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QUANTITATIVE ASSESSMENT OF ANISOCOTYLY IN *HABERLEA RHODOPENSIS* AND *RAMONDA MYCONI*

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Anisocotyly, the unequal development of cotyledons post germination, is a unique trait observed only in Old World Gesneriaceae (Lamiales). New World Gesneriaceae have isocotylous seedlings. In both Old and New World Gesneriaceae, cotyledons initially grow equally for a short period just after germination. In the New World species, both cotyledons cease their growth at the same time early on, whereas in Old World species one cotyledon continues to expand to become a macrocotyledon while the other withers away. In this study, cotyledon growth was observed in two European Old World Gesneriaceae: *Haberlea rhodopensis* and *Ramonda myconi*. The results were compared with those for the typical anisocotylous species *Streptocarpus rexii* and the typical isocotylous species *Corytoplectus speciosus*. We found that the cotyledon growth patterns in *Haberlea rhodopensis* and *Ramonda myconi* were intermediate between the typical anisocotylous or isocotylous species. *Haberlea rhodopensis* and *Ramonda myconi* showed irregular growth patterns, with some plants being slightly anisocotylous but most being isocotylous. The developmental basis for the residual anisocotyly, the extended basal meristem activity in the macrocotyledon, appeared to be identical in the European species to that in the typical Old World *Streptocarpus rexii* but weakly expressed, rare and terminated early. In conclusion, European Gesneriaceae retain a reduced anisocotylous growth that may be linked to their early plumule development.

Keywords. Anisocotyly, cotyledon, Gesneriaceae, *Haberlea rhodopensis*, meristem, *Amonda myconi*.

Introduction

Old World species in the family Gesneriaceae (Lamiales, Eudicots) are well known for their unorthodox cotyledon development, termed anisocotyly (Jong & Burtt, 1975). During germination just after cotyledon unfolding, the two cotyledons are equal in size, as in most other dicotyledonous species. However, soon afterwards the two cotyledons develop at different rates, with one cotyledon eventually being significantly larger than the other (Nishii *et al*., 2017 and references therein). This phenomenon was first reported in the mid-nineteenth century in *Streptocarpus* Lindl., an African genus (Caspary, 1858; Crocker, 1860).

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In previous studies, the mechanism behind anisocotyly was investigated mainly in *Monophyllaea* R.Br. and *Streptocarpus* species (Oehlkers, 1923; Jong, 1970; Jong & Burtt, 1975; Tsukaya, 1997; Imaichi *et al*., 2000, 2001; Nishii *et al*., 2004). It has been found that unequal cotyledon development is caused by differential meristem activity in the proximal part of the lamina of the two cotyledons, the so-called basal meristem. Just after germination, both cotyledons are equal in size and both show cell division in the proximal region of the lamina. Soon afterwards, the basal meristem activity in one cotyledon ceases and it becomes the microcotyledon, while in the other the basal meristem activity continues to form the macrocotyledon. It has been suggested that cotyledon fate is determined after germination (Oehlkers, 1923; Tsukaya, 1997; Nishii *et al*., 2004; Saueregger & Weber, 2004).

Burtt (1963) suggested that anisocotyly is characteristic of Old World Gesneriaceae, but not many species had been studied at that time. In addition, it seems there are large variations in the expression of anisocotyly between species. In the unifoliate *Streptocarpus* (so-called one-leaf plants), the macrocotyledon can sometimes grow up to 1 m in length (Hilliard & Burtt, 1971). In contrast, some species in other genera, such as *Aeschynanthus* Jack or the former *Chirita* Buch.-Ham. (now split into five different genera; see Weber *et al*., 2011) show only moderate anisocotyly (Hill, 1938; Burtt & Woods, 1958).

Although mostly tropical and subtropical Gesneriaceae genera have been previously been studied for anisocotyly, only brief reports on the European *Haberlea* Friv. and *Ramonda* Rich. have been published. Fritsch (1904) reported simply that the cotyledons of *Ramonda myconi* (L.) Rchb. were unequal. Hill (1938) reported that *Ramonda serbica* Pančić and *Haberlea rhodopensis* Friv. "have seedlings with unequal cotyledons, though less markedly so than in *Streptocarpus*". In *Haberlea*, apparently, the larger one is 1.00–1.25 mm in length, whereas the smaller one is 0.85–0.95 mm long. No further observations or images were presented and no detailed study of the involvement of meristem activity was undertaken. Therefore, whether the same meristem activity observed in *Streptocarpus* and *Monophyllaea* underlies this mild anisocotyly is unknown.

The underlying mechanisms of weak anisocotyly have received little attention thus far, and this study presents quantitative data relevant to the subject. The cotyledon development of the two European Gesneriaceae species *Haberlea rhodopensis* and *Ramonda myconi* was studied in detail, assessing basal meristem activity and growth of the cotyledons in particular. The results were compared with those for typical anisocotylous Old World species and typical isocotylous New World species.

Materials and Methods

Plant material

Besides the European *Haberlea rhodopensis* (RBGE accession no. 19281002) and *Ramonda myconi* (19710970), the plant material used in this study included the typical anisocotylous Old World *Streptocarpus rexii* (Bowie ex Hook.) Lindl. (20080299) and the isocotylous New World *Corytoplectus speciosus* (Poepp.) Wiehler (19540131). Seeds

were sown on soil (vermiculite to peat moss, 3:1) in pots or on filter paper in 9-cm Petri dishes and cultivated in a growth room at the National Taiwan University (NTU), Taipei, Taiwan, at 23°C, with 16 h of light and 8 h of darkness.

Morphological observations

To observe the process of germination, seeds sown on filter paper were observed under a stereomicroscope, SMZ-10 (Nikon, Tokyo, Japan). To be able to comparatively observe the consecutive seedling development, the first day of cotyledon expansion was defined as 1 day after cotyledon unfolding (1 DAU). Cotyledon area was measured from images obtained using ImageJ (Schneider *et al*., 2012). Student's paired *t*-tests were performed with Microsoft Excel (Microsoft Corporation, Washington, USA) to statistically detect significant size differences between larger and smaller cotyledons in a seedling.

For scanning electron microscopy (SEM), seedlings were fixed in FAA (50% ethanol, 10% acetic acid, 3.7% formaldehyde) under vacuum. The samples were dehydrated in an ethanol series, followed by treatment with acetone–ethanol (1:1) and then 100% acetone. Following critical-point drying with an HCP-2 (Hitachi, Tokyo, Japan), the samples were coated with gold palladium in a IB-2 ion sputter coater (Eiko Engineering, Tokyo, Japan) and observed under a Quanta 200 scanning electron microscope (FEI, Hillsboro, Oregon, USA) at either the Institute of Plant and Microbial Biology, Academia Sinica (Taipei, Taiwan) or the TC5-Bio-Image group, Tech-comm, College of Life Science, NTU (Taipei, Taiwan).

To observe the epidermal cell size, seedlings were fixed in ethanol–acetic acid (3:1) and hydrated in an ethanol series. The hydrated seedlings were cleared with chloral hydrate (Nishii *et al*., 2004) and observed under Nomarski optics on a BX51 microscope (Olympus, Tokyo, Japan). Epidermal cell size was measured from SEM or light microscopy images of cleared samples using ImageJ.

Observation of meristem activity

Cell division activity was detected by aniline blue staining (Nishii *et al*., 2004). Briefly, aniline blue stains callose (β-1,3-glucans), which exists mainly in the cell plane of freshly divided cells but rapidly degrades after division. Seedlings were fixed in ethanol–acetic acid (4:1) and further treated with 100% ethanol then ethanol plus 100 mM pH 9.0 phosphate buffer (1:1) before finally being transferred to pH 9.0 phosphate buffer. The seedlings were stained with 0.02% aniline blue in 100 mM phosphate buffer (v/v) and observed under ultraviolet excitation with a fluorescence microscope BX51 (Olympus); images were taken with a DP71 camera (Olympus). The images were analysed in ImageJ and XY coordinates of the cotyledon contour and aniline blue–stained cell planes obtained. The XY data were transferred into Microsoft Excel to produce graphs by using the scatter graph function.

Results

Seedling development of European Gesneriaceae

The initial stages of seed germination were similar in the four species examined (Fig. 1). Between 10 and 15 days after sowing, the seed coat was ruptured at one end by the hypocotyl, pushed out by the expanding cotyledons and hypocotyl. The radicle emerged earlier in New World *Corytoplectus speciosus* than in Old World species. Shortly afterwards, the cotyledons unfolded (1 DAU). At this point both cotyledons were equal in size (see Fig. 1).

Anisocotyly became statistically established in *Streptocarpus rexii* at 9–12 DAU (Table 1) and visually apparent between 21 and 30 DAU (Figs 2A, 3B). In contrast, *Haberlea rhodopensis* and *Ramonda myconi* seedlings did not show clear anisocotyly (see Table 1; Figs 2B,C, 3D–I). Most seedlings remained isocotylous, with a few seedlings showing weak anisocotyly (Figs 2B2,C2, 3J,K) but with no statistical significance (see Table 1). In *Corytoplectus speciosus*, all seedlings remained isocotylous, with very little variation between the two cotyledons (see Table 1; Fig. 2D).

To illustrate the differences between the studied species, a linear trend line was fitted to the scatter plots of seedling cotyledon sizes in relation to time, from the folded cotyledon stage to 20 DAU (Fig. 2A3–D3). *Corytoplectus speciosus* showed a very high level of linear fit (R^2, 0.99; Fig. 2D3), whereas in *Streptocarpus rexii* the correlation was not strong (R^2, 0.86; Fig. 2A3); the results for the European species were somewhat in-between (*Haberlea rhodopensis*; R^2, 0.90; Fig. 2B3; *Ramonda myconi*, R^2, 0.93; Fig. 2C3). Furthermore, the trend line in *Corytoplectus speciosus* sloped in almost perfect correlation between the smaller and larger cotyledons ($y = 0.95x + 0.00$, Fig. 2D3), whereas in *Streptocarpus rexii* it was strongly inclined towards the larger cotyledon ($y = 0.63x + 0.07$, Fig. 2A3). In *Haberlea rhodopensis* and *Ramonda myconi*, the slope of the trend line was between those for *Corytoplectus speciosus* and *Streptocarpus rexii* (*H. rhodopensis*, $y = 0.87x + 0.01$, Fig. 2B3; *R. myconi*, $y = 0.84x + 0.02$, Fig. 2C3).

Plumules started to be observed in *Corytoplectus speciosus* at 5 DAU, in *Haberlea rhodopensis* at 6 DAU and in *Ramonda myconi* at 15 DAU (see Table 1). The morphology of seedings and plumules is shown in Fig. 3. In *Streptocarpus rexii*, instead of plumules, leaf primordia formed at a much later stage of about 50 DAU (65 days after sowing; see Nishii & Nagata, 2007; Nishii *et al*., 2010). In all species, stomata were found only on the underside of cotyledons.

In seedlings with folded cotyledons, dividing cells were observed more or less scattered across both cotyledons (Fig. 4A). Later, at 1 DAU, the arrest front moved towards the base and the dividing cells were focused in the proximal area of the cotyledons (Fig. 4B). At 6 DAU (10 DAU for *Corytoplectus speciosus*) cell division had ceased in all species except for *Streptocarpus rexii*, in which cell division was observed continuously in one cotyledon (Table 2, Fig. 4C).

In *Streptocarpus rexii*, lateral veins were formed in cotyledons at 6 DAU. At 30 DAU, about 3 or 4 veins (mean, 3.6 ± 0.7) were observed in the larger cotyledon and 1 or 2 veins (mean, 1.4 ± 0.4) in the smaller cotyledon (Table 3). In contrast, in *Haberlea rhodopensis*

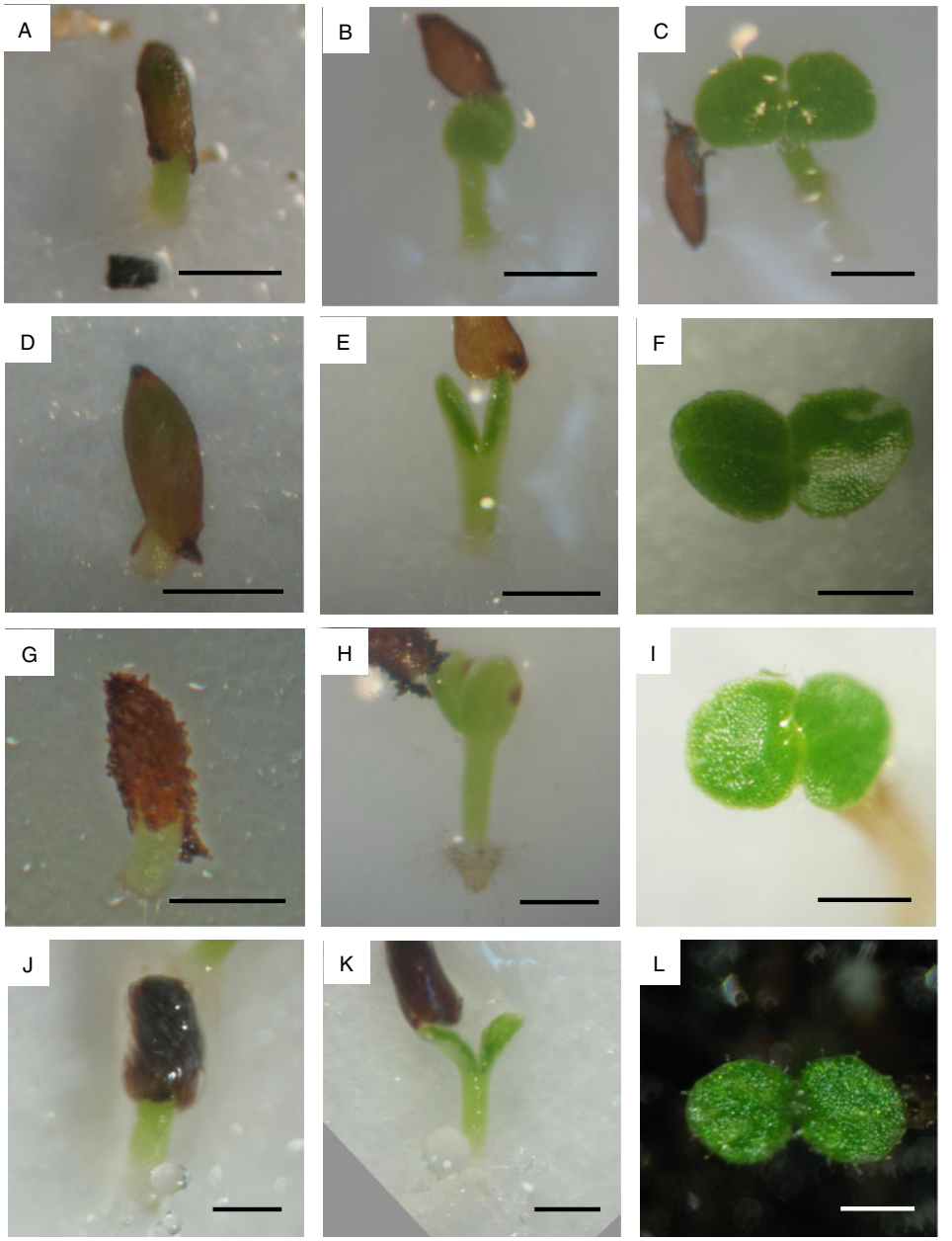

FIG. 1. Germination in *Streptocarpus rexii* (A–C), *Haberlea rhodopensis* (D–F), *Ramonda myconi* (G–I) and *Corytoplectus speciosus* (J–L). A, D, G and J, Germinated seeds; B, E, H and K, seedlings with folded cotyledons; C, F, I and L, seedlings with unfolded cotyledons. Scale bars, 500 µm.

TABLE 1. Descriptive statistics for cotyledons during seedling development

Species and DAU	LC area (mm^2)[a]	SC area (mm^2)[a]	LC/SC[a]	P[b]	*NP*	*N*
Streptocarpus rexii						
FC	0.12 ± 0.01	0.12 ± 0.01	1.07 ± 0.05	0.47	0	3
1	0.17 ± 0.00	0.16 ± 0.01	1.06 ± 0.04	0.40	0	3
3	0.52 ± 0.04	0.48 ± 0.04	1.07 ± 0.01	0.59	0	3
6	0.79 ± 0.07	0.60 ± 0.15	1.45 ± 0.29	0.33	0	3
9–12	0.61 ± 0.04	0.43 ± 0.03	1.44 ± 0.12	0.02*	0	4
20	0.86 ± 0.05	0.60 ± 0.05	1.44 ± 0.04	0.01*	0	4
40	7.23 ± 1.29	0.97 ± 0.10	7.34 ± 0.77	0.02*	0	4
Haberlea rhodopensis						
FC	0.19 ± 0.03	0.17 ± 0.03	1.10 ± 0.02	0.70	0	3
1	0.25 ± 0.03	0.22 ± 0.02	1.12 ± 0.05	0.51	0	3
3	0.31 ± 0.03	0.29 ± 0.03	1.08 ± 0.04	0.64	0	4
6	0.36 ± 0.02	0.33 ± 0.02	1.10 ± 0.07	0.31	4	4
15	0.41 ± 0.03	0.38 ± 0.02	1.08 ± 0.05	0.42	4	4
40	0.58 ± 0.10	0.54 ± 0.13	1.12 ± 0.08	0.80	3	3
Ramonda myconi						
FC	0.18 ± 0.01	0.16 ± 0.01	1.15 ± 0.08	0.19	0	3
1	0.23 ± 0.02	0.21 ± 0.02	1.09 ± 0.04	0.59	0	3
3	0.35 ± 0.02	0.34 ± 0.02	1.05 ± 0.01	0.66	0	3
6	0.48 ± 0.01	0.42 ± 0.02	1.15 ± 0.07	0.09	0	4
15	0.52 ± 0.02	0.47 ± 0.02	1.13 ± 0.03	0.06	4	5
40	0.53 ± 0.11	0.44 ± 0.08	1.20 ± 0.05	0.23	3	4
Corytoplectus speciosus						
FC	0.15 ± 0.01	0.14 ± 0.01	1.05 ± 0.02	0.73	0	4
1	0.22 ± 0.01	0.22 ± 0.01	1.04 ± 0.02	0.64	0	5
5	0.40 ± 0.04	0.37 ± 0.04	1.08 ± 0.02	0.62	1	8
10	0.75 ± 0.05	0.72 ± 0.04	1.05 ± 0.02	0.63	5	5
20	1.10 ± 0.03	1.06 ± 0.03	1.04 ± 0.01	0.33	5	5
30	1.03 ± 0.07	0.99 ± 0.07	1.04 ± 0.01	0.72	5	5

DAU, days after cotyledon unfolding; FC, folded cotyledon stage; LC, larger cotyledon in a seedling; LC/SC, ratio of area of larger cotyledon to area of smaller cotyledon in a seedling; *N*, total number of seedlings examined; *NP*, number of seedlings forming a plumule; SC, smaller cotyledon in a seedling.

[a] Values represent means ± standard errors.

[b] For size differences between larger and smaller cotyledons (determined by Student's paired *t*-test).

* $P < 0.05$.

and *Ramonda myconi*, most seedlings did not develop lateral veins, only a few showing 1 or 2 veins in cotyledons at 30–40 DAU, similar to *Corytoplectus speciosus* (see Table 3).

Measurement of epidermal cell size during cotyledon development showed that in both cotyledons of all four species at 3–5 DAU, the proximal region had smaller cells compared with the distal region (Fig. 5). At 15 DAU and 45 DAU in *Streptocarpus rexii*, only the larger cotyledon still had smaller cells ($< 500\ \mu m^2$) in the proximal region, these not being found in the proximal region of the smaller cotyledon (Fig. 5A). In *Haberlea rhodopensis* and *Ramonda myconi*, cell size in the proximal region of both cotyledons had

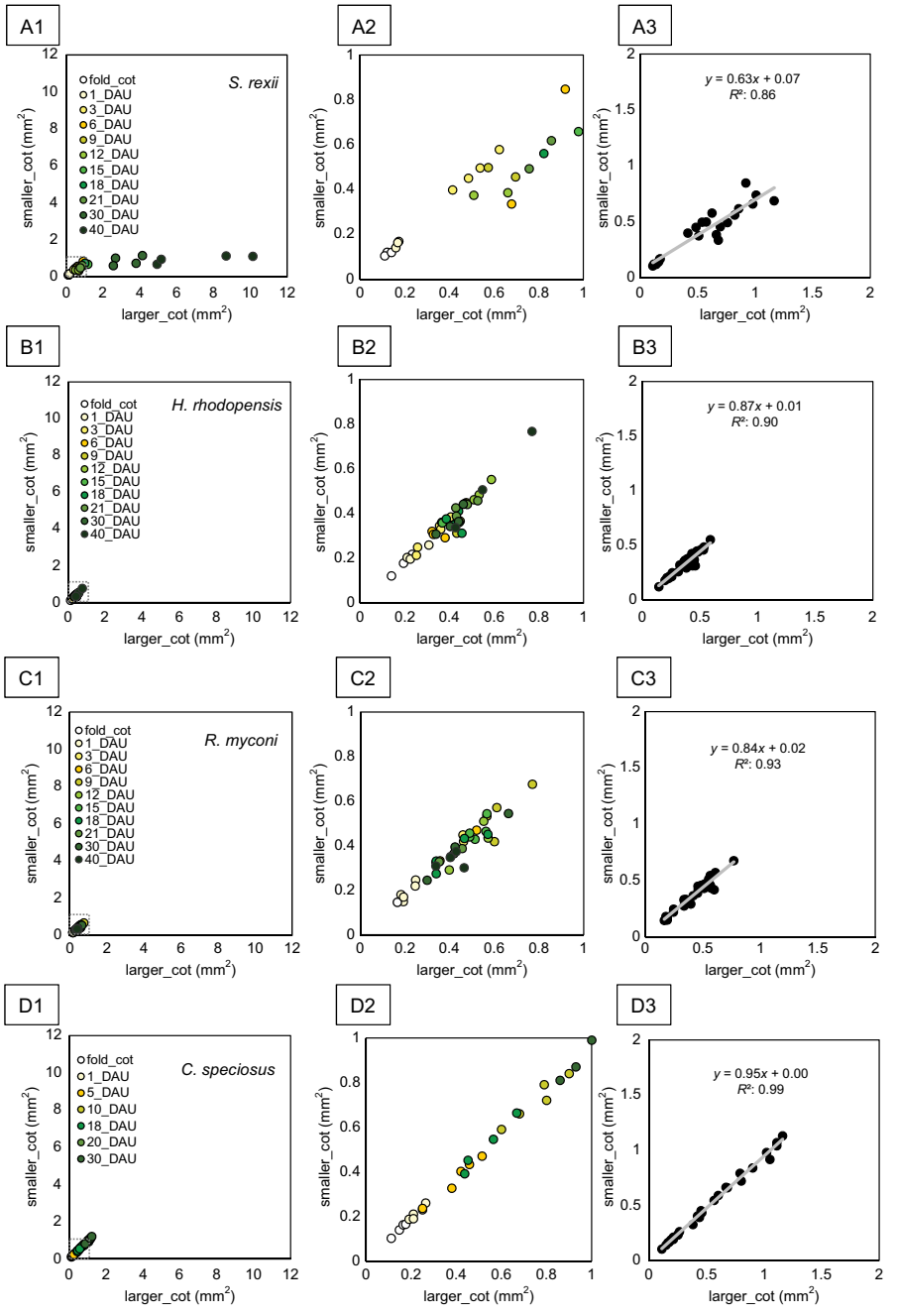

FIG. 2. Growth patterns of cotyledon area. A1–3, *Streptocarpus rexii* with anisocotylous growth; B1–3, *Haberlea rhodopensis*; C1–3, *Ramonda myconi*; D1–3, *Corytoplectus speciosus* with isocotylous growth. A1, B1, C1 and D1, Scatter plots of cotyledon area from folded cotyledon stage to 40 days after cotyledon unfolding (DAU); A2, B2, C2, and D2, magnified views of 1×1 mm^2 plots of A1, B1, C1 and D1, respectively, indicated by stippled boxes; A3, B3, C3 and D3, scatter plots of cotyledon area from folded cotyledon stage to 20 DAU and linear trend lines.

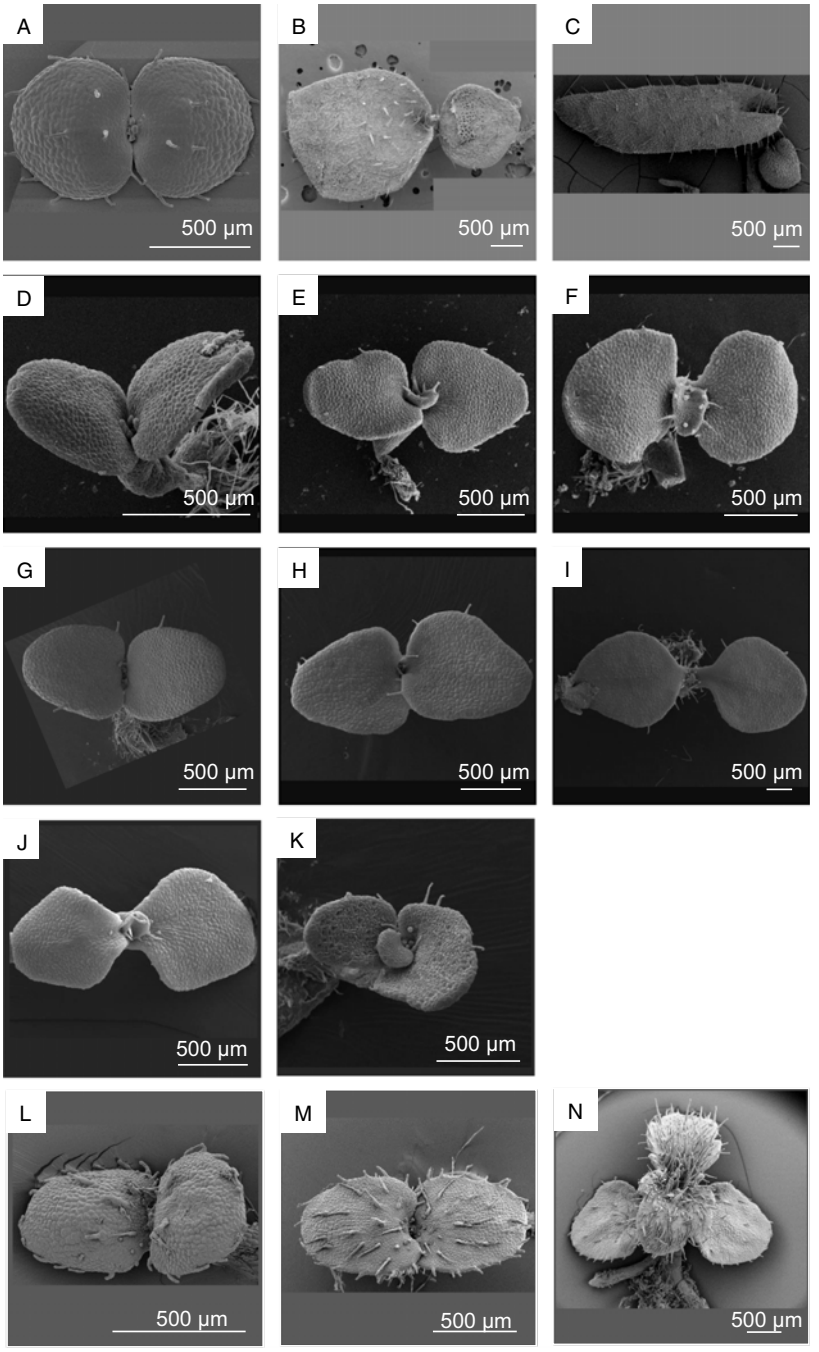

FIG. 3. Scanning electron micrographs of seedlings. A–C, *Streptocarpus rexii*: A, 5 days after cotyledon unfolding (DAU); B, 25 DAU; C, 40 DAU. D–F, J, *Haberlea rhodopensis*: D, 5 DAU; E, 15 DAU; F, 25-DAU seedling with equal cotyledons; J, 25-DAU seedling with slightly unequal cotyledons. G–I, K, *Ramonda myconi*: G, 5 DAU; H, 15 DAU; I, 25-DAU seedling with equal cotyledons; K, 25-DAU seedling with slightly unequal cotyledons. L–N, *Corytoplectus speciosus*: L, 1 DAU; M, 5 DAU; N, 20 DAU.

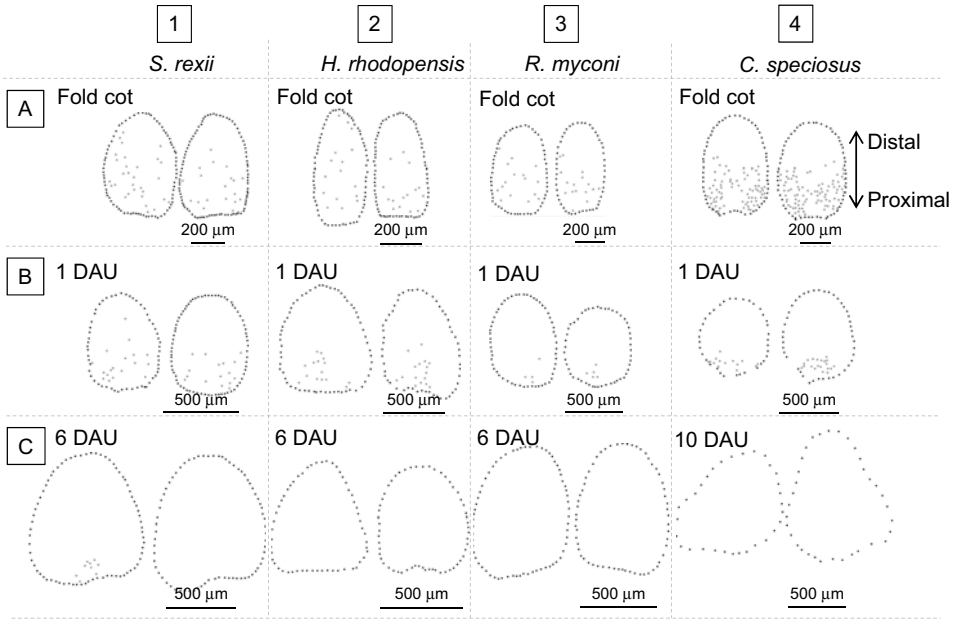

FIG. 4. Distribution of planes of newly divided cells stained with aniline blue. A, Folded cotyledon stage; B, cotyledons 1 day after cotyledon unfolding (DAU); C, cotyledons 6 DAU (10 DAU for *Corytoplectus speciosus*). 1, *Streptocarpus rexii*; 2, *Haberlea rhodopensis*; 3, *Ramonda myconi*; 4, *Corytoplectus speciosus*.

increased at 15 DAU compared with 5 DAU and had further expanded at 45 DAU. Some variation in cell size was observed between the proximal and distal regions (Fig. 5B,C). Cell size in cotyledons of *Corytoplectus speciosus* at 20 DAU was uniform across and between the cotyledons (Fig. 5D).

DISCUSSION

In this study, we investigated the phenomenon of anisocotyly in two European Gesneriaceae, *Haberlea rhodopensis* and *Ramonda myconi*, through the localisation and characterisation of meristematic activities in cotyledons of developing seedlings.

Our results show a cotyledon growth behaviour of the European species that is in-between the typical anisocotyly present in the Old World *Streptocarpus rexii* and the typical isocotyly of the New World *Corytoplectus speciosus*. Previous studies of *Haberlea* and *Ramonda* have reported their less marked anisocotyly (Hill, 1938), but without detailed analysis of the underlying developmental cascades. In our studies, many seedlings of *Haberlea rhodopensis* and *Ramonda myconi* showed no anisocotyly and a plumule was observed at an earlier stage than in *Streptocarpus rexii*. About one-fifth of the seedlings of the European genera showed size differences between the cotyledons in a pair, albeit weak. This unequal size *per se* would not qualify as anisocotyly. Anisocotyly involves several

TABLE 2. Detection of cell planes in newly divided cells by aniline blue staining

Species and DAU	Larger cotyledon[a]	Smaller cotyledon[a]
Streptocarpus rexii		
Folded cotyledon stage	3/3[b]	3/3[b]
1	3/3[b]	3/3[b]
3	3/3[b]	2/3[b]
6	3/3[b]	0/3
20	2/2[b]	0/2
40	2/2[b]	0/2
Haberlea rhodopensis		
Folded cotyledon stage	3/3[b]	3/3[b]
1	3/3[b]	3/3[b]
3	3/3[b]	3/3[b]
6	1/3[b]	1/3[b]
15	0/2	0/2
40	0/2	0/2
Ramonda myconi		
Folded cotyledon stage	3/3[b]	3/3[b]
1	3/3[b]	3/3[b]
3	0/3	0/3
6	0/3	0/3
15	0/2	0/2
40	0/3	0/3
Corytoplectus speciosus		
Folded cotyledon stage	5/5[b]	5/5[b]
1	7/7[b]	7/7[b]
5	2/4[b]	2/4[b]
10	0/5	0/5
20	0/5	0/5

DAU, days after cotyledon unfolding.

[a] Numbers before the slash are the number of cotyledons showing divisions, and those after the slash are the number of seedlings analysed.

[b] Stages during which meristems are present in the cotyledons.

features, such as continued meristematic activity in a narrowly defined basal region of the cotyledon and its limitation to only one of the two cotyledons in a pair (Burtt, 1970; Jong, 1970).

Aniline blue staining showed that all four species initially possessed meristematic activity in the basal region of both their cotyledons, where the cells were, typically for a meristem, very small. Cell sizes in the New World *Corytoplectus speciosus*, which lacks anisocotyly, became uniform across and between both cotyledons over time, whereas the European species retained smaller cells in the proximal area of cotyledons and, at least in *Haberlea*, more so in one than in the other cotyledon at 25 DAU (see Figs 4, 5). This may indicate the presence of typical anisocotyly in the European species, although weak and

TABLE 3. Number of lateral veins

Species and DAU	Larger cotyledon[a]	Smaller cotyledon[a]	No. of seedlings examined
Streptocarpus rexii			
1	0.0 ± 0.0	0.0 ± 0.0	3
3	0.0 ± 0.0	0.0 ± 0.0	5
6	0.5 ± 0.3	0.0 ± 0.0	4
12	2.0	0.0	2
30	3.6 ± 0.7	1.4 ± 0.4	5
Haberlea rhodopensis			
1	0.0 ± 0.0	0.0 ± 0.0	3
3	0.0 ± 0.0	0.0 ± 0.0	5
6	0.0 ± 0.0	0.0 ± 0.0	3
15	0.0	0.0	2
30–40	0.8 ± 0.3	0.5 ± 0.3	4
Ramonda myconi			
1	0.0 ± 0.0	0.0 ± 0.0	3
3	0.0 ± 0.0	0.0 ± 0.0	3
6	0.0 ± 0.0	0.0 ± 0.0	3
15	0.0	0.0	2
30–40	0.6 ± 0.4	0.0 ± 0.0	5
Corytoplectus speciosus			
1	0.0 ± 0.0	0.0 ± 0.0	7
5	0.5 ± 0.5	0.5 ± 0.5	4
10	0.0 ± 0.0	0.0 ± 0.0	5
20	0.6 ± 0.4	0.4 ± 0.2	5

DAU, days after cotyledon unfolding.
[a] Numbers represent the mean number of lateral veins per cotyledon ± standard error.

persisting for only a short period. This was reflected in the small size of the cotyledons (<1 mm^2) and small size difference between the macrocotyledon and the microcotyledon. In *Streptocarpus rexii* the ratio can be greater than 5–7 times in seedlings at 40 DAU (see Table 1; Nishii *et al.*, 2017). Considering all the data together, it appears that at least some seedlings of *Haberlea rhodopensis* and *Ramonda myconi* possess an anisocotylous phase but of very short duration, and thus anisocotyly is not completely lost in these species, although it is greatly reduced (Fig. 6).

Three Gesneriaceae genera are found in Europe on the Balkan Peninsula and in the Pyrenees: *Haberlea*, *Ramonda* and *Jancaea* Boiss. In recent phylogenetic studies, *Jancaea* was nested within *Ramonda* (Petrova *et al.*, 2015). *Jancaea* seedlings also appear to show weak anisocotyly for some seedlings within a batch (K. Nishii, personal observation). Thus, all three European Gesneriaceae genera may share a similar cotyledon development with residual anisocotyly.

Anisocotyly has been proposed to be potentially beneficial for the quick expansion of photosynthetic area in the dark forest conditions that many Old World Gesneriaceae inhabit (Burtt, 1970). The European genera, however, occupy alpine habitats and much cooler

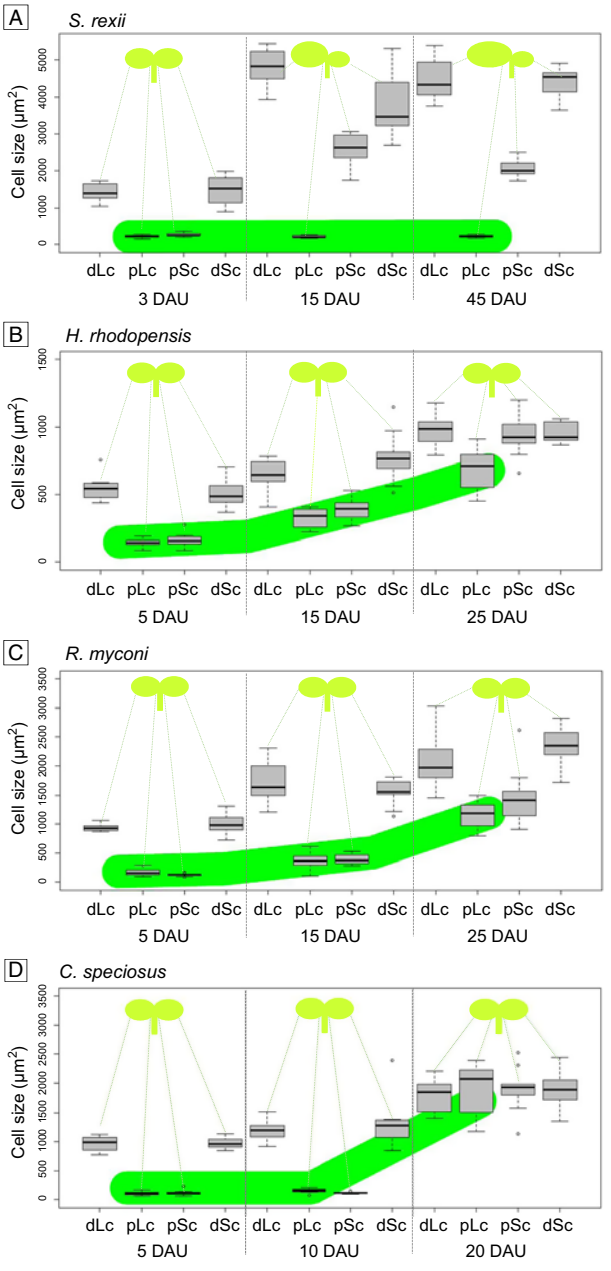

FIG. 5. Box plots of epidermal cell sizes in cotyledons ($n = 10$). Cells in proximal and distal positions in the smaller and larger cotyledon were plotted separately. A, *Streptocarpus rexii*; B, *Haberlea rhodopensis*; C, *Ramonda myconi*; D, *Corytoplectus speciosus*. DAU, days after cotyledon unfolding; dLc, distal part of larger cotyledon; dSc, distal part of smaller cotyledon; pLc, proximal part of larger cotyledon; pSc, proximal part of smaller cotyledon. Broad green lines highlight the cells observed in the proximal region of the larger cotyledons, tracking their cell sizes over time.

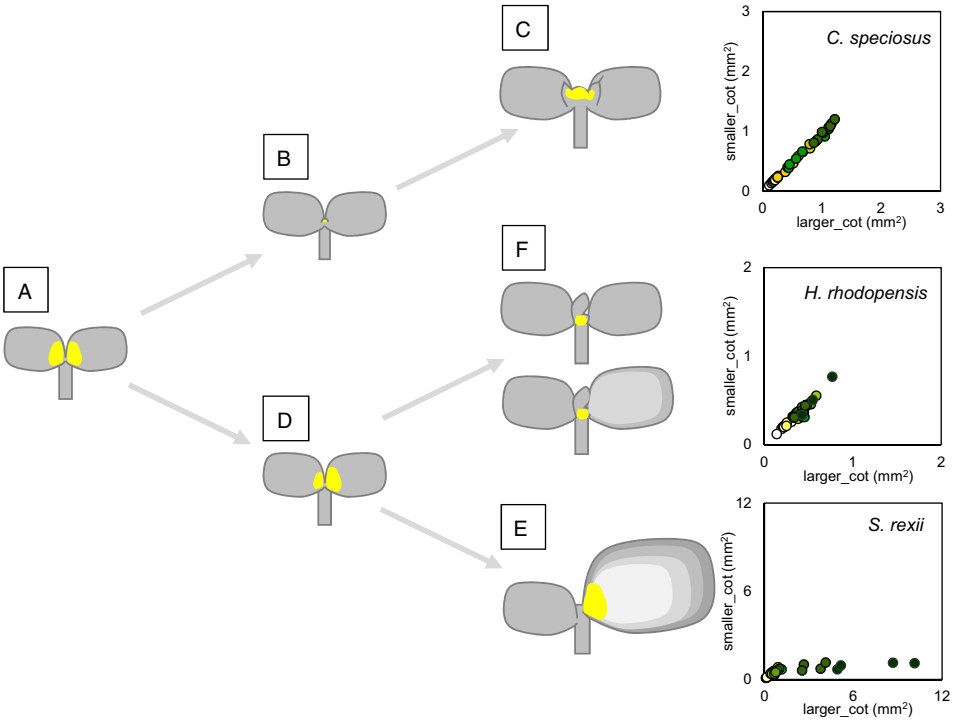

FIG. 6. Seedling development of typical isocotylous (*Corytoplectus speciosus*) and anisocotylous (*Streptocarpus rexii*) Gesneriaceae species, and European Gesneriaceae (*Haberlea rhodopensis*). Yellow indicates areas of detected meristem activity. A → B → C, New World Gesneriaceae. A basal meristem exists in both cotyledons just after germination (A) but ceases simultaneously in both cotyledons (B). A plumule develops and a shoot apical meristem produce leaves (C). A → D → E, Old World Gesneriaceae. Both cotyledons maintain cell-division capabilities until the cotyledon fates are determined (A → D). Later, the basal meristem becomes persistent in one cotyledon, which develops into the macrocotyledon (E). A → D → F, European Gesneriaceae. Both cotyledons initially show a basal meristem with variable and limited activities (A → D), which ceases simultaneously soon afterwards, at which point plumule development occurs, creating seedling populations with variable and limited anisocotyly or no anisocotyly (F).

environments. It is possible that the production of plumules and leaves at an early stage may be beneficial in protecting the shoot apical meristem from damage by low temperatures, reminiscent of winter buds tightly wrapping shoot apical meristems (Moore, 1909). However, this hypothesis requires further investigation.

This study was limited to a few Old World genera exhibiting weak anisocotyly and showed that cotyledon size differences, even when small, were based on basal meristem activity in the macrocotyledon. It should be seen as an initial contribution to future broader studies across the subfamily Didymocarpoideae. Such research would allow detailed analyses of the evolutionary history of anisocotyly in the family Gesneriaceae.

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