^{2+} flux kinetics (influx positive) measured from broad bean mesophyll tissue in response to addition of various levels of Mg to the bath solution. **b** A short-term fragment of Ca^{2+} flux response kinetics (as shown in **a**) depicting valid measurement intervals (denoted as steady-state and transient response kinetics, respectively) as well as the peak value used for data analysis. Note that the first 40–50 s immediately after Mg addition were discarded, as unstirred layer conditions are not met over this period

The above protocol was used to measure light-induced kinetics of H^+, K^+, and Ca^{2+} fluxes from leaf segments growth at various Mg supplies. Measurements of Mg^{2+} fluxes, however, were significantly complicated by the confounding effects of Ca^{2+} on Mg^{2+} LIX selectivity. As all commercially available Mg LIX are very sensitive to Ca^{2+} (Fluka 1996 Selectophore catalogue), there was a great danger of misinterpreting measured Mg^{2+} flux in response to light treatment when Ca^{2+} was present in the measuring solution. To avoid this problem, Ca^{2+} was omitted from the solution during Mg^{2+} flux measurements, Although some Ca^{2+} may have leaked from the tissue, its overall concentration

was too small to significantly affect Mg^{2+} flux measurements (<8 μM Ca^{2+} compared with 200 μM Mg^{2+} in the measuring solution). It should be noted that omitting Ca^{2+} from the measuring solution caused no significant changes in either H^+ of K^+ flux responses to light (data not shown), making light-induced Mg^{2+} transients comparable with the rest of the data.

Results

Nutrient uptake kinetics

Each cell possesses a sophisticated network of ion transporters, with numerous interactions and feedbacks between them. This significantly complicates the interpretation of long-term kinetics of ions in response to a particular stimulus. One of the objectives of this study was to characterize the effects of Mg availability on nutrient uptake kinetics of various plasma-membrane transporters in bean mesophyll cells. The experimental design allowed this to happen, with a (relatively) rapid equilibration of ions in solution taking place (see "Materials and methods"). As a result, the time required to achieve the unstirred layer conditions was minimized to just 30–40 s (Fig. 1). Consequently, it became possible to measure a "peak flux value" of the ion of interest (Fig. 1b). This is a significant improvement compared with previous publications, where measurements were either made in larger chambers (Shabala 2000; Shabala et al. 2000) or the solution was replaced gradually (Garnett et al. 2003). In those studies, 2–3 min immediately after the treatment were usually discarded from the analysis.

Generally, fluxes of all ions across the plasma membrane of bean mesophyll cells were affected by the addition of Mg to the bulk solution. In general, the higher the amount of added Mg, the larger the magnitude of flux changes (Fig. 2a). The most affected were Mg fluxes, followed by Ca^{2+}. K^+ flux was next, and H^+ flux had the lowest sensitivity to added Mg. When the peak magnitude (see Fig. 1b) of ion flux change was plotted versus Mg concentration in the bulk solution, several specific regions were present in the dose response curves:

1. Addition of 10–30 μM of external Mg caused a very small (<10 nmol m^{-2} s^{-1}) uptake of Mg. Net H^+ flux "mirrored" changes in net Mg^{2+} flux (H^+ efflux of about the same magnitude; Fig. 2b), with stoichiometry between 1:1 and 2:1 H^+/Mg^{2+}. Much more significant were changes in Ca^{2+} and K^+ fluxes. At least an order of magnitude higher net Ca^{2+} influx and net K^+ efflux were observed (Fig. 2b). Calculated stoichiometry values between Mg and these ions were as high as 25–30 (for 10 μM Mg, Fig. 3).
2. Concentration range 30–1,000 μM Mg. For these concentrations, there was almost a linear increase in net Mg^{2+} uptake accompanied by a progressive efflux of K^+ and Ca^{2+} (Fig. 2) Stoichiometry values approach some "reasonable" physiological values (Fig. 3).
3. A "plateau" region in a 3–30 mM range. The magnitude of Mg^{2+} flux responses remains steady; so are the changes in net H^+ and K^+ fluxes. Ca^{2+} shows progressive leakage. For this region, there is almost a 1:1 stoichiometry between Ca, K and Mg flux changes (Fig. 3).
4. Further dramatic increase in Mg^{2+} uptake ("low affinity" range in Epstein's terms), accompanied by significant (although not very dramatic) Ca^{2+} efflux. Both H^+ and K^+ fluxes are not responding any further to increasing Mg^{2+} concentrations.

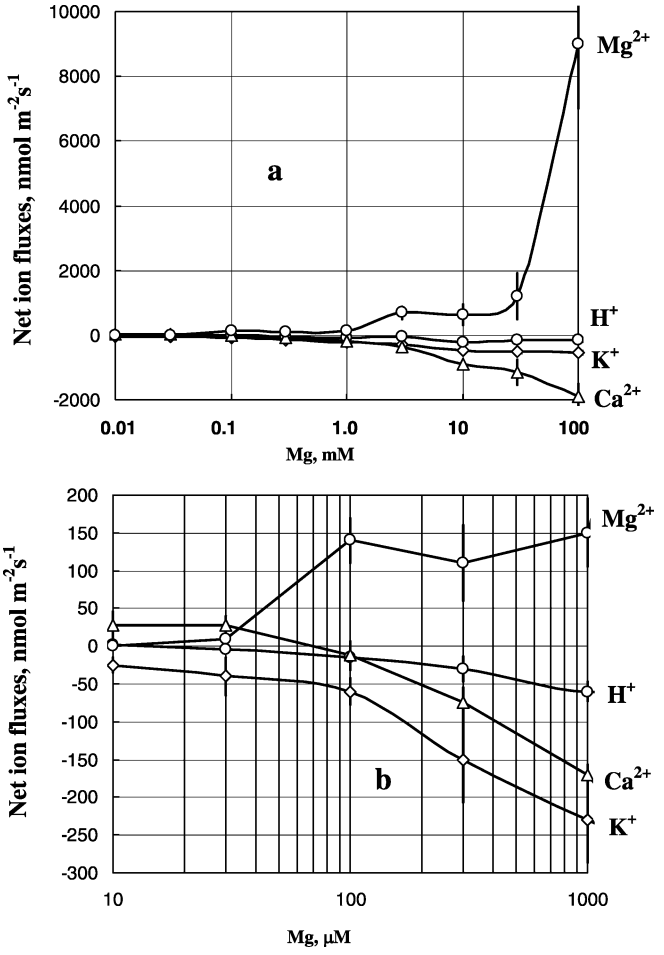

Fig. 2 Ion uptake kinetics as a function of Mg^{2+} concentration in bath solution. Peak values of Mg^{2+}, H^+, Ca^{2+} and K^+ fluxes were measured and plotted against concentration of Mg added to mesophyll tissues of Mg-deficient plants. Data are average fluxes of four to six plants (±SEM)

Pharmacology and effect of leaf "growth history"

In order to provide some insights into the underlying ionic mechanisms of the above responses, a series of pharmacological experiments were undertaken. Leaf

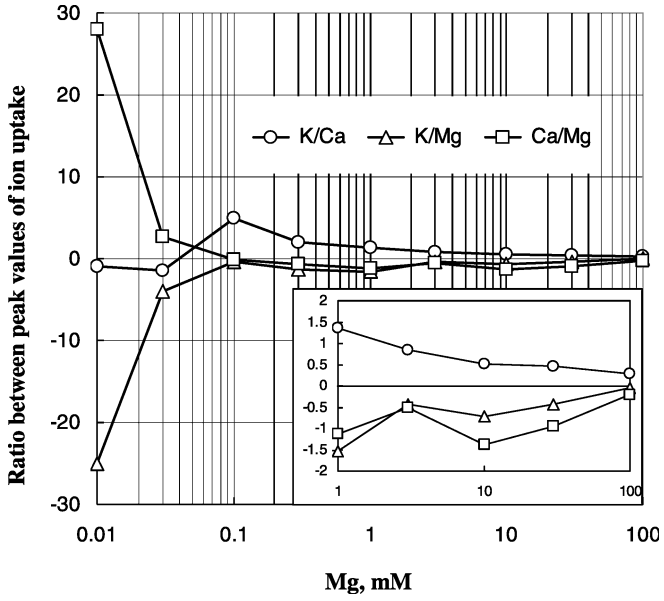

Fig. 3 Stoichiometry between peak values of fluxes of basic cations (Ca^{2+}, Mg^{2+}, and K^{+}) in response to added Mg. The *inset* gives a close-up of the stoichiometry between ion fluxes in the higher concentration range (above 1 mM of Mg)

incubation in 50 μM $GdCl_3$, a known blocker of non-selective cation channels (NSCC), led to a significant reduction in the magnitude of both Mg^{2+} and Ca^{2+} flux responses (Fig. 4). At the same time, 20 μM verapamil (specific Ca^{2+} channel blocker) had no effect on either Ca^{2+} or Mg^{2+} flux kinetics. Leaf incubation in 1 mM vanadate significantly ($P = 0.05$) suppressed both H^{+} and Mg^{2+} flux responses (Fig. 5a, b).

When Mg^{2+} uptake kinetics was compared between leaves from Mg-deficient (Mg1) plants and those grown at optimal (Mg50) supply, Mg1 plants were generally more responsive to supplemental Mg (Fig. 6).

Steady-state fluxes

With the only exception of the data in Fig. 6, the majority of the above nutrient uptake kinetics experiments were undertaken using leaves from Mg-deficient (Mg1) plants. The second question addressed in this study was to what extent Mg availability during plant growth would affect the activity of plasma-membrane transporters in steady-state conditions. Consequently, steady-state fluxes of K^{+}, Ca^{2+}, H^{+}, and Mg^{2+} were measured from dark-adapted leaves of bean plants, grown for 4 weeks in deficient (Mg1), optimal (Mg50), and excessive (Mg200) conditions. Results are shown in Figs. 7 and 8 (time intervals 0–2 min). Several distinct features were observed.

There was a pronounced effect of Mg availability during leaf growth on the magnitude of steady-state H^{+}-flux (Fig. 7a). H^{+} flux values were significantly more negative (net efflux) for Mg-sufficient variants, with the increase in H^{+} efflux almost directly proportional to Mg availability during plant growth. Calcium

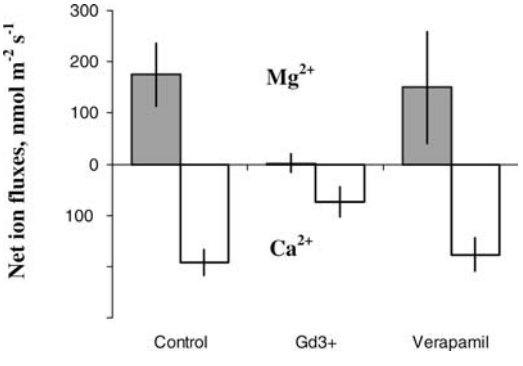

Fig. 4 Effect of specific channel blockers (50 μM Gd^{3+} and 20 μM verapamil) on the magnitude of net Ca^{2+} and Mg^{2+} fluxes in response to added Mg (1 mM). Data are average ± SEM ($n = 4$–5)

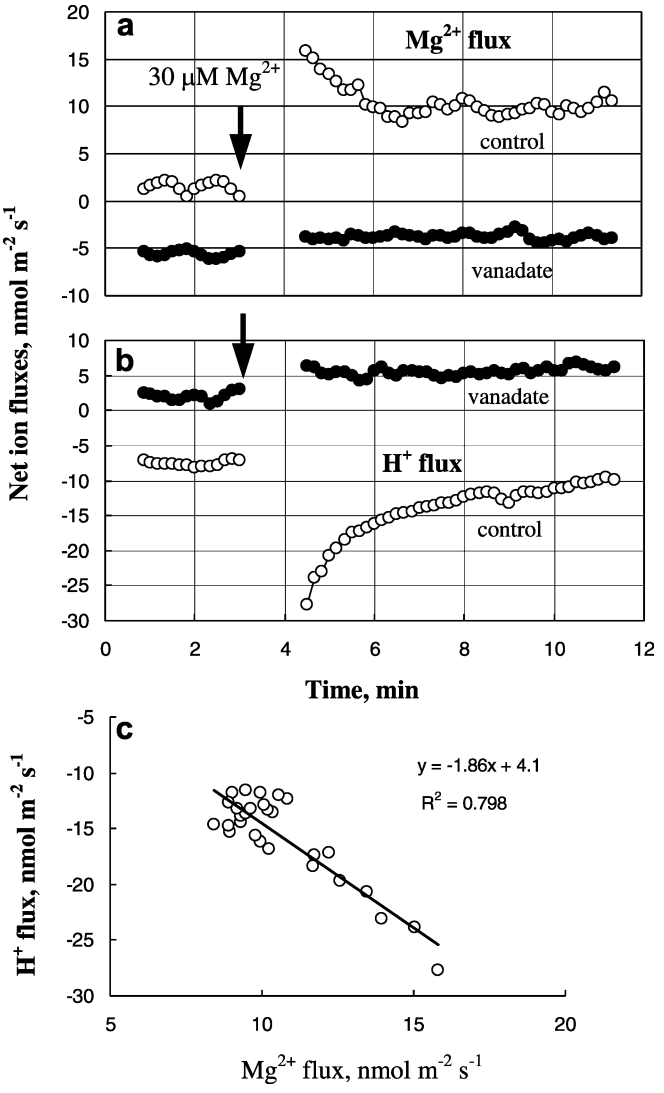

Fig. 5 Effect of 1 mM sodium orthovanadate on net Mg^{2+} (**a**) and H^{+} (**b**) flux responses from bean leaf mesophyll to added Mg (30 μM at 3 min). One typical example (out of four) is shown. (**c**) Correlation between net Mg^{2+} and H^{+} flux changes in response to 30 μM Mg^{2+} from control (no inhibitors) leaf samples

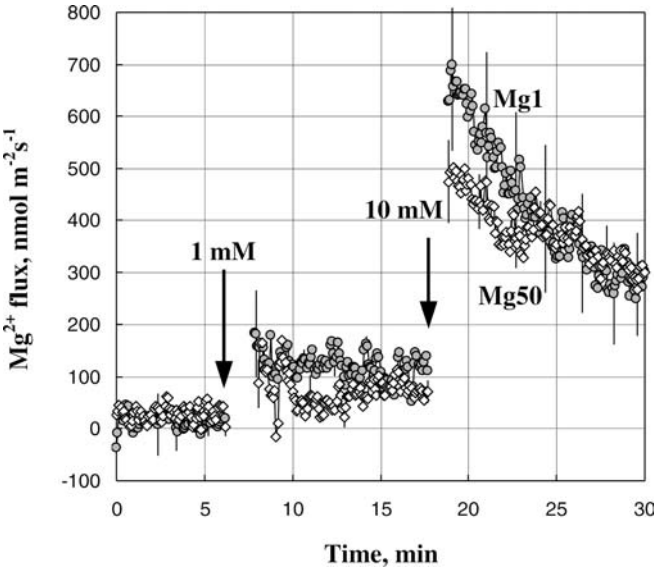

Fig. 6 Comparison of transient Mg^{2+} flux kinetics from Mg-deficient (Mg1, *closed circles*) and Mg-sufficient (Mg50, *open diamonds*) leaves. Data are average ± SEM ($n = 4$)

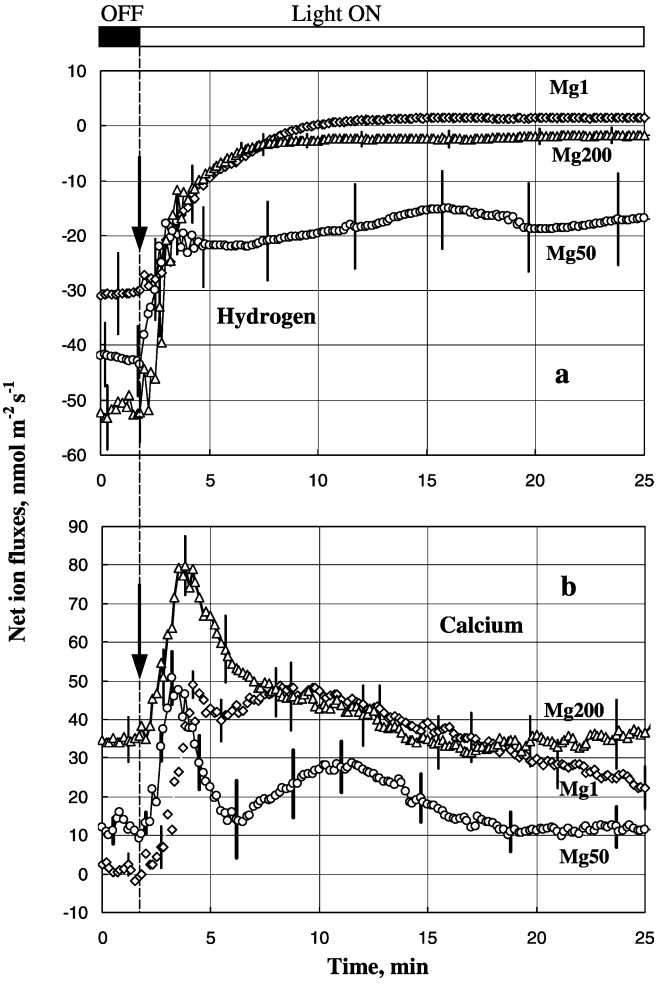

Fig. 7 Effect of Mg availability on light-induced kinetics of net H^+ (**a**) and Ca^{2+} (**b**) fluxes from bean mesophyll tissue. Leaves were grown for 4 weeks at various levels of Mg supply: Mg1 (1 ppm)—deficient; Mg 50 (50 ppm)—optimal; Mg200 (200 ppm)—excessive. Data are average ± SEM ($n = 8$–12)

fluxes essentially "mirrored" those of Mg. Higher Mg supply resulted in higher net Ca^{2+} influx in the dark (0, 10 and 35 nmol m^{-2} s^{-1}, respectively; Fig. 7b). Both H^+- and Ca^{2+}-flux differences were statistically significant at the $P = 0.05$ level. Net magnesium fluxes were near-zero for Mg1 and Mg50, with a significant ($P = 0.001$) Mg^{2+} efflux for excessive (Mg200) treatment (Fig. 8a). Finally, there were near-zero net K^+ fluxes for Mg1 and Mg50 leaves, while a large K^+ efflux was observed for Mg200 treatment (Fig. 8b). In general, K^+ data largely "mirrored" the Mg^{2+}-flux data.

Light-induced transients

The onset of illumination triggers a cascade of electrical events in thylakoid and plasma membranes of green plant tissues. As can be seen from our data, Mg availability has a significant impact on the activity of plasma-membrane transporters (not only for Mg^{2+} but also those for H^+, K^+, and Ca^{2+} ions) upon illumination (Figs. 7, 8). The highest magnitude of light-induced H^+-flux response was for Mg200 treatment (Fig. 7a). After stabilization, Mg50 (optimal) plants retained H^+ efflux, while in two other treatments, fluxes shifted to near zero.

Light-induced Ca^{2+}-flux kinetics was generally consistent with previous observations (Shabala and Newman 1999). The onset of illumination caused immediate and rapid influx of Ca^{2+} (Fig. 7b), peaking within 1–2 min after the light treatment, and then gradually decreasing over the next 20 min. As for the effect of Mg availability on Ca^{2+} flux transients, there was a significant ($P = 0.05$) difference in the temporal characteristics of light-induced Ca^{2+} uptake between treatments. In plants grown under optimal Mg supply (Mg50), Ca^{2+} influx peaked at about 1 min after onset of illumination, while in Mg1 and Mg200 treatments it was delayed for an additional 1–1.5 min (Fig. 7b). Also, Mg-deficient (Mg1) plants retained Ca^{2+} influx for much longer compared with the other two treatments.

The onset of illumination caused rapid and prolonged net Mg^{2+} uptake for all treatments (Fig. 8a). Regardless of Mg availability during plant growth, light-induced Mg^{2+} influx peaked at about 2 –2.5 min after light exposure, and gradually wound down. As a general rule, net Mg^{2+} fluxes in the light were significantly ($P = 0.05$) higher than those in the dark for each of the treatments. The magnitude of Mg^{2+}-flux response was proportional to the level of Mg available during growth (Fig. 8a). Light-induced K^+-flux kinetics was also consistent with our previous observations (Shabala and Newman 1999). Net K^+ efflux was measured after some lag (of about 1 min).

There was a significant difference in light-induced K^+-flux responses as a function of Mg availability

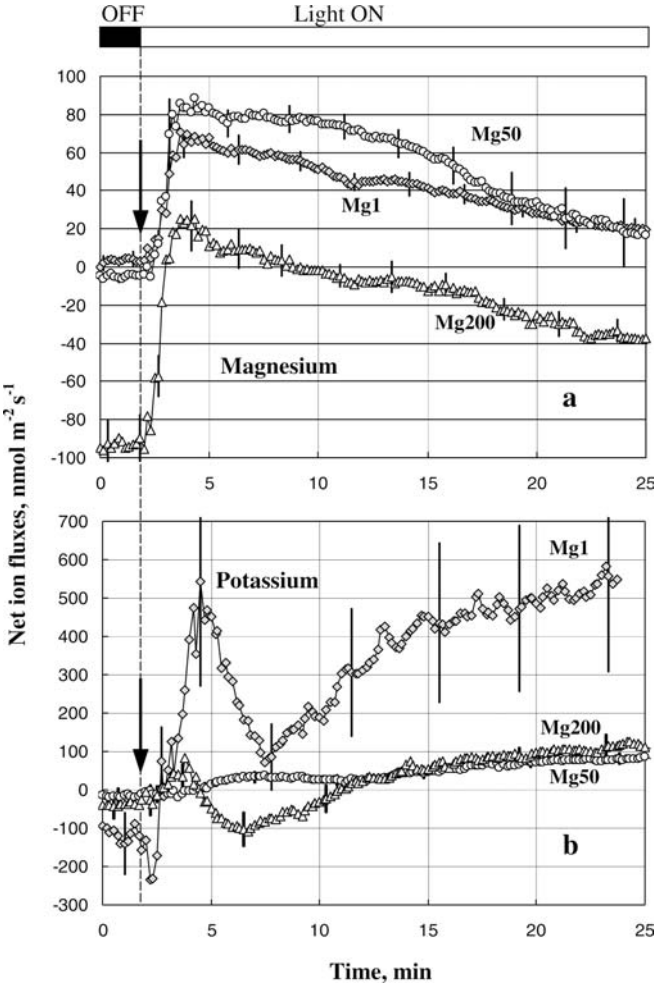

Fig. 8 Effect of Mg availability on light-induced kinetics of net Mg^{2+} (**a**) and K^+ (**b**) fluxes from bean mesophyll tissue. Data are average ±SEM (n = 8–12)

during plant growth. Mg-deficient plants showed the biggest magnitude of response (Fig. 8b), followed by excessive (Mg200) plants. The smallest K^+-flux changes were measured from leaves grown in the optimal Mg50 conditions.

Discussion

Our data represent the first real-time measurements of Mg^{2+} flux across the plasma membrane of leaf mesophyll cells and their changes in response to light.

Nutrient uptake kinetics

The pathway(s) of Mg^{2+} entry through the plasma membrane of plant cells have not been clearly determined (Shaul 2002). From our data, it is likely that at least two mechanisms are involved in Mg^{2+} uptake across the plasma membrane of bean mesophyll cells. At low Mg^{2+} concentrations (< 30 μM), there was no apparent correlation between the uptake of Mg^{2+} and Ca^{2+} (Fig. 2). Both Mg^{2+} and Ca^{2+} ions were transported in the same direction, but whilst Mg^{2+} uptake increased proportionally with Mg^{2+} concentration, Ca^{2+}-flux responses remained more or less unchanged. Thus, it is unreasonable to suggest that any causal relationship exists between transport of Ca^{2+} and Mg^{2+} ions at low (< 30 μM) Mg^{2+} concentration. In contrast, a high correlation ($R^2 = 0.8$) between H^+ and Mg^{2+} transport, "physiologically reasonable" stoichiometry (between 1:1 and 1:2) and the fact that both Mg^{2+} and H^+ fluxes were suppressed by vanadate (Fig. 5) suggests that in this concentration range, transport of Mg^{2+} and H^+ are coupled (Fig. 9). There are very few molecules known to transport Mg^{2+} in eukaryotes (Haynes et al. 2002; Shaul 2002; Gardner 2003). Recent work by Li et al. (2001) suggested that among the ten members of the AtMGT family of transporters that are involved in Mg^{2+} acquisition from the soil and/or in Mg^{2+} transport in arabidopsis, at least one (AtMGTl) was localized at the plasma membrane.

The stoichiometry of this transporter is not clear. Although, due to the small magnitude of net Mg and H^+ fluxes at low Mg concentration in our experiments, as well as because of the masking activity of H^+-ATPase pump, the unequivocal deduction of the porter mechanism from our data is not possible. Our data (Figs. 2, 5) does allow us to speculate (at least qualitatively) that the Mg^{2+}/H^+ exchanger may be involved in Mg^{2+} transport across the plasma membrane of bean mesophyll cells, with stoichiometry between 1:1 and 1:2 (Fig. 5c). So far, Mg^{2+}/H^+ exchangers have been reported only at the tonoplast in *Arabidopsis* (AtMHX, Shaul et al. 1999) and *Hevea* (Amalou et al. 1994) vacuoles, with 1:2 and 1:3 stoichiometry, respectively. AtMHX is the first Mg^{2+} transporter to be cloned from a multicellular organism, and it shares limited sequence homology (36% identity) with NCX1, a mammalian plasma membrane Na^+/Ca^{2+} exchanger (Nicoll et al., 1990) as well as with the Mg^{2+} permeable channel in *Paramecium* (Haynes et al. 2002).

At higher Mg concentrations (> 30 μM), there was apparent competition between Ca^{2+} and Mg^{2+} for uptake. Judging by K^+ data and stoichiometry between all three cations (Ca, K, and Mg) it is likely that such a transporter could be a NSCC (Fig. 6). This is further supported by pharmacological experiments (Fig. 4), which showed that both Mg^{2+} and Ca^{2+} fluxes were strongly inhibited by Gd^{3+}, a known blocker of NSCC. At the same time, neither Ca^{2+} nor Mg^{2+} fluxes were affected by verapamil, a specific Ca^{2+} channel blocker. In general, an almost electroneutral exchange of Mg^{2+} for K^+ and Ca^{2+} could be observed in the 1–100 mM range of Mg concentration (Fig. 3). Some observed deviation from electroneutrality may be explained by the fact that a small fraction of ion fluxes may originate from the cell wall, thus masking the activity of plasma-membrane transporters.

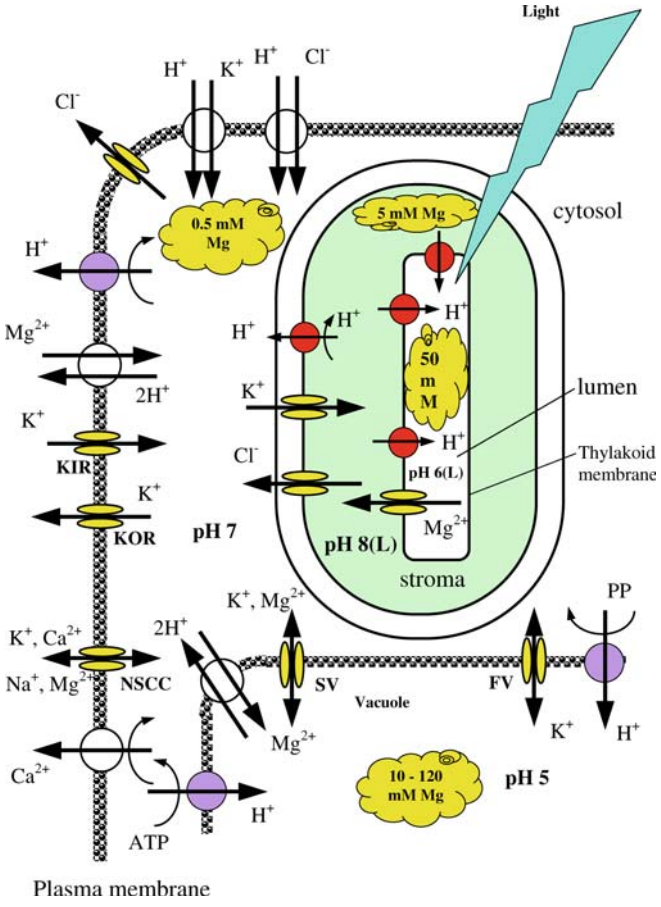

Fig. 9 Summary of membrane transporters involved in regulation of Mg homeostasis in leaf cells. Abbreviations used: *KIR* potassium inward rectifying channel, *KOR* potassium outward rectifying channel, *NSCC* non-selective cation channel, *SV* slow vacuolar channel, *FV* fast vacuolar channel. Intracellular compartmentation of Mg is achieved by at least two known Mg^{2+} transport systems at the tonoplast membrane (SV channel and $2H^+/Mg^{2+}$ antiporter) and one at the thylakoid membrane (Mg^{2+} permeable non-selective cation channel). Magnesium entry into the cell is mediated by the non-selective cation channel (*NSCC*) and some high affinity Mg^{2+} transporter (suggested H^+/Mg^{2+} antiporter). Please note that no information about free vacuolar Mg^{2+} concentrations is available in the literature, while the total Mg^{2+} content in this organelle was reported to be in the range 10–120 mM (see Shaul 2002)

Importantly, the fraction of K^+ tends to decrease with the increase in Mg concentration, which is expected under the condition of saturation of divalent cation binding sites within the NSCC. The molecular identity of the suggested Mg^{2+}-permeable NSCC channel remains unknown. It has been shown in some species (such as wheat) that one possible route of Mg^{2+} entry may be through putative homologs of the *rca* channel (White et al. 2000). The *rca* channel is defined as a calcium channel, but is also permeable to a wide variety of monovalent and divalent cations, including Ca^{2+}, Mg^{2+}, K^+, and Na^+ (Pineros and Tester 1997; White et al. 2000). As this channel opens upon plasma membrane depolarization (Pineros and Tester 1997), light-induced Ca^{2+} uptake (Fig. 7) leading to rapid initial depolarization of the plasma membrane (Spalding et al. 1992; Elzenga et al. 1995; Shabala and Newman 1999) may be responsible for the observed Mg^{2+} influx (Fig. 8). As repolarization occurs (several minutes after light exposure; Shabala and Newman 1999), this channel will be closed, and Mg influx will gradually decrease (Fig 8a).

Steady-state fluxes

The observed dependence of H^+ efflux as a function of Mg availability during plant growth (Fig. 4a; steady-state flux values before light treatment) is consistent with the role of Mg as the activator of plasma membrane H^+-ATPase (Shaul 2002). Yazaki et al. (1988) showed that about 90% of cytosolic ATP is complexed to Mg. It may be suggested that, despite the large storage capacity of the vacuole (between 10 and 120 mM of total Mg; Dietz et al. 1992; Shaul 2002) and a strict Mg homeostasis (Gardner 2003), cytosolic-free Mg^{2+} levels differ significantly between Mg-deficient and Mg-sufficient leaves. Some additional support for this fact might be found in Fig. 6, showing that plants grown at sufficient (Mg50) magnesium supply showed smaller responses to supplemental Mg^{2+} compared with deficient Mg1 plants. Assuming that under these conditions Mg^{2+} transport is mediated by the NSCC, reduced ability to take up Mg^{2+} in Mg50 plants (Fig. 6) may be indicative of higher cytosolic free Mg^{2+} concentrations. Even higher cytosolic free Mg^{2+} levels are expected to be present in "excessive" Mg200 plants, explaining the observed significant net Mg^{2+} efflux in steady-state conditions from Mg200 leaves (Fig. 8a).

Light-induced transients

Mg availability had a significant impact on light-induced changes in net ion fluxes of Mg^{2+}, H^+, K^+, and Ca^{2+} across the plasma membrane of bean mesophyll cells (Figs. 4, 5). Interpretation of the H^+ data is complicated by the fact that H^+ fluxes were measured from the actively photosynthesizing mesophyll tissues. Upon illumination, the cytosolic CO_2 pool is quickly depleted, leading to a significant influx of CO_2 from external media into the cell (Hansen et al. 1993). That shifts the equilibrium between HCO_3^- and CO_2 in the apoplastic solution towards CO_2 formation and results in significant alkalinization of the measuring solution (Yin et al., 1996), which is interpreted by the MIFE technique as an apparent influx (Shabala and Newman 1999). As a result, activation of plasma membrane H^+ pump, known to occur in response to light (Elzenga 1997), is masked by this phenomenon. Nonetheless, steady-state H^+ flux values in the light (20 min after onset of illumination) showed the largest efflux for Mg50 leaves. This is consistent with the role of Mg as the activator of plasma membrane H^+-ATPase (Shaul 2002).

Light-induced Ca^{2+} uptake into the leaf cell is one of the most rapid events, which is believed to be responsible for membrane depolarization (Elzenga et al. 1997; Shabala and Newman 1999) and be involved in the signal transduction process (Babourina et al. 2002). Similar to our previous publication (Shabala and Newman 1999), the onset of illumination caused immediate and rapid influx of Ca^{2+} (Fig. 7b), peaking within 1–2 min after the light treatment, and then gradually decreasing over the next 20 min. Our data also suggest that Mg availability during plant growth may significantly affect light-induced Ca^{2+} flux "signatures" and thus regulate (directly or indirectly) signal transduction between light photoreceptors and plasma membrane effectors (ion channels). In plants grown under optimal Mg supply (Mg50) Ca^{2+} influx peaked at about 1 min after the onset of illumination, while in Mg1 and Mg200 treatments it was delayed for an additional 1–1.5 min (Fig. 7b).

The peak of light-induced Mg^{2+} uptake occurred at 2 min after the onset of illumination, at about the same time as the peak of Ca^{2+} influx was reached (Figs. 7b, 8a) and thus this peak coincides with a peak of the plasma membrane depolarization (Shabala and Newman 1999). Therefore, it is unlikely that light-induced Mg^{2+} influx may be explained by light-induced changes in membrane potential. It is reasonable to suggest that Mg^{2+} influx may be another factor responsible for plasma-membrane depolarization within the first minutes of light treatment (Spalding et al. 1992). Other physiological roles of net Mg^{2+} influx across the plasma membrane (Fig. 5a) remain obscure. In chloroplasts, there is a significant change in free Mg^{2+} concentrations in both thylakoid lumen and stroma upon illumination. In intact, dark-kept spinach chloroplasts, internal [Mg^{2+}] was estimated to be 0.5 mM, and illumination caused an increase in [Mg^{2+}] to 2.0 mM in the stroma (Ishijima et al. 2003). Such light-induced Mg^{2+} efflux from lumen to stroma is essential for regulation of the activity of the thylakoid ATPase complex (ATP synthase, CF^0F_1) as well as for the activity of key stromal enzymes, including Rubisco and Dl protein of photosystem II (Horlitz and Klaff 2000; Shaul 2002). It is unclear if the same scenario is applicable to light-induced Mg^{2+} uptake into the cytosol (Fig. 5a). The most obvious function of elevated cytosolic Mg^{2+} would be to "fuel" the plasma membrane H^+ pump, responsible for the overall membrane hypopolarization in the light (Elzenga et al. 1995; Shabala and Newman 1999).

The effects of Mg availability on light-induced K^+ fluxes were rather unexpected. The smallest K^+ flux changes were measured from leaves grown in optimal Mg50 conditions, while Mg-deficient plants showed much greater responses (at least one order of higher magnitude; Fig. 5b). Therefore, it is unlikely that light-induced K^+ fluxes are relevant to cell turgor regulation and, ultimately, leaf expansion growth. The charge-balancing role of light-induced K^+ flux is more likely.

Acknowledgements This work was supported by an ARC Large Grant (A00001144) to Dr S. Shabala. My sincere thanks to Dr Richard Gardner and Prof. Igor Pottosin for helpful discussion and suggestions.

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