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News and Views

Breaking the barrier: Mg hyperaccumulation in *Brassica rapa* endodermal mutants

Elisa Dell'Aglio [1]

1 Institut National des Sciences Appliquées de Lyon, 69100 Villeurbanne, France

Just as a country border regulates the movement of goods and people from two neighboring countries, the Casparian strip limits the passage of solutes from the root periphery to the central vessels and vice versa (Barbosa et al., 2019). Molecularly, the Casparian strip is a lignified, vertical diffusion barrier that surrounds the endodermal cells, thus blocking exchanges through the cell wall (the apoplastic route) and forcing all movement to pass through the endodermal cytoplasm (the symplastic route), meaning that substances must pass through selective transporters and ion channels.

This is why a group of Arabidopsis (*Arabidopsis thaliana*) genes whose mutations disrupt the correct assembly of Casparian strips have been named "Schengen" (*SGN*), after the 1985 commercial treaty that facilitates trades between various European countries by abolishing border checks. They include a receptor-like cytoplasmic kinase (*AtSGN1*, Alassimone et al., 2016), a tyrosylprotein sulfotransferase (*AtSGN2*, Doblas et al., 2017), a leucine-rich repeat receptor kinase (*AtSGN3*, Pfister et al., 2014), and the Respiratory Burst Oxidase Homolog F (*AtSGN4*, Lee et al., 2013).

In particular, Pfister et al. (2014) demonstrate the AtSGN3 receptor kinase localizes at the plasma membrane and delimits the borders of the Casparian strip, thus ensuring the formation of a single contiguous band. In *Atsgn3* mutants, the Casparian strip is still present but appears patchy, interrupted by irregular holes. As a consequence, *Atsgn3* mutants grown in various conditions show higher levels of some minerals, especially magnesium (Mg) and cesium (Cs), while they show decreased levels of potassium (K) and often zinc (Zn). Mutant plant growth is also mildly compromised in conditions not stressful to wild-type plants, for example, at 25°C, especially under long-day photoperiod.

In this issue of *Plant Physiology*, by combining EMS screening with ionomic profiling, Alcock and colleagues identified an Mg-hyperaccumulating *Brassica rapa* mutant called *braA.sgn3.a*, which contains a mutation in a gene homolog of *AtSGN3* (Alcock et al., 2021). The causative mutation was localized in the *BrSGN3* kinase domain, in close proximity with Arabidopsis mutations that disrupt the gene function. The gene identification was confirmed by complementing the mutants with the wild-type *BrSGN3* sequence and by comparison with other *BrSGN3* mutants publicly available (Stephenson et al., 2010).

In agreement with previous results in Arabidopsis (Pfister et al., 2014) and tomato (*Solanum lycopersicum*; Li et al., 2018), the *braA.sgn3.a* mutant showed propidium iodide stained xylem vessels and higher propidium iodide penetration through the root tip (in average, up to 139 cells above the root tip vs. an average of 26 cells for the wild type). Since propidium iodide apoplastic diffusion is blocked by the Casparian strip, these results, as well as the patchy lignin deposition at the endodermis typical of *Atsgn3*, indicate impairments in root permeability. Furthermore, while in wild-type plants exogenous application of the SGN3 ligand the Casparian strip Integrity Factor 2 (CIF2; Doblas et al., 2017; Okuda et al., 2020) leads to ectopic lignin deposition, no response was observed in *braA.sgn3.a*.

The similarities between *Atsgn3* and *braA.sgn3.a* also concerned solute accumulation in leaves. In particular, in *braA.sgn3.a*, the leaf accumulation of Mg was approximately twice as high as in wild type (30.5 g kg-DW^{-1} vs. 13.6 g kg-DW^{-1}) and, to a lesser extent, sodium (Na), phosphorus (P), and sulfur (S) were elevated in the mutant while K was depleted compared to wild type (41.8 g kg-DW^{-1} vs. 52.6 g kg-DW^{-1}). In addition, *braA.sgn3.a* accumulated about a third more calcium (Ca) than wild type (28.5 g kg-DW^{-1} vs. 21.4 g kg-DW^{-1})—a drastic increase that was not highlighted in Arabidopsis (Pfister et al., 2014). Vice versa, *braA.sgn3.a* did not show signs of Cs hyperaccumulation which were reported in *Atsgn3*, either suggesting that Ca and Cs accumulate differently in Arabidopsis and *B. rapa* or that their accumulation depends on specific growth

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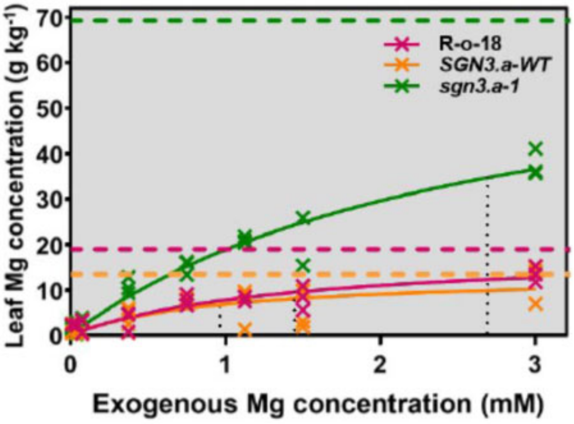

Figure 1 Leaf Mg concentrations of plants grown under different exogenous Mg concentrations. The figure shows Michaelis–Menten kinetics curves for wild-type *B. rapa* (R-o-18), *sgn3.a-1* mutants, and wild-type-like segregants (*SGN3.a-WT*), with Vmax and Km lines shown as dashed and dotted lines, respectively (from Figure 6 of Alcock et al., 2021).

parameters. Microparticle-induced X-ray emission showed several of these elements were not evenly distributed in the leaves. Rather, Na and P were elevated in leaf epidermal tissues, while Mg and Ca accumulated both in the epidermis and in the mesophyll. The increase in Mg was also observed in other tissues such as flowers, mature pods, and roots, while no differences were detected in seeds.

To further characterize the increased permeability of *braA.sgn3.a*, the authors measured leaf Mg concentration in plants grown in hydroponic conditions using the nutrient film technique, testing concentrations of Mg spanning from 0.0075 to 3 mM (in this system, the optimum concentration for plant growth is 0.75 mM). While a positive association between solution Mg concentration and leaf Mg concentration was always present, the wild-type plant uptake soon reached a plateau, with a theoretical maximum leaf Mg concentration calculated at around 20 g/kg. Conversely, *braA.sgn3.a* theoretical maximum leaf Mg concentration was more than 3 times higher (Figure 1). These results clearly show to what extent Mg levels can be perturbed in *braA.sgn3.a*.

The present work confirms previous results obtained in Arabidopsis and tomato regarding the effect of the *sgn3* mutation (Pfister et al., 2014, Li et al., 2018), while expanding the knowledge about metal accumulation in these mutants from both a quantitative and a spatial perspective. In addition, this work raises the possibility of achieving biofortification of Mg in crop plants by exploiting Casparian strip mutants that accumulate higher levels of Mg. Future studies will enlighten the best growth conditions for these plants, as well as their responses to other nutrient stress, such as high levels of Ca or K deficiency.

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