

Starch and sugar accumulation in *Sulla carnosa* leaves upon Mg^{2+} starvation

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Abstract In the present work, magnesium deficiency effects were studied in *Sulla carnosa* plants grown in nutrient solution containing 1.50, 0.05, 0.01, and 0.00 mM Mg^{2+} . After 5 weeks of treatment, fully expanded leaves were harvested to study their morphological and ultrastructural changes, as well as their carbohydrate, pigment, and Mg^{2+} concentrations. In control plants, leaves were well developed with remarkable green color. Down to 0.05 mM Mg^{2+} , no chlorosis symptom was recorded, but below this concentration, mature leaves showed an appearance of interveinal chlorosis that was much more pronounced at 0.00 mM Mg^{2+} with the development of necrotic spots. Optima of chlorophyll *a*, chlorophyll *b*, and carotenoid concentrations were observed at 0.05 and 1.50 mM Mg^{2+} ; leaf magnesium concentration was severely reduced at 0.05 mM Mg^{2+} . A significant decrease in pigment concentrations was noticed at 0.01 mM Mg^{2+} , but the lowest values were recorded at 0.00 mM Mg^{2+} . Enzymatic assays showed an increase in the accumulation of soluble sugars and starch with decreasing Mg^{2+} concentration. These results were in accordance with those of ultrastructural studies that revealed a marked alteration of chloroplasts in leaves of deficient plants. These chloroplasts were round and bigger as a result of a massive

accumulation of oversized starch grains with disrupted thylakoids. As a whole, 1.50, 0.05, and 0.01 mM Mg^{2+} were found optimal, suboptimal, and deficient concentrations, respectively, the latter showing no significant difference with absolute Mg^{2+} absence (0.00 mM Mg^{2+}).

Keywords Deficient concentration · Optimal concentration · Suboptimal concentration · Soluble sugars · Starch

Introduction

Plants need magnesium in harvesting solar energy and in driving photochemistry (Hermans et al. 2010). Moreover, it was established that magnesium is a crucial component in plant cells since it is involved in the activation of more enzymes than any other mineral nutrient (Epstein and Bloom 2004). Among Mg-activated enzymes, the following are included: ATPases, RNA polymerase, 1,5-biphosphate carboxylase/oxidase (Rubisco), and protein kinases (Marschner 1995; Shaul 2002). In addition, being a central atom in the chlorophyll molecule (Beale 1999), magnesium plays a key role in the utilization of light energy in photosynthesis (Cakmak and Kirkby 2008). Plants suffering from magnesium deficiency constitute a worldwide problem (Mitchell et al. 1999). It was revealed that before any change in shoot growth, photosynthetic activity, and leaf morphology, the most harmful effect of magnesium starvation is sugar accumulation in source leaves (Cakmak et al. 1994a, b; Hermans and Verbruggen 2005). Therefore, this accumulation of sugar was more related to magnesium availability decline than to chlorophyll content decrease (Hermans et al. 2004; Hermans and Verbruggen 2005).

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According to Hermans et al. (2010), plant growth reduction appears as a later response to magnesium deficiency.

Recently, the relationship between sugar partitioning and magnesium deficiency was studied by Hermans et al. (2005). According to these authors, a high accumulation of starch and sucrose in rosette leaves was concomitant with a marked inhibition of sucrose export from these leaves into roots under magnesium deficiency. However, sucrose accumulation seems to be the major growth constraint especially for roots (Ericsson and Kahr 1995). Different explanations for this accumulation were proposed: Fischer and Bremer (1993) explained it by an interruption of sucrose transport from source to sink leaves, while Cakmak et al. (1994a, b) supposed that such increase in assimilate content is due to a sensitivity of phloem sucrose loading under magnesium limitations in *Phaseolus vulgaris*. At the morphological level, magnesium deficiency can be detected through interveinal chlorosis in fully expanded leaves (Cakmak and Kirkby 2008). This constitutes a symptom of damages in chloroplast ultrastructure as demonstrated with several plant species by transmission electron microscopy (Thomson and Weier 1962; Vesik et al. 1966).

The scarcity of data on magnesium deficiency in wild plants, in particular in halophytes, encouraged us to study the effects of decreasing magnesium availability on the indifferent halophyte *Sulla carnosa*. This legume was recently proposed to be grown on salt-affected soils as forage and phytodesalination plant (Jlassi et al. 2012). In the present work, we investigated the morphology of fully expanded leaves, changes in chloroplast ultrastructure, and sugar content (sucrose, glucose, fructose, and starch) in *S. carnosa* as a function of magnesium availability in the medium.

Materials and methods

Plant material and culture conditions

After three days of germination in Petri dishes, *S. carnosa* seedlings were transferred into plastic containers with aerated nutrient solution of Hewitt (1966): 3.0 mM KNO₃, 3.5 mM Ca(NO₃)₂, 1.6 mM KH₂PO₄, 0.6 mM K₂HPO₄, 2.0 mM NH₄NO₃, 3.0 μM Fe-EDTA, 0.5 μM MnCl₂, 0.04 μM CuSO₄, 0.05 μM ZnSO₄, 0.05 μM H₃BO₃ and 0.02 μM Mo₇O₂₄(NH₄). Plants were separated into four groups. The first one was considered as control treatment; thus, plants received a complete nutrient solution (1.50 mM MgSO₄). Three deficient treatments were considered: 0.05, 0.01, and 0.00 mM MgSO₄. The experiments were performed under controlled conditions: 25/20 °C day/night temperatures, a light intensity of 300 mmol m⁻² s⁻¹

PAR, a relative humidity of 60–80 %, and a photoperiod 16 h (lighting was controlled by a timer). After five weeks of treatment, fully expanded leaves were harvested for transmission electron microscopy and sugar, pigment, and Mg²⁺ analysis.

Transmission electron microscopy

Observations of the effects of Mg²⁺ deficiency on the structure of *S. carnosa* were made on leaf mid-region obtained from three plants of *S. carnosa*. Samples were cut into small pieces (1–2 mm²) and fixed in 2 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 1 h according to Sabatini et al. (1963). Postfixation was performed with 1 % osmium tetroxide in 20 mM phosphate buffer (pH 6.8), followed by three washing baths with bi-distilled water. Specimens were dehydrated in ethanol series (50–100 %) and embedded in Spurr resin (1969). Ultrathin sections were stained with 2 % uranyl acetate and lead citrate and then observed by a transmission electron microscope. After observation of ultrathin sections, at least ten micrographs were taken and analyzed for each treatment.

Iodine staining

Starch was visualized by iodine staining. Three fresh plants of *S. carnosa* treated with 1.50, 0.05, 0.01, and 0.00 mM Mg²⁺ were bleached in 80 % (v/v) boiling ethanol and then stained with Lugol solution (2 mM I₂, 6 mM KI). Thereafter, plants were briefly destained with distilled water (Hermans et al. 2005).

Soluble sugar assays

Sample preparation

Extraction of frozen leaf material (100 mg) was realized in deionized water. Obtained solution was heated (up to about 60 °C) to aid extraction of soluble sugars. Glucose, sucrose, and fructose concentrations in the supernatant were determined by a spectrophotometric enzymatic assay, recording the reduction of NAD⁺ at 340 nm. All sugar assays were made in five replicates from five different plants.

Glucose determination

A vial of glucose assay reagent kit was reconstituted with 20 mL H₂O. The obtained assay buffer used to measure glucose contained: 1.5 mM NAD, 1.0 mM ATP, 1.0 unit mL⁻¹ of hexokinase, and 1.0 unit mL⁻¹ of glucose-6-phosphate dehydrogenase. 1 mL of glucose assay

reagent was added to a sample volume of 10–200 μL . Tubes were mixed and incubated for 15 min at room temperature (18–35 $^{\circ}\text{C}$). Absorbance was measured at 340 nm versus deionized water for glucose determination (Bondar and Mead 1974; Southgate 1976).

Fructose determination

For the determination of fructose concentration, an enzyme suspension for fructose assay containing approximately 600 units mL^{-1} of phosphoglucose isomerase (PGI) was used. To 100 μL of sample volume were added 20 μL of PGI and 2 mL of glucose assay reagent. After 15 min of incubation at 18–35 $^{\circ}\text{C}$, absorbance was measured at 340 nm for fructose determination (Bergmeyer and Bernt 1974; Southgate 1976).

Sucrose determination

For sucrose determination, an enzyme suspension (2 mL) contained 150 units mL^{-1} of invertase was used. A suspension of 100 μL of the invertase was added to 100 μL of the sample. Tubes were incubated for 10 min at 18–35 $^{\circ}\text{C}$. After that, an aliquot of 2 mL of glucose assay reagent was added to each tube. After incubation at 18–35 $^{\circ}\text{C}$ for 15 min, absorbance was measured at 340 nm and sucrose determination was obtained according to Bergmeyer and Bernt (1974) and Southgate (1976).

Starch assay

For starch determination, 200 mg frozen material were used. To solubilize the sample, 20 mL DMSO (dimethyl sulfoxide) and 5 mL HCl (8 N) were added to it for 30 min at 60 $^{\circ}\text{C}$ in a shaking water bath. Thereafter, 50 mL deionized water was added, and the pH was adjusted to 4.4–4.6 by NaOH (5 N). Then, the solution was diluted with deionized water to a final volume of 100 mL. To measure starch concentration, a vial reconstituted with 20 mL water containing 50 units mL^{-1} of amyloglucosidase was used. Hence, to 1 mL of the sample, 1 mL of the starch reagent assay was added. Tubes were mixed and incubated for 15 min at 60 $^{\circ}\text{C}$ in a shaking water bath. After that, tubes were removed from water bath and cooled. From each tube, an aliquot of 10–200 μL was used with 1 mL of glucose reagent assay. After incubation of tubes for 15 min at 18–35 $^{\circ}\text{C}$, absorbance was measured at 340 nm (Beutler 1984; Southgate 1976; Thivend et al. 1972).

Chlorophyll and carotenoid concentrations

For pigment analysis, 100 mg fresh fully expanded leaves were extracted in 80 % (v/v) acetone. Chlorophyll and

carotenoid concentrations were determined spectroscopically according to the method of Lichtenthaler (1987). The absorbance of the cleared extract incubated for 72 h in the dark at 4 $^{\circ}\text{C}$ was measured at 663, 647, and 470 nm for chlorophyll *a*, chlorophyll *b*, and total carotenoids, respectively.

Mg^{2+} extraction and analysis

For mineral analysis, leaves were oven-dried at 60 $^{\circ}\text{C}$ for one week. After that, dry matter was ground and samples wet-digested by adding a mixture of nitric and perchloric acids (3:1, v:v), heating at 150 $^{\circ}\text{C}$ until dryness using the method described by Grusak (1995). Digests were thereafter dissolved in 30 mL HNO_3 (0.5 %). Magnesium concentrations were measured by a VARIAN 220 FS atomic absorption spectrometer.

Statistical analysis

Data were subjected to a one-way ANOVA test using SPSS 11.0 for Windows, and means were compared according to Duncan's multiple-range test at 5 % level of significance.

Results

Morphological aspects

The first visual symptoms of magnesium deprivation were seen after three weeks of treatment (slight interveinal chlorosis started to appear) in old leaves of plants fed with 0.00 mM Mg^{2+} . After 5 weeks, when the symptoms were more obvious, plants were harvested. Those treated with 1.50 (control) and 0.05 mM Mg^{2+} remained similar: They were green (without any chlorosis) and well developed (Fig. 1). At 0.01 mM Mg^{2+} , the leaves were invaded by



Fig. 1 Morphological aspect of mature leaves of *S. carnosia* plants grown for 5 weeks in glasshouse in the presence of 1.50, 0.05, 0.01, or 0.00 mM Mg^{2+} in the medium

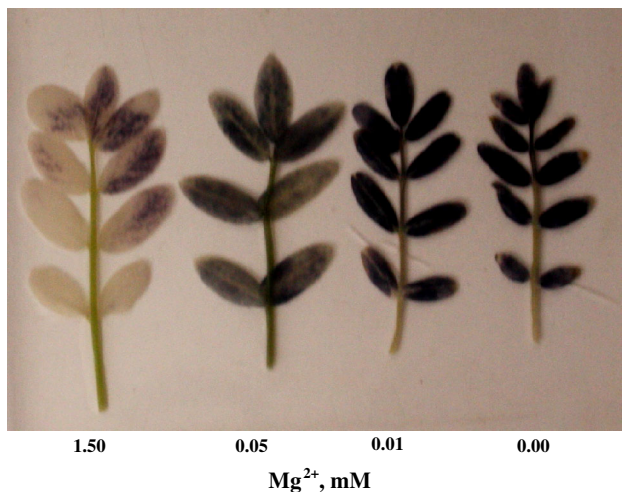


Fig. 2 Iodine staining of starch in mature leaves of *S. carnosa* plants grown for 5 weeks in glasshouse in the presence of 1.50, 0.05, 0.01, or 0.00 mM Mg^{2+} in the medium

chlorotic areas; these phenomena were further enhanced in the total absence of Mg^{2+} .

Starch accumulation

Iodine staining of *S. carnosa* leaves treated with different magnesium concentration showed a high starch accumulation in leaves of deficient ones (Fig. 2). Indeed, 0.00 and 0.01 mM Mg^{2+} -treated leaves exhibited a dark color indicating high starch concentration as compared to the control that showed a clear color, a sign of much less starch. At 0.05 mM Mg^{2+} , leaves of *S. carnosa* were only slightly affected as compared to control ones.

Chloroplast ultrastructure

Fully expanded leaves showed a variation both in size and shape of the chloroplasts depending on Mg^{2+} availability (Fig. 3). This variation was due to the accumulation of large starch grains that increased upon decreasing Mg^{2+} concentration in the medium. At 1.50 mM Mg^{2+} , the optimal concentration for growth, leaves exhibited lenticular chloroplasts with typical thylakoid system. Granum thylakoids were well arranged and aligned, and some of them contained few and small starch grains. Decreasing Mg^{2+} concentration resulted in drastically altered chloroplasts. At 0.05 mM Mg^{2+} , an accumulation of small starch grains was observed, while at 0.01 mM Mg^{2+} , the chloroplasts became round and bigger as a result of a massive accumulation of oversized starch grains. The number of grana was increased, and thylakoids were swollen. Under complete magnesium deficiency, chloroplasts were severely disrupted. Starch grains were bigger and more in number.

Sugar concentrations

The carbohydrate assay showed the lowest values of glucose, fructose, and sucrose at 1.50 and 0.05 mM Mg^{2+} (Fig. 4). However, in plants fed with 0.01 mM Mg^{2+} or with Mg^{2+} -free nutrient solution (0.00 mM Mg^{2+}), their concentrations were, respectively, 21, 11, and 7 times higher than those of the control. The sum of the three soluble sugars showed the same trend with no significant variation at 0.05 mM Mg^{2+} and a marked increase below this magnesium level, the effect being significantly higher at 0.00 than at 0.01 mM Mg^{2+} (Fig. 5).

A significant accumulation of starch was also noticed under magnesium deficiency conditions (Fig. 5). The lowest values were recorded at 1.50 and 0.05 mM Mg^{2+} . Starch concentration was more than 2 times higher in plants treated with 0.01 mM Mg^{2+} and about 4 times higher at complete magnesium deprivation (0.00 mM Mg^{2+}).

Pigment concentrations and patterns

For all pigment concentrations and patterns, no significant difference was recorded between 0.05 mM Mg^{2+} treatment and the control (Table 1). At 0.01 mM Mg^{2+} , differences began to appear; chlorophyll *a* (Chl*a*), chlorophyll *b* (Chl*b*), and their sum were reduced to the 35 % of the control. As regards Chl*a*/Chl*b* ratio, a slight increase was found in leaves under absolute Mg^{2+} absence, which suggests that Chl*b* production was slightly more affected than that of Chl*a*. Carotenoid (Car) concentration ranged from 0.09 mg g^{-1} FW at 1.50 mM Mg^{2+} to 0.03 mg g^{-1} FW at 0.00 mM Mg^{2+} . Thus, Chl/Car ratio exhibited a slight increase under absolute magnesium absence, suggesting that chlorophyll biosynthesis was more affected than that of carotenoids.

Magnesium concentration

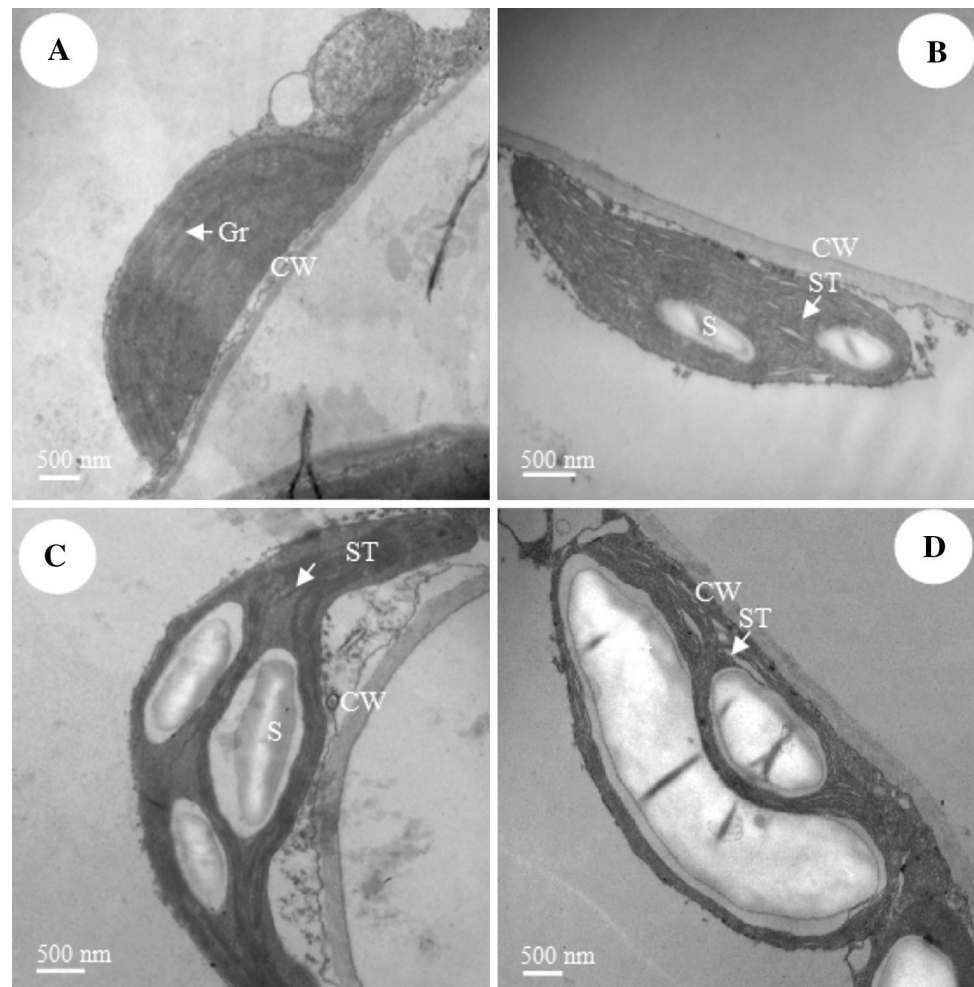
The highest magnesium concentration was noticed in control plants (0.49 mmol g^{-1} dry weight (DW); Fig. 6). With decreasing magnesium concentration in the medium, its concentration in leaves was significantly reduced. Decreases were to 29, 10, and 4 % of the control at 0.05, 0.01, and 0.00 mM Mg^{2+} , respectively.

Discussion

Optimal (1.50 mM) and suboptimal (0.05 mM) Mg^{2+} concentrations

According to Marschner et al. (1996), mineral nutrition is a major determinant of biomass allocation among environmental factors. In the present work, no difference was

Fig. 3 Ultrastructural changes in chloroplasts of mature leaves of *S. carnosus* plants grown for 5 weeks in glasshouse in the presence of 1.50 (a), 0.05 (b), 0.01 (c), or 0.00 (d) mM Mg^{2+} in the culturing medium. CW cell wall, Gr granum, S starch grain, ST swollen thylakoid. For each treatment, at least 10 micrographs were taken for 10 different chloroplasts, but only one was presented



observed in morphological aspect of *S. carnosus* plants grown at 1.50 (control) and 0.05 mM Mg^{2+} ; leaves were green (without any chlorosis) and well developed. Indeed, although their magnesium concentration was reduced to the 29 % of the control at 0.05 mM Mg^{2+} , it was maintained above the critical deficiency level of 0.05 mmol g^{-1} DW (Ding et al. 2006), which may explain the absence of visible symptoms of magnesium deficiency application. Hence, pigment concentrations in these two treatments were similar and much higher than those of the two other treatments. According to mineral profiles, Hermans et al. (2004) found that magnesium concentration in shoots of sugar beet could fall to 0.12 mmol g^{-1} DW without chlorosis development and with no effect on photosynthesis parameters. In contrast, Ding et al. (2006) noticed that under 0.05 mM Mg^{2+} , an enhancement of interveinal chlorosis was observed in leaves of rice plants with a dramatic reduction of net photosynthetic rate and total chlorophyll as compared to control ones (1.00 mM Mg^{2+}). Cakmak and Marschner (1992) explained such differences between plant species in the expression of visual

magnesium deficiency symptoms or in critical deficiency concentrations of magnesium in leaves by constitutive differences in generation or detoxification of harmful O_2 species in the chloroplasts. But in fact, chlorosis is a result of several factors that are in relation to growth rate and chlorophyll synthesis/degradation balance. If the resulting quantity of chlorophylls per leaf area is more affected than the leaf expansion, a phenomenon of dilution occurs for these pigments, leading to the appearance of chlorosis symptoms (Abadía et al. 2000). This indirectly includes magnesium mobility from old to young leaves as well as its use efficiency for chlorophyll synthesis and biomass production as it was shown for iron (Rabhi et al. 2007).

In most of the higher plants, the principal end products of leaf photosynthesis are starch and sucrose. Hence, different environmental factors, in particular macronutrient (N, P, K and Mg) deficiencies (Druege et al. 2000; Hermans et al. 2006; López-Bucio et al. 2003; Marschner 1995; Marschner et al. 1996), were supposed to affect partitioning of sucrose and starch (Ding and Xu 2011). In the last decade, magnesium deficiency was reported to

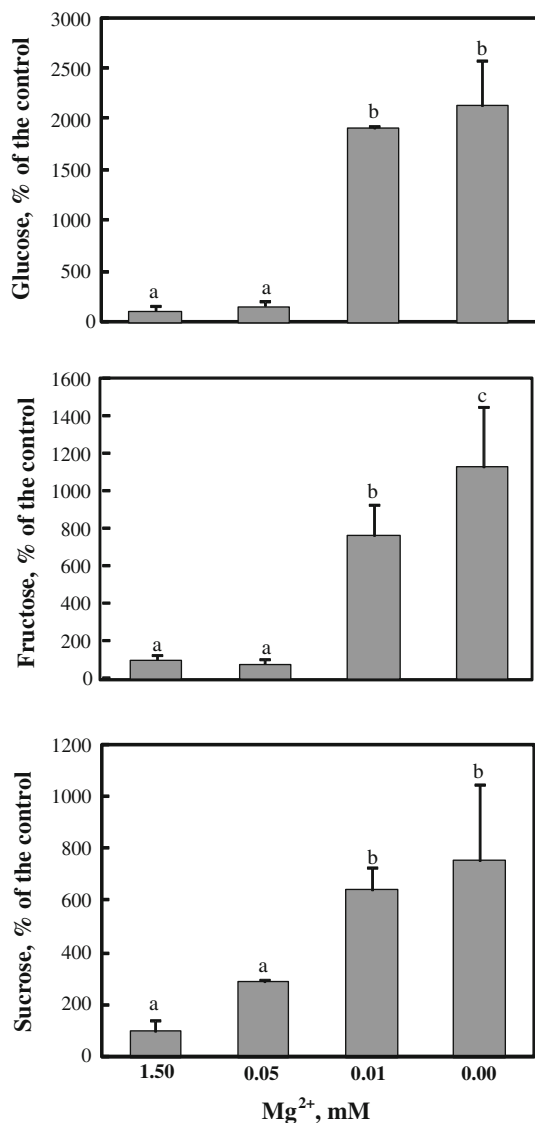


Fig. 4 Soluble sugar (glucose, sucrose, and fructose) concentrations in mature leaves of *S. carnosia* plants grown for 5 weeks in glasshouse in the presence of 1.50, 0.05, 0.01, or 0.00 mM Mg²⁺ in the medium. Values are means of five measurements. Values followed by *different letters* are significantly different according to Duncan's test at $P \leq 0.05$

affect carbon partitioning (Hermans et al. 2005). Our data showed that the lowest concentrations of soluble sugars and starch were obtained in leaves of *S. carnosia* plants treated with 1.50 and 0.05 mM Mg²⁺, and no difference was noticed between the two treatments. These results are in accordance with those of pigment concentrations, which suggest that sugars acted as signals in the regulation of their own partitioning as well as that of other plant functions, in particular photosynthesis (Chiou and Bush 1998; Rook and Bevan 2003). In contrast to our results, Ding et al. (2006) found that leaves of rice plants treated with 0.05 mM Mg²⁺ showed higher concentrations of both

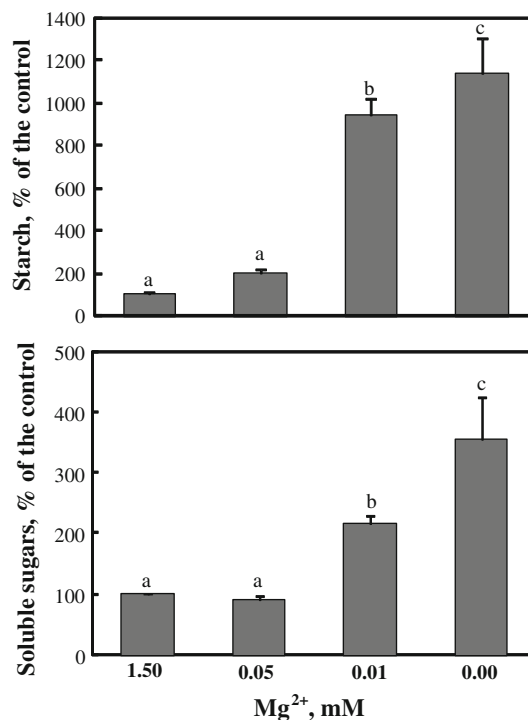


Fig. 5 Starch and sum of soluble sugar (glucose, sucrose, and fructose) concentrations in mature leaves of *S. carnosia* plants grown for 5 weeks in glasshouse in the presence of 1.50, 0.05, 0.01, or 0.00 mM Mg²⁺ in the medium. Values are means of five measurements. Values followed by *different letters* are significantly different according to Duncan's test at $P \leq 0.05$

Table 1 Pigment concentrations and patterns in fully expanded leaves of *S. carnosia* plants grown for 5 weeks in glasshouse in the presence of 1.50, 0.05, 0.01, or 0.00 mM Mg²⁺ in the medium

Mg (mM)	1.50	0.05	0.01	0.00
Chla (mg g ⁻¹ FW)	0.77b	0.81b	0.27a	0.18a
Chlb (mg g ⁻¹ FW)	0.43c	0.43c	0.15b	0.08a
Chla + Chlb (mg g ⁻¹ FW)	1.20b	1.24b	0.42a	0.26a
Chla/Chlb ratio	1.79a	1.88ab	1.81a	2.38b
Car (mg g ⁻¹ FW)	0.09b	0.1b	0.03a	0.03a
Car/Chl ratio	0.07a	0.08a	0.08a	0.11a

Values are means of six replicates. For each parameter, values followed by different letters are significantly different according to Duncan's test at $P \leq 0.05$

starch and soluble sugars as compared to the control. These authors related such accumulation to an impairment of carbohydrate export from source to sink sites.

Under control treatment (1.50 mM Mg²⁺), no structural changes were found in chloroplasts. Hence, these plants exhibited a normal lenticular shape and typical thylakoidal system, which can explain the highest values obtained for pigment concentrations since the structure of the chloroplast is known to have a relationship with photochemistry

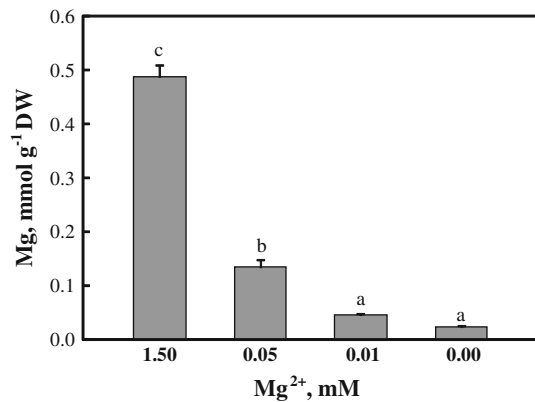


Fig. 6 Mg²⁺ concentrations in mature leaves of *S. carnosia* plants grown for 5 weeks in glasshouse in the presence of 1.50, 0.05, 0.01, or 0.00 mM Mg²⁺ in the medium. Values are means of three experiments. Values followed by *different letters* are significantly different according to Duncan's test at $P \leq 0.05$

activity (Jiang et al. 2007). However, at 0.05 mM Mg²⁺, a slight alteration of chloroplasts was noticed; small starch grains were found.

Deficiency (0.01 mM) and absolute absence (0.00 mM) of Mg²⁺

After 3 weeks of treatment, symptoms of magnesium deficiency started to appear with development of interveinal chlorosis in old leaves of plants grown under complete magnesium deprivation (0.00 mM Mg²⁺). After 5 weeks of treatment, these symptoms were obvious at low magnesium level (0.01 mM Mg²⁺), the effect being more pronounced in plants grown in Mg²⁺-free medium. Hence, under absolute absence of Mg²⁺, many necrotic spots appeared not only in old leaves but also in young ones. These observations were in accordance with those of Hermans and Verbruggen (2005) who described slight interveinal chlorosis in *Arabidopsis thaliana* plants after 15 days of Mg²⁺ removal that were intensified into necrotic spots with time. Hermans et al. (2004) noticed also a development of chlorotic areas in sugar beet plants under magnesium starvation. Hence, photosynthesis inhibition was explained as a result of chlorosis (Asada 1999; Foyer and Noctor 2000) since magnesium is a key constituent of chlorophyll molecule (Cakmak and Kirkby 2008). According to Bergmann (1992), deficiency symptoms appear first in old leaves due to magnesium high mobility. In fact, just 20 % of the total magnesium is bound to chlorophyll while the remaining fraction (80 %) is present in mobile forms (Marschner 2012). In this work, a significant reduction of Chla and Chlb was found upon 0.01 mM Mg²⁺ treatment in which further increased at 0.00 mM Mg²⁺. Therefore, total chlorophyll concentration

was reduced, respectively, to the 34 and 21 % of the control. As described earlier, we can conclude that morphological aspect reflects pigment contents. Under such nutritional stress, Tewari et al. (2006) noticed in mulberry plants a reduction to the 54 and 76 % of the control in chlorophyll and carotenoid concentrations, respectively. Also, a gradual loss of photosynthetic pigments was reported in sugar beet plants under magnesium starvation (Hermans et al. 2004).

Several authors explained the decline in chlorophyll concentrations and the development of chlorosis and necrosis by a decrease in leaf magnesium concentrations under its deficiency conditions (Cakmak and Marschner 1992; Cakmak 1994). The mineral analysis carried out on leaves of *S. carnosia* plants can explain this fact. Indeed, leaf Mg²⁺ concentration significantly decreased with the decreasing Mg²⁺ concentration in the medium. Hence, Mg²⁺ concentration decreased from 0.49 mmol g⁻¹ DW in leaves of control plants to 0.02 mmol g⁻¹ DW in leaves of plants grown in Mg²⁺-free solution (0.00 mM Mg²⁺), which was below the critical deficiency level of 0.05 mmol g⁻¹ DW (Ding et al. 2006). According to Cakmak and Marschner (1992), under magnesium deficiency, its concentrations declined with leaf age and such decline is related to magnesium re-translocation to young leaves, "dilution effect" due to carbohydrate accumulation, or both. In addition, Hermans et al. (2005) noticed that under these conditions, the most dramatic decrease in magnesium concentrations of sugar beet plants was found in leaves. This resulted in a decline in chlorophyll *a* and *b* concentrations (Hermans et al. 2004) and an impairment of sugar metabolism (Hermans et al. 2005).

Our results showed a marked accumulation of soluble sugars (glucose, fructose, and sucrose) in leaves of plants treated with 0.01 and 0.00 mM Mg²⁺. Ding and Xu (2011) also reported a significant soluble sugar accumulation in leaves of rice plants when treated with 0.01 mM Mg²⁺. Hermans and Verbruggen (2005), who found a high sugar accumulation in *Arabidopsis thaliana* plants under magnesium deficiency, suggested a disruption of balance between sucrose synthesis and its utilization or by an interference of magnesium deficiency with sucrose export from the source leaf or both simultaneously. Besides, a massive accumulation of sucrose and starch was noticed under such conditions in the leaves of rosette of sugar beet plants. This increase was in accordance with severe inhibition of sucrose export from these leaves into roots (Hermans et al. 2005). Cakmak et al. (1994a) found that magnesium deficient plants can experience a 3-to-12-fold sucrose concentration than plants fed with sufficient magnesium concentration. This marked increase was considered as a direct response to magnesium deficiency before any adverse effect on morphological aspect, growth,

chlorophyll concentration, or photosynthetic activity (Cakmak et al. 1994a, b; Hermans and Verbruggen 2005). In our present experiments, massive starch accumulation was also noticed under magnesium deficiency and absolute absence conditions. Similar results were found by Hermans et al. (2005) in sugar beet plants, who noticed an accumulation of soluble sugars and starch before the development of chlorosis and necrosis. These authors considered such increase in sugar concentrations as an intermediate sink in photosynthetic leaves when sucrose accumulation and its utilization are not equilibrated. Many different explanations were given to this accumulation such as an antisense expression of sucrose symporters (Rook et al. 2001), a co-suppression of the plasma membrane H^+ -ATPase (Zhao et al. 2000), and sink consumption reduction (Ntsika and Deltrot 1986; Grusak et al. 1990; Paul and Foyer 2001).

At the ultrastructural level, we found that at 0.01 and 0.00 mM Mg^{2+} , plants showed chloroplasts with disrupted thylakoids that were reduced to a few isolated membranes and an accumulation of large starch grains that changed chloroplast shape from oblong to round. Similar findings were noticed in conifer needles under Mg and K deficiencies by Fink (1991), who described these changes as indicators of a strongly reduced photosynthetic activity. In fact, starch accumulation was found under several biotic and abiotic stresses (Morales et al. 1997; Zhang et al. 2010).

Conclusion

Although Mg^{2+} concentration in leaves of plants fed with 0.05 mM Mg^{2+} was significantly reduced, it did not cause morphological symptoms, although plant growth was moderately decreased (data not shown). The absence of magnesium deficiency symptoms was confirmed by the maintenance of pigment concentrations and patterns, as well as soluble sugar and starch concentrations. At the ultrastructural level, however, a slight alteration was noticed in chloroplast. Below 0.05 mM Mg^{2+} , leaves experienced deficiency symptoms (development of chlorosis and even necrosis), a decrease in pigment concentrations and high sugar accumulation that was detected through both chloroplast ultrastructure and sugar analysis. Moreover, for the majority of the studied parameters, the effect was significantly more severe in 0.00 mM Mg^{2+} treatment than in 0.01 mM Mg^{2+} one. In conclusion, we found that in the 1.50–0.05 mM Mg^{2+} concentration range, fully expanded leaves of *S. carnosus* seem to manage well the available quantity of Mg^{2+} to maintain the integrity of their chloroplasts, which constitutes an adaptive trait to low availability of this macronutrient.

Author contribution Miss Nèjia Farhat was responsible for the work from experiment conception to manuscript submission. Dr. Mokded Rabhi contributed to the experiment conception, plant culture, sample preparation for TEM analysis, data analysis, and manuscript preparation. Dr. Marianna Krol and Dr. Zouhaier Barhoumi contributed to the ultrastructural study. Dr. Alexander, G. Ivanov, and Mr. Avery McCarthy helped in biochemical assays. Prof. Chedly Abdelly, Prof. Abderrazak Smaoui, and Prof. Norman P. A. Hüner are the supervisors of the work.

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