



Ecological significance of determinate primary root growth: inter- and intra-specific differences in two species of *Gymnocalycium* (Cactaceae) along elevation gradients

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ABSTRACT

Determinate primary root growth in Cactaceae has been interpreted as an adaptation to dry environments. However, little information is available regarding ecological patterns of this process. Our research question was whether primary root length is affected by the determinate growth pattern of the plant or following predictions from resource allocation theories. We analyzed the presence of apical meristem exhaustion and patterns of primary root length (PRL), days until the end of growth (day's post-germination, DPG) and seed mass in *Gymnocalycium monvillei* and *G. quehlianum*, which present different elevation distributions and wide elevation ranges. We analyzed five elevation provenances for *G. monvillei* (878, 1250, 1555, 1940 and 2230 m a.s.l.), and three for *G. quehlianum* (610, 950 and 1250 m a.s.l.). One hundred seeds per species per altitude were set to germinate in vertical petri dishes. We measured PRL and DPG in each seedling and also seed mass. Both species present determinate growth and PRL varied between species and among populations. PRL was higher in *G. quehlianum*. DPG was related to differences between species in PRL: roots of *G. quehlianum* grow for a longer period. In both species we found differences among elevation provenances, with higher PRL at the extremes of the distribution. Among elevation provenances, DPG was significantly related to PRL in *G. monvillei*, and marginally significantly related in *G. quehlianum*. Seed size was not related to differences in PRL between species or among elevation provenances. The comparison between species and among elevation provenances suggests that a higher PRL would be related to more extreme environments; this assumption agrees with plant resource allocation theories, which predict a lower shoot : root ratio with increasingly stressful environments.

1. Introduction

For most plant species, primary root can grow indefinitely under environmental optimum conditions. However, some plant species, particularly members of the Cactaceae family, present determinate growth (Dubrovsky, 1997; Shishkova et al., 2008), which is the termination of the primary root growth even when environmental conditions are optimum for growth (Dubrovsky, 1997). Previous studies also demonstrated that this process is genetically determined (Shishkova et al., 2008, 2013), although the causes that lead to the exhaustion of the root apical meristem are still not well understood (Shishkova et al., 2013).

In angiosperms, determinate growth has been observed only in the Cactaceae family, particularly in most genera of the Cactoideae subfamily, and in some of the Opuntioideae (Dubrovsky, 1997; Dubrovsky

and Gómez-Lomelí, 2003; Shishkova et al., 2013), and was interpreted as an adaptation to dry environments, since plants would anticipate dry conditions. This anticipation would have positive effects on seedlings, increasing their establishment and likely their long-term survival and growth (Dubrovsky, 1997). In agreement with this hypothesis, Shishkova et al. (2013) found that determinate growth is mainly present in cactus species from arid ecosystems, whereas those species from mesic or humid ecosystems mainly exhibit indeterminate growth. However, although the genetic and physiological mechanisms behind determinate growth are well understood (Dubrovsky, 1997; Shishkova et al., 2008, 2013), there is still a lack of information about the ecological patterns and significance of this process.

Although not quantitatively analyzed, previous studies also found that the length of the primary root until growth stops (PRL) also differs among species or growing conditions (Dubrovsky and Gómez-Lomelí,

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2003; Shishkova et al., 2013), but its ecological significance is not clear. On the one hand, PRL could be related to a degree of determinate growth, e.g. species or populations from drier environments should present lower PRL values than species from more humid environments. In a study on *Pachycereus pringlei*, Dubrovsky and Gómez-Lomelí (2003) found a relationship between PRL and water potential of the seed growth medium. PRL decreased as the germination medium presented lower water potential. If PRL patterns among species or populations follow environmental gradients (e.g. higher PRL under more mesic conditions), PRL might be used as a quantitative indicator of determinate growth. On the other hand, plant biomass allocation theories suggest that this pattern could be the opposite (Mokany et al., 2006; Poorter et al., 2012). It is well known that species or individuals growing under more mesic conditions (e.g. higher levels of water or nutrients) present a lower root:shoot ratio than species growing under less mesic conditions, since roots do not need a higher allocation in order to tap resources. This would mean that even if a species presented determinate growth, species or populations growing at more mesic sites would present lower PRL than those growing under opposite conditions (Poorter et al., 2012).

To date, patterns of determinate growth and PRL have not been related to species characteristics. Among plant characteristics, seed size is likely to be related to PRL. Seed size has been related to seedling size (Seiwa and Kikuzawa, 1996), including cactus species (Ayala Cordero et al., 2006; Sosa Pivatto et al., 2014). A positive relationship between PRL and seed size might be expected, since the amount of reserves is essential for early plant growth (Leishman et al., 2000), especially in cactus species whose seedlings present a limited photosynthetic surface (Sosa Pivatto et al., 2014). Another variable that could be related to PRL is the time elapsed until the termination of root growth (DPG). Although previous studies (Shishkova et al., 2013) reported that in seeds germinating under standard and optimal conditions, DPG can vary from 1 to as many as 30 days, its relationship with PRL was not analyzed. In a study of seedlings of *Pachycereus pringlei*, Dubrovsky and Gómez-Lomelí (2003) found that DPG was positively related to PRL.

In mountain environments, different cactus species present different elevation distribution, and a single species can present wide elevation ranges (Gurvich et al., 2014; Bauk et al., 2015, 2017). The different distributions of species or populations impose very different environmental conditions for plant growth. In a cactus species that inhabits a wide elevation range, *G. monvillei*, Bauk et al. (2015, 2017) found differences in some traits, such as seedling shape, total germination, and germination response to different water potentials, indicating the pressure imposed by environmental conditions, particularly temperature and humidity, as well as the capacity of the species to adapt to those situations. If determinate growth were of ecological importance, differences between species growing at different elevations and among populations occurring at different elevations would be expected. So far, only seven species of the tribe Trichocereae were analyzed regarding the presence or not of determinate growth, and its presence was confirmed in all of them (Shishkova et al., 2013).

Gymnocalycium, tribe Trichocereae, is a genus of globose species composed of approximately 55 species native to southern South America (Kiesling et al., 2008; Charles, 2009). The mountains of Central Argentina are especially rich in species of this genus. Only one species of the genus *Gymnocalycium*, *G. gibbosum*, was studied and was found to have determinate growth (Shishkova et al., 2013). *Gymnocalycium monvillei* and *G. quehlianum* are endemic to the Central Argentina Mountains (Kiesling et al., 2008; Gurvich et al., 2014) and present different elevation ranges: from 800 to 2300 m a.s.l. and from 600 to 1200 m a.s.l. for *G. monvillei* and *G. quehlianum*, respectively (Charles, 2009; Gurvich et al., 2014). In both gradients, the lower sites are warmer and drier than the higher sites. The aims of this study were to analyze 1) if *G. monvillei* and *G. quehlianum* present determinate growth, 2) differences in PRL between these species, which differ in elevation distributions, and among altitudinal populations within

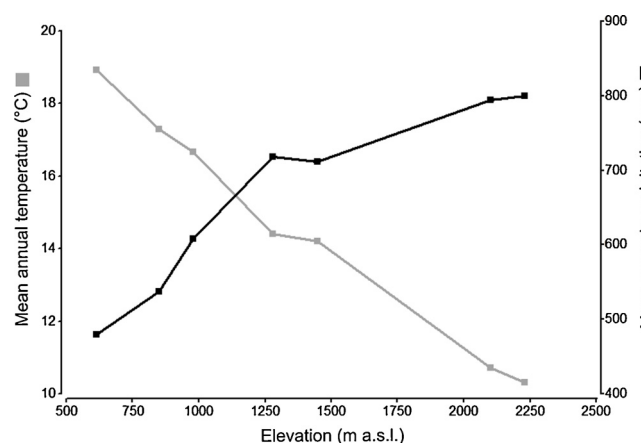


Fig. 1. Mean annual temperature and mean annual precipitation in relation to elevation in the Córdoba Mountains, Central Argentina. Data taken from De Fina (1992).

species, and 3) if patterns of PRL are related to elevation, DPG and seed mass. Particularly we want to test two contrasting assumptions: 1) that PRL could be used as a quantitative indicator of determinate growth (in this case, we expect a lower PRL under more stressful conditions) and 2) that PRL is governed by resource allocation theories (in this case, we expect a higher PRL under more stressful conditions).

2. Materials and methods

2.1. Studied species and study area

We studied five populations of *G. monvillei* (hereafter altitudinal provenances) located along an altitudinal gradient between the localities of Cuesta Blanca (31° 28' S, 64° 34' W) and Pampa de Achala (31° 41' S, 64° 50' W), at 878, 1250, 1555, 1940 and 2230 m a.s.l. In *G. quehlianum*, which presents a narrower elevation distribution, we studied three altitudinal provenances located between the localities of San Marcos Sierras (30° 44' S, 64° 34' W) and Camino del Cuadrado (31° 7' S, 64° 25' W), at 615, 948 and 1257 m a.s.l. Climate differs markedly along the gradient, with mean annual temperatures varying from 18.7 to 10.3 °C and mean annual precipitation from 480 to 800 mm (Fig. 1). In all the area the precipitations occur during the warm season, from October to April (de Fina, 1992). Frost can occur all year round and snow events are common during winter at sites above 1900 m a.s.l. Vegetation varies from subtropical dry forest at lower altitudes to cold-temperate grasslands at higher ones (Giorgis et al., 2017).

2.2. Measurements

Mature fruits from 20 individuals of similar size were collected in each altitudinal provenance. Seeds were air-dried and stored in the laboratory at ambient conditions (25 °C and 25% air relative humidity) until the start of experiments (Gurvich et al., 2008). One hundred seeds per species / altitude were set to germinate in Petri dishes. Seeds were no more than two years old at the beginning of the experiments.

Seeds were pre-sterilized for 10 min in 70% ethanol, sterilized for 20 min in 60% commercial bleach supplemented with 0.08% Triton X-100, and washed in sterile distilled water four times for 10 min each. Sterilized seeds were germinated in Petri dishes containing 0.2 × LS (Linsmaier and Skoog, 1965; Phyto Technology Laboratories, Lenexa, KS, USA) medium buffered to pH 5.7 and solidified with 0.8% bacto-agar. Plants were cultivated in vertically oriented Petri dishes maintained at 28 °C, with a 12 h light/dark photoperiod. Primary root length was recorded until it stopped growing, as well as time elapsed until root stopped growing (DPG, days post-germination). Roots that either were

submerged in the medium or had a root tip that did not make contact with the medium surface were disregarded. Petri dishes with seedlings were photographed under an Olympus SZX16 (Olympus, Tokyo, Japan) stereoscope equipped with an Olympus DP71 camera software. Measurements were taken with the free software ImageJ (<http://rsbweb.nih.gov/ij/>). Roots were cleared using the method of Malamy and Benfey (1997), with minor modifications (Dubrovsky et al., 2006), and observed under microscope Olympus BX61 camera JAI model CV-M4 + CL. Because not all the seeds germinated, and not all the seedlings survived, the number of replicates for each treatment varied between 10 and 18.

We estimated seed mass by determining the weight of 50 dry seeds per population with a precision balance (Ohaus Corp. Pine Brook, NJ; 0.0001 g). The 50 seeds were weighed in five pools of 10 seeds because of their low weight. Seeds were air-dried at 25% air humidity for at least 5 months.

2.3. Data analyses

To compare species, we performed ANOVAs for PRL, DPG and seed mass with species as factor. To analyze the effect of altitude and DPG on PRL of each species we generated linear models using altitudinal provenance as factor and DPG as covariate. The interaction between the variables was non-significant, so we removed it from the final model (as assumed by ANCOVA). To analyze seed mass response of each species to altitude we performed ANOVAs with altitudinal provenance as factor. We made separate analysis for comparing species and altitudinal provenances, since the species ranges are different. DPG was square-root transformed to meet normality and in every model, if necessary, we added variance functions to cope with heteroscedasticity. Data analyses were performed with the program INFOSTAT version 2012 (Di Rienzo et al., 2015).

3. Results

In both species, the primary root stopped growing during the first month after germination. The analyses of roots confirmed the exhaustion of the meristem (Fig. 2). *Gymnocalycium quehlianum* presented a higher PRL than *G. monvillei* ($F_{(1,106)} = 7.43$, $p = 0.0075$; Fig. 3a). DPG was also higher in *G. quehlianum* than in *G. monvillei* ($F_{(1,107)} = 26.24$, $p < 0.0001$; Fig. 3b). *Gymnocalycium quehlianum* presented lighter seeds than *G. monvillei* ($F_{(1,165)} = 132.12$, $p < 0.0001$; Fig. 3c).

The analysis of differences among populations of *G. quehlianum* showed a significant effect of altitudinal provenance on PRL ($F_{(2,41)} = 8.64$, $p = 0.0007$) and a marginal and positive effect of DPG on PRL ($F_{(1,41)} = 3.25$, $p = 0.0788$, $\beta = 0.08 \pm 0.04$ s.e.; Fig. 4a). For *G. monvillei*, we found a significant effect of altitudinal provenance ($F_{(4,58)} = 5.37$, $p = 0.0009$) and a significant and positive effect of DPG ($F_{(1,58)} = 5.17$, $p = 0.0267$, $\beta = 0.05 \pm 0.02$ s.e.; Fig. 4b) on PRL. The pattern of altitudinal provenances was similar between species: populations at the extremes of the elevation range presented higher PRL, while the intermediate ones presented the lower PRL (Fig. 4a, b).

Altitudinal provenances had a significant effect on seed mass in *G. quehlianum* ($F_{(2,39)} = 4.30$, $p = 0.0205$): seed mass was lowest at the intermediate altitude (Fig. 4c). We also found a significant effect of altitudinal provenances for *G. monvillei* ($F_{(4,120)} = 39.02$, $p < 0.0001$), but without showing a clear pattern between elevations and seed mass (Fig. 4d). In both species the altitudinal provenance with the lowest seed mass was also the one with lowest PRL (Fig. 4).

4. Discussion

Determinate primary root growth has been regarded as an adaptation of cactus species to arid environments, but little information is

available regarding its ecological patterns and significance (Dubrovsky, 1997; Shishkova et al., 2013). In the present article we present novel results about the ecological patterns of determinate growth, with a more detailed analysis of other variables PRL, DPG and seed mass, comparing two *Gymnocalycium* species and different altitudinal provenances.

Both species present determinate growth, which agrees with previous studies in the Trichocereae tribe (Shishkova et al., 2013). We also found differences between and within species. *Gymnocalycium monvillei* presented a lower PRL than *G. quehlianum* although the former species presented heavier seeds. Patterns between species were not related to seed mass, but to DPG. *Gymnocalycium quehlianum*, the species with a higher PRL, present lighter seeds, but higher DPG. This species inhabits warmer and drier habitats than *G. monvillei*, suggesting that in more stressful environments, PRL would indicate a higher allocation to roots in order to tap resources than in suitable environments (Poorter et al., 2012).

We also found differences between altitudinal provenances in PRL for both species, with a similar pattern: PRL was higher at the extremes of the altitudinal distribution, and lower at intermediate sites. Coincidentally, the sites where both species presented their lowest PRL coincide with sites of greatest abundance (K. Bauk, P. Martino, unpublished data). These results suggest that at the optimal altitude for species growth, seedlings would allocate fewer resources to roots, which is expected according to resource allocation theories (Poorter et al., 2012). Our results from the comparison between species and among altitudinal provenances suggest that PRL is regulated following predictions from allocation theories: higher allocation to root biomass for species or populations growing in more stressful, drier or colder, environments than for species growing in suitable environments. Our results also discard that PRL could be used as a quantitative indicator of determinate growth. Anyway, studies involving a higher number of species, belonging to a wider taxonomic representation of the family, and inhabiting wider environmental conditions, should be necessary to confirm this pattern.

Contrary to our expectations, seed mass was not related to PRL, either in the comparison between species or among altitudinal provenances. Only in *G. quehlianum* were seed size patterns similar to PRL patterns. In a comparison of 17 cactus species, Sosa Pivatto et al. (2014) found that seed mass was positively related to seedling size. Despite this relationship, our results show that seed mass does not affect PRL. DPG was related to PRL in both species, but not so clearly in *G. quehlianum*. Seed mass is usually larger in species occurring in drier environments; this fact has been related to a high seedling survival (Jurado and Westoby, 1992), a pattern already found in cacti (Ayala-Cordero et al., 2006; Guillén et al., 2015). However, we found the opposite trend in seed mass in the comparison between the studied *Gymnocalycium* species. Studies encompassing more species of the genus, or of the family, are necessary to understand these patterns.

Determinate root growth has been indicated as an important trait affecting establishment and growth in cactus species (Dubrovsky, 1997; Shishkova et al., 2013), but almost no work has analyzed its ecological significance. The present study provides evidences that inter- and intraspecific differences in PRL among elevation provenances are important, even though the experiments were performed under controlled and standard conditions. Our results suggest that studies on determinate growth should be integrated to resource allocation theories for a better understanding of this process (Jurado and Westoby, 1992; Poorter et al., 2012). DPG clearly determined PRL between species, but its effect within species was not so clear.

Even though determinate root growth was described 20 years ago (Dubrovsky, 1997), little progress has been made in relation to its ecological significance. Determinate growth has only been observed in Cactaceae; to our knowledge, however, no other plant groups (succulents of other families, or other plants from arid environments) were analyzed. It is interesting to note that many succulent species from the

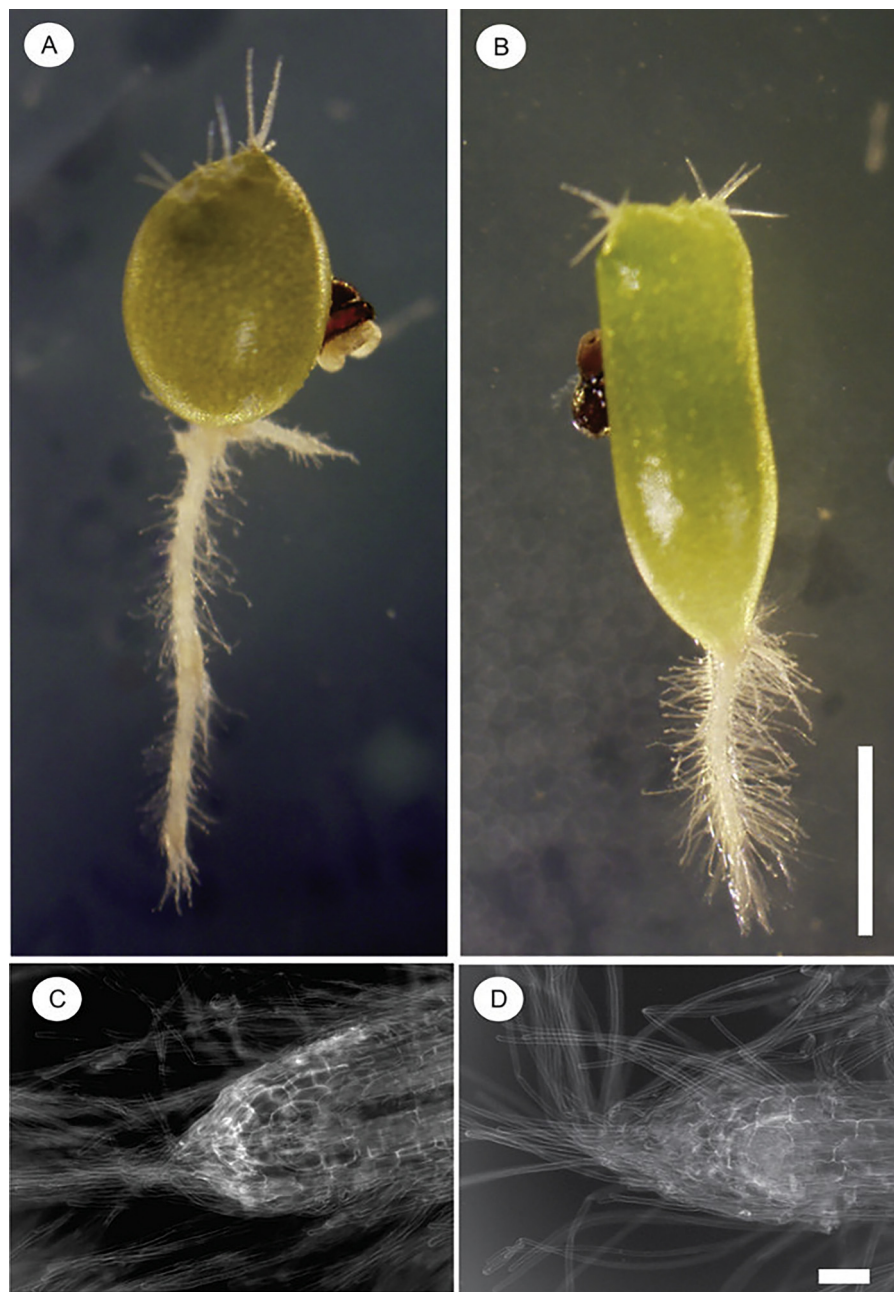


Fig. 2. Seedlings of (A) *Gymnocalycium quehlianum* and (B) *G. monvillei* showing the primary root with the apical meristem already exhausted (bar 5 mm). Cleared primary root tips showing the exhaustion of the apical meristem in (C) *G. quehlianum* and (D) *G. monvillei* (bar 0.01 mm).

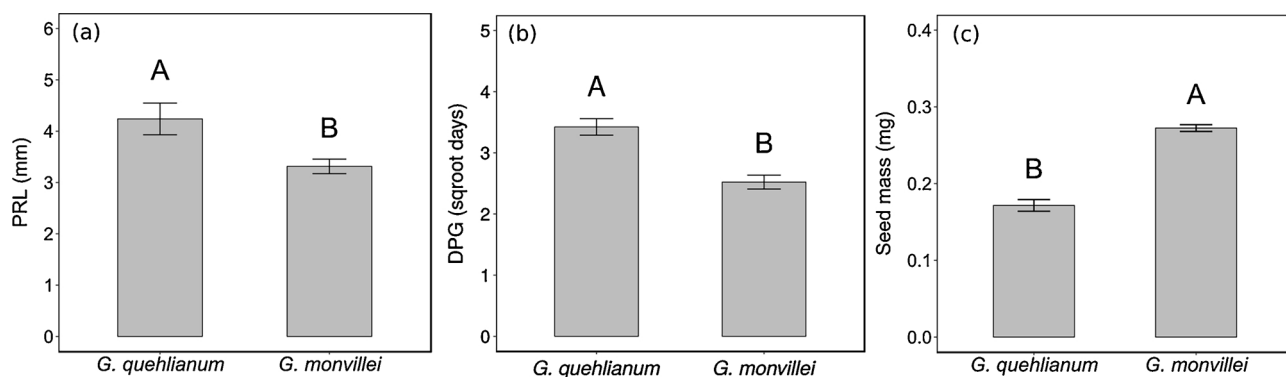


Fig. 3. Estimated mean \pm standard error values for (A) primary root length (PRL), (B) days post germination (DPG, square root of days) and (C) seed mass of the two cacti species *Gymnocalycium quehlianum* and *G. monvillei*. Different letters indicate statistically significant differences between species (LSD-test, $p < 0.05$).

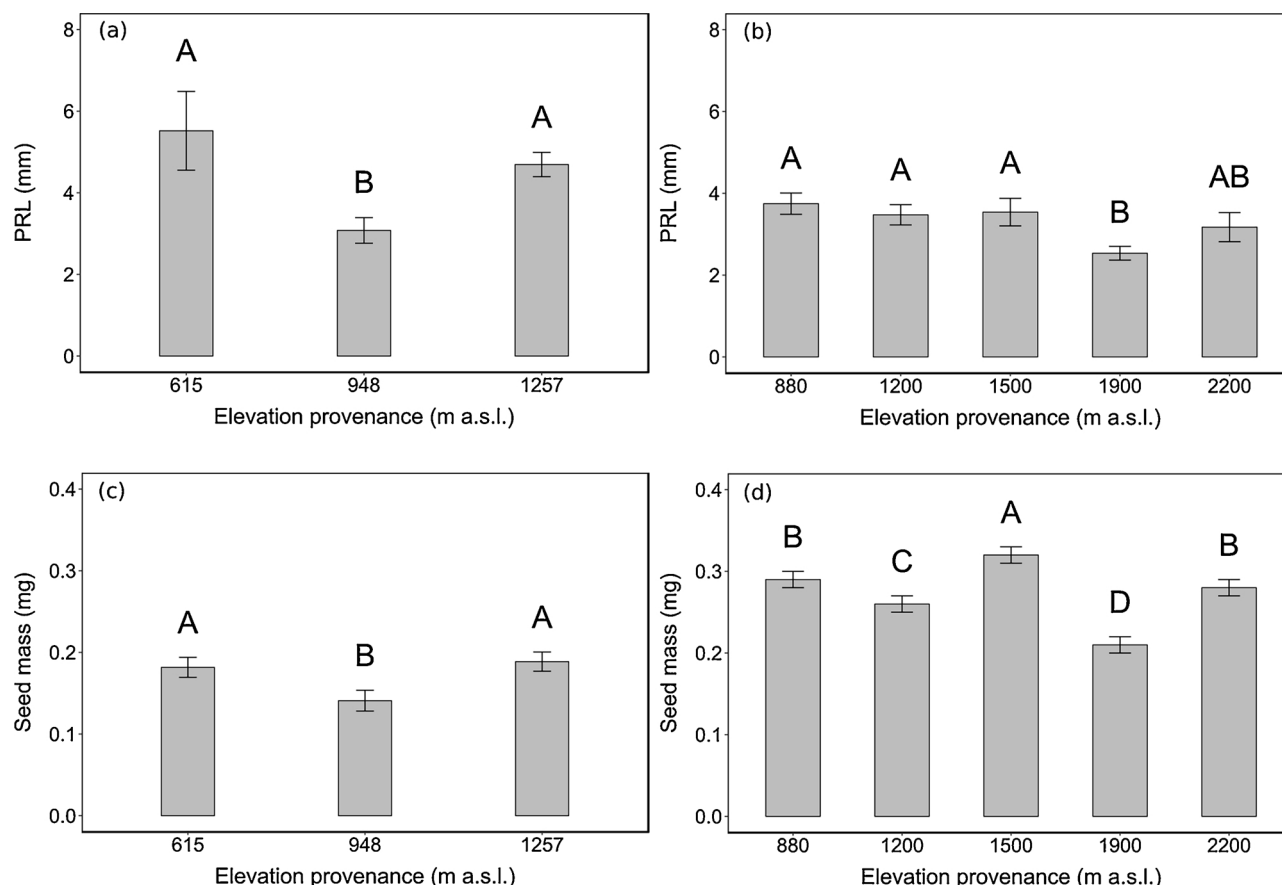


Fig. 4. Mean \pm standard error values of estimated altitudinal provenances of (A) *Gymnocalycium quehlianum* primary root length (PRL), (B) *G. monvillei* PRL, (C) *G. quehlianum* seed mass and (D) *G. monvillei* seed mass. Different letters in each graph indicate statistically significant differences among elevation provenances of the species (LSD-test, $p < 0.05$). Effects of days post-germination (DPG) on PRL are included in the statistical model (see the text for the explanation).

Apocynaceae family (e.g. genera *Stapelia* and *Huernia*) present stems that seem to grow in a determinate way: new stems arise from the base of old ones and grow until a given size is reached. The growth of the cladodes of most Opuntioideae species (prickly pears cactus) can also be regarded as a type of determinate growth. The presence of this process in other plant families, and also in other organs of the plants, would indicate the ecological importance of this process and would open future research agendas.

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