Nutritional Disorders of Macronutrients in *Bletia catenulata*

Carlos Henrique Oliveira de David

Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, Brazil

Vespasiano Borges de Paiva Neto

Universidade Federal do Vale do São Francisco, Petrolina, Brazil

Cid Naudi Silva Campos, Priscilla Maria da Silva Liber Lopes,
and Paulo Eduardo Teodoro

Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, Brazil

Renato de Mello Prado

Universidade Federal de Mato Grosso do Sul, Chapadão do Sul, Brazil;
Department of Soils and Fertilizers, Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP), Jaboticabal, Brazil

Additional index words. Orchidaceae, nutritional disorder, plant nutrition

Abstract. This study evaluated the impact of macronutrient omission on nutritional disorders and the in vitro growth of *Bletia catenulata*. The experiment was performed in a growth room, with a controlled environment, in the biotechnology laboratory of the Campus of Chapadão do Sul (CPCS/UFMS). The experiment consisted of a completely randomized design, with seven treatments and four replications, corresponding to the following treatments: complete treatment (N, P, K, Ca, Mg, S, B, Mn, Zn, Cu, Fe, and Mo), nitrogen omission (–N), phosphorus omission (–P), potassium omission (–K), calcium omission (–Ca), magnesium omission (–Mg), and sulfur omission (–S) in *B. catenulata*. After detecting the symptoms, plant height, leaf area, dry weight, micronutrient content in the shoot, micronutrient accumulation in the shoot, and visual symptoms of nutritional deficiency were evaluated. The –N, –P, –Ca, and –Mg treatments hindered plant growth and dry weight yield. The deficiency of each nutrient resulted in morphological changes, which were verified by typical visual symptoms of nutritional disorder for each nutrient.

The genus *Bletia* Ruiz & Pav. is composed of more than 35 species. Only two have been registered in Brazil, and one of them is *Bletia catenulata*. This species has a terrestrial habit and sympodial growth, presenting commercial potential as a result of the rare beauty of its leaves and flowers.

Reports of this species occurring in different locations in the Brazilian territory, such as Tocantins and Maranhão (Silva et al., 1995), Minas Gerais (Araújo et al., 2002), Distrito Federal (Batista et al., 2005), São Paulo (Ferreira et al., 2010), and Mato Grosso do Sul (Barros et al., 2018), as well as in other countries such as Bolivia (Vásquez et al., 2003) and Paraguay (Schinini, 2010) have been documented.

B. catenulata was found in four different sites in the northeastern region of the Mato Grosso do Sul State, always with low frequency. In some sites, it is exposed to

environments that undergo human disturbance. In addition, this orchid has terrestrial habits and superficial roots, and often occurs near watercourses (Paiva Neto et al., 2015).

However, in the literature we only found one study involving aspects related to the cultivation of this species, carried out by Paiva Neto et al. (2015). These authors demonstrated that the in vitro germination of seeds of all fruit resulting from different pollinations was low, with 8.6% being the greatest percentage value of protocorms obtained. Therefore, *B. catenulata* is self-compatible, generating viable seeds, but is pollinator dependent for sexual reproduction. In addition, this species has specific conditions for growing because the plant is a geophyte and has reserve organs, where gems are found and where food is stored, that allow these plants to survive in adverse weather conditions.

Hence, it is clear there is a need for information on the cultivation of *B. catenulata*, including nutritional requirements and the most limiting nutrients for this plant. Each nutrient has a specific function in the plant's metabolism, and all of them are essential to the plant cycle. The lack of an unsatisfactory amount of a particular nutrient promotes metabolic changes in the plant (Prado and Vidal, 2008), causing visual symptoms in plant tissues, depending on the missing nutrient.

Thus, the deficiency symptoms of a particular nutrient are typical and may appear in several plant organs, such as leaves, stems, roots, and fruit. These symptoms assist in the nutritional evaluation of plants (Gontijo et al., 2007). However, mineral deficiency symptoms present particular inter- and intraspecies responses as a result of gene expression and environmental factors (Hawkesford et al., 2012).

The knowledge of the symptomatology caused by the deficiency of a specific nutrient is fundamental to the use of this method of plant nutritional evaluation. Thus, the cultivation of plants in protected systems using culture medium is an essential tool for plant nutrition studies, especially those that induce nutritional deficiency (Prado, 2008).

Studies involving nutritional disorders in orchids are still incipient in the literature, specifically for *B. catenulata*. Therefore, nutritional management of this species has not yet been consolidated, which can affect plant yield and quality. Therefore, work in this field must be developed. We evaluated the impact of macronutrient omission on nutritional disorders and in vitro growth of *B. catenulata*.

Material and Methods

B. catenulata plants were collected in Dec. 2013 in Cachoeira da Rapadura (lat. 18°25'36.54"S, long. 52°56'57.23"W) in Costa Rica, Mato Grosso do Sul State, Brazil. The experiment was carried out in a growth room for 111 d, in a controlled environment, in the biotechnology laboratory of CPCS/UFMS. Growth room conditions were maintained constant at 27 ± 2 °C, with 16/8 h light/dark, and an irradiance of 30 m²·s⁻¹, from Osram® fluorescent tubes, 40 W daylight.

The experiment consisted of a completely randomized design, with seven treatments and four replications, corresponding to the following treatments: complete (N, P, K, Ca, Mg, S, B, Mn, Zn, Cu, Fe, and Mo), –N, –P, –K, –Ca, –Mg, and –S. Each experimental unit was composed of a glass bottle (height, 13.5 cm; diameter, 8 cm) containing 70 mL nutrient solution (Hoagland and Arnon, 1950) adjusted to pH 5.8 before the autoclaving process at 120 °C, 1 atm, and 20 min. The prepared solution was semisolid with nutrients and agar. For the Fe supply, we used the complexed form with Fe-EDDHMA (YaraVita Rexolin®), and the water used was deionized. To each bottle, *B. catenulata* seedlings were added and the bottle sealed with transparent film paper. The process for obtaining the seedlings followed the methodology recommended by Paiva Neto et al. (2015).

The seedlings were selected according to uniformity and were transplanted into the culture medium (using a pair of tweezers in a Filter Flux® horizontal laminar flow hood) with the respective treatments, and were cultivated until deficiency symptoms appeared. The plants transferred to the nutrient-deficient solution came from a culture medium with all nutrients available.

All plants were analyzed daily for visual symptoms of nutritional disorders related to

Received for publication 13 June 2019. Accepted for publication 17 July 2019.

This study was funded in part by Federal University of Mato Grosso do Sul and Higher Education Personnel Improvement Coordination - Brazil (CAPES) - Finance Code 001.

P.E.T. is the corresponding author. E-mail: eduteodoro@hotmail.com.

Table 1. Macronutrient content shoot dry weight of *Bletia catenulata* plants as a function of the treatments.

Treatments	N	P	K	Ca	Mg	S
	Shoot (g·kg ⁻¹)					
Complete	13.10 c	1.85 ab	19.95 ab	10.25 a	2.05 b	2.95 a
–N	8.60 d	1.05 bc	12.30 ab	3.25 b	0.80 c	0.80 b
–P	17.20 b	0.55 c	19.58 ab	11.28 a	2.30 b	2.08 ab
–K	23.25 a	0.95 bc	5.25 c	10.80 a	2.05 b	3.15 a
–Ca	—	2.15 a	26.35 a	2.00 b	3.85 a	3.35 a
–Mg	18.55 b	2.30 a	23.88 a	11.00 a	0.45 c	3.25 a
–S	13.30 c	1.68 ab	18.98 ab	9.33 a	1.78 b	0.75 b
F test	112.32**	9.22**	11.03**	25.92**	32.63**	7.21**
MSD	3.32	1.02	10.06	3.60	0.90	1.99
cv (%)	10.6	28.9	23.9	18.6	20.4	36.5

**Significant at 1% probability by F test. Means followed by different letters in the columns differ by the Tukey test ($P < 0.05$). —, the plant material was not enough for chemical analysis.

Table 2. Shoot dry weight macronutrient accumulation in *Bletia catenulata* plants as a function of the treatments.

Treatments	N	P	K	Ca	Mg	S
	Shoot (mg/plant)					
Complete	1.14 a	0.16 a	1.73 a	0.89 a	0.18 ab	0.26 a
–N	0.32 c	0.05 bc	0.77 cd	0.17 c	0.04 cd	0.06 bc
–P	0.78 abc	0.03 c	0.89 bcd	0.52 b	0.11 bc	0.09 bc
–K	1.46 a	0.06 bc	0.25 d	0.68 ab	0.13 ab	0.20 ab
–Ca	0.38 bc	0.11 ab	1.33 abc	0.09 c	0.19 a	0.17 abc
–Mg	0.88 abc	0.11 ab	1.14 abc	0.53 b	0.02 d	0.16 abc
–S	1.08 ab	0.14 a	1.52 ab	0.75 ab	0.15 ab	0.04 c
F test	7.39**	10.75**	11.03**	18.31**	16.19**	6.74*
MSD	0.71	0.07	0.71	0.32	0.08	0.14
cv (%)	35.4	32.6	27.8	26.6	28.1	43.8

*, *Significant at 1% and 5% probability, respectively, by F test. Means followed by different letters in the columns differ by the Tukey test ($P < 0.05$).

each treatment. At 34, 89, 110, 110, 110, and 110 d after treatment application, the symptoms of –N, –Ca, –K, –P, –Mg, and –S, respectively, were detected and characterized. Plant height was measured with a ruler (from the stem to the apex of the last fully expanded leaf), and leaf area was measured with a leaf area meter (model LI-3000C; LI-COR). At 110 d after treatment application, based on the appearance of the deficiency symptoms, plants were separated into shoots and roots, washed, stored in paper bags, and then dried in a forced-air circulation oven (65 °C) until a constant weight was reached. Afterward, shoot dry weight was obtained using a digital scale, and the material was then ground in a Willey mill.

Macronutrient contents were determined chemically according to the methodology proposed by Bataglia et al. (1983). After that, macronutrient accumulation was calculated using the dry weight content, based on the values of shoot dry weight.

Results were subject to analysis of variance, followed by the Tukey's test at the 5% probability level for means comparison, using the statistical software ASSISTAT (version 7.6 beta) (Silva, 2013).

Results and Discussion

Nitrogen. *B. catenulata* plants subject to the complete treatment and –N treatment presented N content in the shoot dry weight of 13.10 and 8.60 g·kg⁻¹, respectively, indicating that N omission decreases the element leaf content (Table 1). N accumulation in the plant shoot in nutrient solution with –N decreased 72% when compared with the

complete treatment (Table 2). N omission was the one that decreased plant growth, according to height and leaf area, which reflected on shoot dry weight accumulation in relation to the complete treatment (Table 3). Rodrigues et al. (2011) also verified the importance of N content and sources in orchid nutrition, obtaining a significant reduction in the growth of *Cattleya loddigesii* 'Type' species at low concentrations of this element.

These results are consistent with those reported by Costa et al. (2017), Silva et al. (2011), and Tanemura et al. (2008), who evaluated N omission in watermelon, cucumber, and cabbage plants, respectively, and concluded that the growth variables were affected by the N deficiency. This fact can be attributed to the limiting effect that N exerts on plant growth because it participates in the synthesis and composition of several organic compounds, making part of the structure of amino acids, proteins, N bases, nucleic acids, coenzymes, enzymes, vitamins, pigments, and by-products; and participating in processes such as ion absorption, respiration, photosynthesis, and cell multiplication and differentiation. Therefore, this nutrient is fundamental to the plant cycle (Epstein and Bloom, 2006).

The symptom of N deficiency appeared at the beginning of plant development (34 d after treatment application) and was characterized by growth inhibition and chlorosis in old leaves (Supplemental Fig. 1A). Chlorosis in older leaves is the first typical symptom of N deficiency. This symptom occurs as a result of the high mobility of this nutrient in the phloem and the high demand by developing tissues (Hawkesford et al., 2012). As a

Table 3. Plant height, leaf area, and shoot dry weight of *Bletia catenulata* plants as a function of the treatments.

Treatments	Plant ht (cm)	Leaf area (cm ²)	Dry wt (g)
Complete	19.18 a	21.01 a	0.08 a
–N	3.08 d	2.40 c	0.04 b
–P	11.33 bc	9.86 bc	0.05 b
–K	12.58 bc	14.87 ab	0.06 ab
–Ca	10.60 bc	11.90 b	0.05 b
–Mg	7.48 cd	10.79 bc	0.05 b
–S	15.68 cd	16.95 ab	0.08 a
F test	17.90**	10.46**	8.16**
MSD	5.80	8.55	0.03
cv (%)	21.7	29.2	21.8

**Significant at 1% probability by F test. Means followed by different letters in the columns differ by the Tukey test ($P < 0.05$).

strategy to meet N demands, the plant deteriorates stromal proteins to release N compounds, such as amino acids (Feller et al., 2008). This symptom has also been reported by Ji-Yong et al. (2012) in cucumber.

Phosphorus. The P content in the shoot dry weight was greater in the complete treatment than in the –P treatment (Table 1). Thus, the P content in the shoot dry weight of the –P treatment (0.55 g·kg⁻¹) is less than that of the complete treatment (1.85 g·kg⁻¹), indicating the nutrient deficiency in the plant. The –P treatment reduced P accumulation in the shoot by 81.25% (Table 2).

Growth parameters were affected by the –P treatment, which resulted in shorter plant height, fewer number of leaves, and less leaf area, culminating in lower shoot dry weight yield (Table 3). Similar results were reported by Prado and Vidal (2008). Mengel and

Kirkby (1987) stated that the development of P-deficient plants reduces because several processes are affected, such as the synthesis of proteins and nucleic acids.

P omission led to visual symptoms of deficiency. Plants were a dull dark green, especially the older leaves (Supplemental Fig. 1B). This result was due to the high mobility of this nutrient in the phloem, causing the P-deficient plant to redistribute the nutrient to the developing tissues (Hawkesford et al., 2012).

In addition, older leaves were narrower. The authors studied nutrient omission in millet and noticed that older leaves of plants subject to P omission were dark green and narrower. According to Taiz et al. (2017), P deficiency induces the excessive production of anthocyanins. This phenomenon may lead to the appearance of purple spots, which did not occur in *Bletia catenulata* plants.

Potassium. *B. catenulata* plants subject to the -K treatment and complete treatment presented K content in the shoot dry weight of 5.25 and 19.95 g·kg⁻¹, respectively (Table 1). Thus, K accumulation decreased by 85.55% in relation to the complete treatment (Table 2). K omission significantly affected plant height when compared with the complete treatment (Table 3).

These results can be attributed to the problems caused by K deficiency in metabolic processes because this nutrient is an essential enzymatic activator, acting in photosynthesis and cell osmoregulation, and synthesis of nucleic acids, carbohydrates, and proteins (Hawkesford et al., 2012).

K omission resulted in visual symptoms of deficiency, causing chlorosis and necrosis on the lower third leaves (Supplemental Fig. 1C). These symptoms occurred as a result of the high mobility of this nutrient in the phloem. Therefore, in the absence of this element, K accumulation in older leaves is translocated to younger leaves. Pathak et al. (2014) observed that K-deficient plants usually accumulate soluble N compounds, such as amines, agmatines, and putrescines. The authors also suggest that putrescines might be responsible for the necrotic spots from the chlorosis symptoms on the leaves of K-deficient plants.

Leaves also presented symptoms of shrinkage, with edges facing upward. The cause of this symptom was excessive water loss by the plant, making it flaccid. According to Prado (2008), K-deficient plants have low water use efficiency, causing poor control of the opening and closing of the stomata, and consequently increasing transpiration and water loss rates.

Similar symptoms have been described by Mattos et al. (2002) and Prado and Leal (2006) in a -K treatment carried out with *Brachiaria* and sunflower plants, respectively.

Calcium. The complete treatment resulted in 10.25 g·kg⁻¹ Ca content in the shoot. Conversely, the -Ca treatment presented a Ca content in the shoots of 2.00 g·kg⁻¹ (Table 1). Therefore, Ca accumulation

decreased by almost 90% in relation to the complete treatment (Table 2).

The -Ca treatment affected plant height and leaf area, which decreased by 44.7% and 43.36%, respectively (Table 3), when compared with the complete treatment. Consequently, shoot dry weight production decreased.

Ca omission resulted in the development of symptoms of nutritional disorder in new leaves because the redistribution of this nutrient via the phloem is limited and is characterized by irregular, rough, undulated, and reduced growth (Supplemental Fig. 1D). These symptoms can be attributed to the involvement of Ca in cell structuring, stabilization, expansion, and stretching (Hochmuth et al., 2004).

Magnesium. The Mg content in the shoot was 2.05 and 0.45 g·kg⁻¹ in the complete treatment and -Mg treatment, respectively (Table 1). Therefore, Mg content decreased significantly in the -Mg treatment when compared with the complete treatment. These results led to less Mg accumulation in the -Mg treatment (Table 2). All macro-nutrient contents in the shoot decreased when the -Mg treatment was applied (Table 1).

The -Mg treatment reduced plant growth significantly, resulting in shorter plants and smaller leaf area, leading to lower shoot dry weight accumulation in relation to the complete treatment (Table 3). Moreover, it resulted in symptoms of nutritional disorder in *B. catenulata*, which manifested in the lower third leaves as a result of the high Mg mobility to younger tissues of active growth.

Symptoms were characterized by a slight yellowing between veins as Mg is a component of the chlorophyll molecule (Verbruggen and Hermans, 2013). The chlorophyll content might have decreased, causing chlorosis in these leaves, leading to a thick reticulate aspect, with leaf blade wrinkling. These symptoms were similar to those observed by Prado and Leal, (2006) and Silva (2013), who investigated Mg omission in sunflower and pepper, respectively.

Sulfur. The S content in the shoot of the complete treatment was 2.95 g·kg⁻¹. The -S treatment presented an S content in the shoot of 0.75 g·kg⁻¹ (Table 1). Thus, S accumulation decreased by 84.6% when the -S treatment was applied (Table 2).

S omission significantly reduced plant height. However, it did not affect shoot dry weight yield and shoot leaf area when compared with the complete treatment (Table 3). The treatment resulted in the development of nutritional disorder symptoms in *B. catenulata* plants, with new leaves that were light green (Supplemental Fig. 1E), demonstrating the main physiological S sink (Silva et al., 2003). This result is consistent with those reported by Malavolta (1997), who confirmed the yellowing of younger leaves as a typical symptom.

Conclusion

The -N, -P, -Ca, and -Mg treatments hindered plant growth and dry weight yield.

The deficiency of each nutrient resulted in morphological changes, which were verified by typical visual symptoms of nutritional disorders for each nutrient.

Literature Cited

- Araújo, G.M., A.A.A. Barbosa, A.A. Arantes, and A.F. Amaral. 2002. Composição florística de veredas no município de Uberlândia, MG. Rev. Bras. Bot. Braz. J. Bot. 25(4):475–493.
- Barros, F., C.F. Hall, V.B. Paiva Neto, and J.A.N. Batista. 2018. Check-list das Orchidaceae do estado de Mato Grosso do Sul, Brasil. Iheringia Ser. Bot. 73(Suppl):287–296.
- Bataglia, O., A. Furlani, J. Teixeira, P. Furlani, and J. Gallo. 1983. Métodos de análise química de plantas. Campinas, Instituto Agrônomo de Campinas, Boletim técnico.
- Batista, J.A.N., J.B. Bianchetti, and K.F. Pellizzaro. 2005. Orchidaceae da Reserva Ecológica do Guará, DF, Brasil. Acta Bot. Bras. 19(2):221–232.
- Costa, L.C., V.M.V. Carmona, A.B. Cecílio Filho, C.S. Nascimento, and C.S. Nascimento. 2017. Symptoms of deficiências macronutrientes in watermelon. Comun. Sci. 8(1):80–92.
- Epstein, E. and A.J. Bloom. 2006. Nutrição mineral de plantas: Princípios e perspectivas. Editora Planta, Londrina.
- Feller, U., I. Anders, and K. Demirevska. 2008. Degradation of rubisco and other chloroplast proteins under abiotic stress. Gen. Appl. Plant Physiol. 34(1–2):5–18.
- Ferreira, A.W.C., M.I.S. Lima, and E.R. Pansarin. 2010. Orchidaceae in the central portion of São Paulo State, Brazil. Rodriguésia 61(2):243–259.
- Gontijo, R.A.N., J.G. de Carvalho, R.J. Guimarães, A.N.G. Mendes, and W.E. de Bastos Andrade. 2007. Faixas críticas de teores foliares de micronutrientes em mudas de café (Coffea arabica L.). Coffee Sci. 2(2):135–141.
- Hawkesford, M., W. Horst, T. Kichey, H. Lambers, J. Schjoerring, I.S. Møller, and P. White. 2012. Functions of macronutrients, p. 135–189. In: Marschner's mineral nutrition of higher plants. 3rd ed. Elsevier.
- Hoagland, D.R. and D.I. Arnon. 1950. The water-culture method for growing plants without soil. 2nd ed. Circular California Agr. Exp. Sta. 347.
- Hochmuth, G., D. Maynard, C. Vavrina, E. Hanlon, and E. Simonne. 2004. Plant tissue analysis and interpretation for vegetable crops in Florida. University of Florida.
- Ji-Yong, S., Z. Xiao-Bo, Z. Jie-Wen, W. Kai-Liang, C. Zheng-Wei, H. Xiao-Wei, Z. De-Tao, and M. Holmes. 2012. Nondestructive diagnostics of nitrogen deficiency by cucumber leaf chlorophyll distribution map based on near infrared hyperspectral imaging. Scientia Hort. 138:190–197.
- Malavolta, E. 1997. Avaliação do estado nutricional das plantas: Princípios e aplicações. 2nd ed. Potafos, Piracicaba.
- Mattos, W.D., A.D. Santos, A.S. Almeida, B. Carreiro, and F. Monteiro. 2002. Aspectos produtivos e diagnose nutricional do capim-Tanzânia submetido a doses de potássio. Magistra 14(1).
- Mengel, K. and E.A. Kirkby. 1987. EA: Principles of plant nutrition. International Potash Institute, Bern, Switzerland.
- Paiva Neto, V.B., A.P. Mezoni, F. Barros, D.R.C. Padilha, and M.C.R.Z. Borges. 2015. The *Bletia catenulata* ornamental orchid is self-compatible but pollinator-dependent for reproduction. Pesqui. Agropecu. Trop. 45(4):473–479.
- Pathak, M.R., J.A.T. Silva, and S.H. Wani. 2014. Polyamines in response to abiotic stress

- tolerance through transgenic approaches. *GM Crops Food* 5(2):87–96.
- Prado, R.M. 2008. Nutrição de plantas. UNESP.
- Prado, R.M. and R.M. Leal. 2006. Desordens nutricionais por deficiência em girassol var. Catissol-01. *Pesqui. Agropecu. Trop.* 36(3).
- Prado, R.M. and A.A. Vidal. 2008. Efeitos da omissão de macronutrientes em solução nutritiva sobre o crescimento e a nutrição do milho. *Pesqui. Agropecu. Trop.* 38(3).
- Rodrigues, F.A., J.D.R. Soares, D.N. Santos, and M. Pasqual. 2011. KNO₃ e NH₄NO₃ no cultivo *in vitro* de orquídea (*Cattleya loddigesii* ‘Tipo’). *Plant Cell Culture Micropropagation* 7(2):61–65.
- Schinini, A. 2010. Orquídeas nativas del Paraguay. *Rojasiana*. 9(1–2):11–316.
- Silva, A.Z. 2013. Sintomas de deficiências de macronutrientes em pimentão. UNESP.
- Silva, D.J., V.H.A. Venegas, H.A. Ruiz, and R. Sant’Anna. 2003. Translocação e redistribuição de enxofre em plantas de milho e de soja. *Pesqui. Agropecu. Bras.* 38(6):715–721.
- Silva, G.F., P.C.R. Fontes, L.P.F. Lima, T.O. Araújo, and L.F. Silva. 2011. Aspectos morfoanatômicos de plantas de pepino (*Cucumis sativus* L.) sob omissão de nutrientes. *Rev. Verde Agroecol. Desenvol. Sustent.* 6(2):13–20.
- Silva, M.F.F., J.B.F. Silva, A.E.S. Rocha, F.P.M. Oliveira, L.S.B. Gonçalves, M.F. Silva, and O.H.A. Queiroz. 1995. Inventário da família Orchidaceae na Amazônia brasileira: Parte I. *Acta Bot. Bras.* 9(1):163–175.
- Taiz, L., E. Zeiger, I.M. Møller, and A. Murphy. 2017. *Fisiologia e desenvolvimento vegetal*. Artmed Editora.
- Tanemura, R., H. Kurashima, N. Ohtake, K. Sueyoshi, and T. Ohyama. 2008. Absorption and translocation of nitrogen in cucumber (*Cucumis sativus* L.) plants using the ¹⁵N tracer technique. *Soil Sci. Plant Nutr.* 54(1): 108–117.
- Vásquez, R., P.L. Ibisch, and B. Gerkmann. 2003. Diversity of Bolivian Orchidaceae: A challenge for taxonomic, floristic and conservation research. *Org. Divers. Evol.* 3(4):93–102.
- Verbruggen, N. and C. Hermans. 2013. Physiological and molecular responses to magnesium nutritional imbalance in plants. *Plant Soil* 368(1–2):87–99.

Supplemental Fig. 1. Visual symptoms of nutritional deficiency in leaves of *Bletia catenulata* cultivated in culture medium with the complete solution (CS) and omissions (–) of nitrogen (A), phosphorus (B), potassium (C), calcium (D), and sulfur (E).