Article

Concentration of macronutrients in organs of three wild *Rhynchostele* species in their natural habitat

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Abstract

In Mexico, there is little research on the nutritional concentration of orchid species developed in their natural habitat. The objective was to determine the macronutrient concentration of the wild orchids *Rinchostele maculata*, *R. rossii* and *R. aptera* in their natural habitat. The collections were made from *R. maculata* in the central zone of Veracruz, *R. rossii* was obtained from the Chichinautzin Biological Corridor in the northwest of Morelos and from the cloud forest of the Cofre of Perote, in Veracruz, the *R. aptera* species was collected in Huitzilac and Tetela of Volcan, Morelos. The nutritional concentration of N, P, K, Ca and Mg was quantified in young leaves and flower buds, new leaves, mature leaves and old leaves (in senescence), new pseudobulbs, mature pseudobulbs, old pseudobulbs; In addition, the same nutrients were quantified in the roots. It was found in *R. rosii*, *R. maculata* and *R. aptera* that the nutrient concentrations, in decreasing order, were N> K> Ca> P> Mg in young leaves, new leaves and mature leaves; with the exception of *R. rosii*, which showed the trend N> Ca> K> Mg> P, only in mature leaves. The nutrient concentration intervals in mature leaves, in percentage, were: N (1.44 - 1.86), P (0.15 - 0.29), K (0.51 - 0.9), Ca (0.32 - 0.65) and Mg (0.11 - 0.25), which could serve as preliminary reference values for nutrimental diagnostic purposes in the three species reported; likewise, these results could be useful in conservation programs and for the agronomic management of these species.

Keywords: *Rhynchostele aptera*, *Rhynchostele maculata*, *Rhynchostele rossii*, mineral nutrition.

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Introduction

Since ancient times orchids have been the most preferred flowers as ornamental plants, this is attributed to its rarity, colorful colors, aromas and shapes. The genus *Rhynchostele* possesses species with ornamental potential; however, in recent years their natural populations have been diminished, due in part to the excessive looting of collectors for sale purposes and collectors of orchids, which has caused that some are subject to special protection according to the Official Mexican Standard NOM-059 (SEMARNAT, 2010).

It has been reported that there are morphological differences within the genera of orchids depending on the collection sites, finding variation in number of leaves, leaf length and width, color tone and number of flowers, among others (Hernández-Muñoz *et al*., 2013), these variations may be due to different factors such as climate, incidence of pests and diseases, lack or excess of nutrients. In relation to the last point, there is scarce information about its nutritional composition in different stages of development and in its natural habitat.

In this context, some authors have suggested more research related to nutrient concentration in orchids (Wang and Konow, 2002), since these studies allow to know the nutritional status, as well as to identify in which organs of the plant and in what magnitude they accumulate within the same (Poole and Sheehan, 1973; Jiménez-Peña *et al*., 2013).

Most studies of mineral nutrition in orchids have focused on commercial hybrids of the *Phalaenopsis* genera (Hinnen *et al*., 1989; Wang, 1996; Hwang *et al*., 2009), *Cymbidium* (Naik *et al*., 2013; Barman and Naik, 2017), *Odontoglossum* (Yoneda *et al*., 1999) and *Dendrobium* (Tse-Leow and Khye-Tan, 2007). In general, these investigations have been carried out in controlled conditions, in soilless cultivation and with the use of nutritive solutions. On the other hand, some studies of mineral concentration in species of wild orchids, *Laelia anceps*, *L. autumnalis* and *Paphiopedilum insgine*, have been carried out in greenhouse, in soilless culture and with nutritive solutions (unpublished data); however, there are few studies that report the nutrimental concentration in wild orchids and in their natural habitat.

This information would be useful to know the nutritional status of species with ornamental potential that serve as a basis to propose conservation strategies and agronomic management (Tejeda-Sartorius *et al*., 2017). In this context, the genus *Rhynchosthele* possesses species with ornamental potential, among which are: *R. maculata* (L. O. Williams), *R. rossii* (Lindl.) and *R. aptera* (Lex.) (Espejo-Serna *et al*., 2002; Hagsater and Soto, 2008; CONABIO, 2015). Therefore, the objective was to determine the concentration of macronutrients of wild orchids *R. maculata*, *R. rossii* and *R. aptera* in their habitat.

Materials and methods

Sixteen *R. maculata* plants were collected in forest areas of the Cofre of Perote or Nauhcampatepetl, in the center of Veracruz, this region belongs to the cloud forest and is located between 1 200 and 2 100 m of altitude; between latitudes 19° 30’ and 19° 45’ north latitude and longitudes 96° 47’ and 97° 01’ west. In the case of *R. rossii*, nine plants were collected in the Chichinautzin Biological Corridor, which is a flora and fauna protection area located in the northwest area of the state of Morelos, located between 18° 50’ 30” and 19° 05’ 40” north latitude; 98° 51’ 50” and 99° 20’ 00” west longitude and the cloud forest of the Cofre of Perote or Nauhcampatepetl. Finally, eight plants of the species *R. aptera* were collected in the municipalities of Huitzilac and Tetela of Volcan, Morelos, located within the aforementioned coordinates. In Figure 1, the morphological characteristics of the three species studied are shown.

Figure 1. Species of orchids collected: a) *Rhynchostele aptera* (Lex.); b) *Rhynchostele rossii* (Lindley); and c) *Rhynchostele maculata* (La Llave & Lex.).

For the plant chemical analysis, the plants were transported in paper bags. The samples were washed with distilled water and placed in perforated paper bags, dried in an oven with circulating air at a temperature of 70 °C. The material was ground in a stainless steel mill. The elements were quantified in leaves, pseudo-elms and roots, in different stages of development, being as follows: young leaves and floral bud, the most recent (in the process of development); new leaves, in development; mature, fully developed leaves and old leaves, that is, in senescence; new pseudobulbs, the most recent ones; mature, fully developed pseudobulbs and old pseudobulbs, in senescence; root, it was considered a sample composed of young, developed and senescent roots.

The total N was determined by the Kjeldahl method by wet digestion with a mixture of sulfuric acid and perchloric acid. Phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) by atomic emission spectroscopy of plasma induction (ICP-AES) (Alcantar and Sandoval, 1999).

Statistical analysis

The data obtained were analyzed by means of an analysis of variance and a comparison test of Tukey means ($p \leq 0.05$) was performed. The statistical program SAS (Statistical Analysis System) version 9.1 (SAS, 2003) was used to analyze the data.

Results and discussion

Nitrogen

There were differences $p \geq 0.5$ between species (Table 1). The percentage of nitrogen varied between the species and the organs of the three *Rhynchostele* orchids. When the species were in a state of development of young leaves and flower buds, the percentage varied from 1.54 to 2.20%. In Table 1, it is observed that the element is required more in the state of young leaves and flower buds than the rest of the stages of development. The N is an essential mobile element in cell division and expansion, as well as in the growth of vegetative structures, such as stems and leaves (Sonneveld and Voogt, 2009).

In leaves, the percentage varied in a range of 1.09 to 2.07%, in the different ages of the leaves (new, mature and old), independently of the species (Table 1). In addition, it was observed that the percentage of N decreased as the leaves senescen. It has been reported that the percentage of N sufficiency in *Cattleya* orchid leaves ranges from 1.50 to 2.5% (Jones *et al*., 1991). In the case of the *Rhynchostele aptera* species, the levels found in leaves were low. However, in the absence of other reports of species similar to the *Rhynchostele*, these values may be sufficient for this species, since no visual symptoms of deficiencies were detected.

In pseudobulbs regardless of the species, the percentages found were from 0.51 to 1.06% (Table 1). In the epiphytic orchids, the storage organs are the pseudobulbs, which have the capacity to store water, minerals and carbohydrates (Ng and Hew, 2000). However, the percentage found is lower compared to the leaves. Poole and Sheehan (1973) mention that in leafs of *Cattleya* the concentration of N foliar decreases with the age of the leaves, since this tends to translocate towards the pseudobulbs of greater age. However, the latter was not observed in *Rhynchostele* species, since the percentage in pseudobulbs was lower, which means that orchids differ in their nutrient distribution in the plant.

The concentration in root varied in a range of 0.76 to 1.15%, indistinctly of the species. The function of the roots of the orchids is to absorb water and nutrients, as well as fix the plant to the substrate. The roots only grow during a vegetative period and suspend their growth with the development of vegetative shoots (Hagsater *et al*., 2005). This is consistent since the N is a mobile element and was found in the organs with higher demand such as buds (new leaves and flower buds) and low levels in roots.

Phosphorus

The concentrations in young leaves and floral buds were higher than in the rest of the organs of the plants. This can be attributed to the fact that the P is a mobile element, and is found in a greater proportion in young or recently matured leaves (demand organs) (Fernández, 2007). The concentration interval was 0.26 to 0.35% regardless of the species evaluated (Table 1).

Table 1. Concentration of macronutrients (%) in three species of *Rynchostele*.

Species	Young leaves and floral buds	Leaves			Pseudobulbs			Roots
		New	Matures	Old	New	Matures	Old	
				Total nitrogen				
R. maculata	2.29 a	1.35 ab	1.73 a	1.89 ab	1.06 a	0.85 a	0.52 b	1.08 a
R. rossii	2.03 ab	2.07 a	1.86 a	1.91 a	0.51 b	0.55 b	0.58 ab	0.76 b
R. aptera	1.54 b	1.4 b	1.44 a	1.09 b	0.87 ab	0.86 a	0.77 a	1.15 a
CV	10.6	21.3	14.6	29.5	29.6	24	26.1	13.9
				Phosphorus				
R. maculata	0.35 a	0.23 a	0.29 a	0.07 b	0.14 a	0.1 ab	0.1 a	0.08 a
R. rossii	0.26 a	0.21 a	0.15 b	0.13 a	0.08 b	0.07 b	0.06 b	0.07 b
R. aptera	0.27 a	0.28 b	0.22 ab	0.12 a	0.13 a	0.14 a	0.09 a	0.08 a
CV	17.1	10.1	27	14.9	25.6	31.5	24.2	8.4
				Potassium				
R. maculata	1.1 a	0.79 b	0.9 a	0.51 ab	0.86 a	0.74 a	0.44 b	0.25 a
R. rossii	0.94 ab	0.9 a	0.51 b	0.56 a	0.51 b	0.47 a	0.61 ab	0.21 ab
R. aptera	0.85 b	0.82 ab	0.69 ab	0.45 b	0.68 ab	0.68 a	0.8 a	0.18 b
CV	8.6	6.3	19.9	7.6	26.3	53.4	28.4	21.5
				Calcium				
R. maculata	0.47 ab	0.47 ab	0.42 a	0.63 a	0.84 a	0.73 a	0.58 a	0.37 a
R. rossii	0.51 a	0.6 a	0.65 b	0.75 a	0.5 b	0.45 b	0.37 b	0.27 b
R. aptera	0.35 b	0.34 b	0.32 a	0.41 b	0.35 b	0.42 b	0.33 b	0.25 b
CV	14.1	16.8	14.9	14.9	22.5	24.9	31.4	13.2
				Magnesium				
R. maculata	0.08 b	0.07 b	0.07 b	0.07 b	0.11 b	0.12 b	0.09 b	0.09 b
R. rossii	0.19 a	0.19 a	0.18 a	0.17 a	0.22 a	0.22 a	0.2 a	0.09 b
R. aptera	0.15 a	0.16 a	0.13 ab	0.12 b	0.25 a	0.27 a	0.26 a	0.13 a
CV	15.3	25.3	15.2	26.7	21.2	27.2	33.9	16.6

CV= coefficient of variation. Values with the same letter within the column are statistically equal, Tukey ($p \leq 0.05$).

The results in leaves varied from 0.12 to 0.29%, independently of the species and the age of the leaf (young, mature and old). Jones *et al*. (1991), for *Cattleya* spp. indicates that the P sufficiency values range from 0.13 to 0.75% in the foliage. Only the case of *R. aptera* in old leaves, is below the sufficiency values, which is attributable to the mobility of the element towards the demand zones (Fernández, 2007).

In general, it was observed that the concentration of P is low in the pseudobulbs of the *Rhynchostele* species, compared with the leaves. Ranges of 0.06 to 0.14% were obtained regardless of the species and the stage of development of the pseudobulb. As mentioned above, the pseudobulbs function as storage organs for water and reserve substances; however, low levels of P were found, which could have been used to support the production of flowers, fruits or the development of new shoots. Bachiega-Zambrosi *et al*. (2012) suggest that remobilization of P reserves is important to meet the demands of new vegetative and reproductive growth. In the roots (0.07 to 0.08% of P) the concentration of P was even lower than leaves and pseudobulbs, which was associated with demand zones such as young growing leaves.

Potassium

In relation to K, the highest concentration was in young leaves and floral buds, as in the previous elements. The concentration of K in young leaves and flower buds was 0.85 to 1.10% (Table 1), which are considered low, compared to the level of foliage sufficiency that ranges from 2.00 to 3.5% in orchids of the *Cattleya* genus (Jones *et al*., 1991). Similarly, concentrations were low in the leaves regardless of the species and morphological development, where the concentration varied from 0.45 to 0.9% (Table 1). However, it is inferred that the concentrations found could be of sufficiency and that the genus *Rhynchostele* could require concentrations lower than 2% of K, since they did not present visual symptoms of deficiency, water stress, cold damage, or diseases (Figure 1).

In this regard, Kumar and Kumar-Sharma (2013) indicates that plants that have an adequate supply of K make more efficient use of water during stress, having a direct relationship with resistance to frost and disease. In addition, K promotes stem stiffness and is involved in cell growth, as well as in the stabilization of the membrane and the cell wall (Armstrong, 2002). The concentration of K in pseudobulbs was from 0.44 to 0.86%, levels slightly below the foliage (Table 1). Likewise, root levels were low from 0.18 to 0.25%, this effect could be due to the mobility of K inside the plant (Marschner, 2012), being translocated towards the aerial part.

Calcium

Ca concentrations of 0.35 to 0.51% were found in young leaves and flower buds (Table 1). In the foliage, values of 0.34 to 0.75% were found regardless of the species and the age of the leaves. However, there is a marked difference between the study species *R. aptera* and *R. macula* had low levels in almost all their leaves, in comparison to the optimal reports of Ca in foliage by Poole and Sheehan (1973) and Jones *et al*. (1991) ranging from 0.5 to 2%. Ca is characterized by low transport ability within the plant because once it is deposited in plant tissues it will be very difficult to remove it. That is why young tissues are the first to be affected when there are deficiencies of this nutrient (Marschner, 2012).

Regarding the pseudobulbs, the concentrations ranged from 0.33 to 0.84% (Table 1). In the root the concentrations were from 0.25 to 0.37%. In this regard, Poole and Sheehan (1973) consider as an optimum level the concentration of 0.1 to 0.4% of Ca in *Cattleya* roots.

Magnesium

It was found that the concentrations of Mg in young leaves and floral buds varied in a range of 0.08 to 0.19% and in young, mature and old leaves from 0.07 to 0.19% regardless of the species. Levels of foliage sufficiency have been reported at concentrations of 0.30-0.70% for *Cattleya*

(Jones *et al*., 1991), so the values found were lower than those reported in the literature. However, the plants did not show visual symptoms of Mg deficiencies, so it is inferred that the Mg level required by *Rhynchostele* may be lower. Additionally, it was observed that the highest accumulation of Mg occurred in the pseudobulbs, where the concentrations were from 0.11 to 0.26%. This may be because the pseudobulbs function as nutrient storage organs Uma *et al*. (2015). In the case of the roots, the values were from 0.09 to 0.13%, similar to the foliage.

In general, the decreasing sequence of nutrient concentration was N> K> Ca> P> Mg. This tendency of nutritional absorption was mainly in the foliage of *R. macula* and *R. aptera*. In *R. rossii* the concentration changed in mature and old leaves in the following way N> Ca> K> Mg> P. In pseudobulbs the tendency of accumulation also changed and was N> K> Ca> Mg> P. The root was the only organ that presented the same tendency in the three species, where its decreasing sequence of accumulation was N> Ca> K> Mg> P. The N and K or Ca were the most required elements in the three species of *Rhynchostele*, the absorption order changed with the stage of development and genotypes. As pointed out by Jiménez-Peña *et al*. (2013), orchids have different nutritional requirements, these same authors point out the importance of providing the nutrients according to the stage of development and of the species, for a better development.

It was observed that the mobile elements were translocated towards the area mainly in leaves and flower buds. This coincides with that reported by Hew and Ng (1996) in a study of O*ncidium goldiana*, where the mineral reserves, mainly in pseudobulbs, are remobilized to support the development of a new outbreak and inflorescence. It has also been reported in *Catasetum viridiflavum* that the minerals in pseudobulb reserves are important in determining the number of flowers produced (Zimmerman, 1990). Therefore, it is recommended that special attention be given to *Rhynchostele* species in the fertilization regime, especially during the development period of new shoots, since in their natural habitat they required smaller amounts than what is reported in orchids such as *Cattleya* and *Phalaenopsis* (Jones *et al*., 1991). It is important to consider the nutritional requirements, since the distribution of these during their development is fundamental to satisfy the specific needs in the periods of highest demand, especially of nutrients such as nitrogen (N) phosphorus (P) and potassium (K).

It should be noted that, in general, low values of nutritional concentration were found in the three species studied compared to those reported in the literature, due in part to the fact that the genotypes that were evaluated grew and developed in their natural habitat. It is inferred that if grown under conditions with optimal fertilization doses and with adequate agronomic management, the nutrient concentration values would be higher as has been found in the *Laelia anceps* orchid (Jiménez-Peña *et al*., 2013); however, despite the fact that the species grew under natural conditions, no visual symptoms of deficiency were detected, probably this response was due to the fact that the Orchidaceae family members form associations with mycorrhizal fungi that give them greater radical exploration and eventually greater capacity of nutrimental absorption, especially in the initial stages of growth and development, as reported in *Eulophia epidendraea* and *Malaxis acuminata* (Uma *et al*., 2015).

Finally, the importance of the results of this research lies in the fact that they constitute the first report on the concentration and distribution of nutrients in the organs of the orchids *R. maculata*, *R. rossii* and *R. aptera* developed under natural conditions, so that the information generated could be useful in conservation programs and for the agronomic management of these species.

Conclusions

Rinchostele rosii, *R. maculata* and *R. aptera* had nutrient concentrations in decreasing order were: N> K> Ca> P> Mg in young leaves, new leaves and mature leaves; with the exception of *R. rosii*, which had nutrient concentrations of N> Ca> K> Mg> P in mature leaves. In new and mature pseudobulbs the order that predominated was N> K> Ca> Mg> P and in the old ones it was K> N> Ca> Mg> P. In the roots the order that prevailed in the three species was N> Ca> K> Mg> P.

The concentrations of nutrients in mature leaves, in percentage: N (1.44 - 1.86), P (0.15 - 0.29), K (0.51 - 0.9), Ca (0.32 - 0.65) and Mg (0.11 - 0.25); in pseudobulbs N (0.55 - 0.85), P (0.07 - 0.14), K (0.47 - 0.74), Ca (0.42 - 0.73) and Mg (0.12 - 0.22); and in root N (0.76 - 1.15), P (0.07 - 0.08), K (0.18 - 0.25), Ca (0.25 - 0.37) and Mg (0.09 - 0.13). Finally, the nutrient concentrations found in mature leaves could serve as reference values for nutritional diagnosis purposes of the three species reported; likewise, these results could be useful in conservation programs and for the agronomic management of *R. rosii*, *R. maculata* and *R. aptera*.

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