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Light as environmental regulator for germination and macrocotyledon development in *Streptocarpus rexii* (Gesneriaceae)

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Abstract

The light requirements for seed germination and seedling development have not been studied widely in Gesneriaceae. Here we report on the effects of light on these aspects in *Streptocarpus rexii* (Gesneriaceae). Seeds did not germinate in the dark but required light, indicating photoblastic seed germination. Light exposure was also required for the establishment of post-germination anisocotylous development, with the seedlings showing the typical basal meristem in the proximal region of the macrocotyledon. In the dark, however, the basal meristem was not established and the seedlings showed two equally sized microcotyledons. Hypocotyl elongation, a typical skotomorphogenesis, was also observed in the dark. Different wavelengths of light, in the red and blue spectrum, differentially affected seedling development. While seedlings exposed to blue light showed typical anisocotyly, seedlings under red light did not and basal meristem activity was not observed. These results suggest that light quality is an important factor for the establishment of anisocotyly in *S. rexii* seedlings. Thus, light plays important roles at different developmental stages of *S. rexii*, which is perhaps linked to adaptation to dense forest habitats where light is marginal. Environmental signals might coordinate endogenous physiological pathways for the extraordinary seedling development in *S. rexii*. Unraveling these interactions requires further studies.

Keywords: Anisocotyly; Blue light; Cotyledon; Germination; Gesneriaceae; Red light; *Streptocarpus*

1. Introduction

Light is an important environmental factor for regulating a range of processes in plants; light signals act through light receptors as environmental cues throughout the life cycle, and light received by chlorophyll is used as an energy source for photosynthesis of the growing plants. Light is also an important signal for seed germination and seedling development. Many plants, such as lettuce, show photoblastic seed germination, requiring light exposure for germination (e.g. Borthwick et al., 1954). Under light, seedlings continue with photomorphogenesis, while seedlings in the dark proceed to skotomorphogenesis (Von Arnim and Deng, 1996).

In plants, light is perceived by several photoreceptors, that can detect different wavelength of light, e.g. red, far-red, blue, UV-A, and these receptors regulate different downstream factors and processes. The light signal is passed to internal physiological pathways that control plant morphogenesis to fit their environment (Wada et al., 2005). Recent detailed molecular studies in *Arabidopsis thaliana* L. revealed genetic players involved in the light signal transduction pathways (Fankhauser and Chory, 1997).

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Although there are many studies on the effects of light on plant growth, few have been carried out on the family Gesneriaceae, even at the descriptive level. This is surprising since seedlings of some Gesneriaceae show a unique seedling development, anisocotyly, that was initially studied over 70 years ago (Hill, 1938). Several anatomical and molecular studies on anisocotyly have recently been carried out in *Streptocarpus* Lindl. and *Monophyllaea* R.Br. (reviewed in Nishii et al., 2010). For instance, in *Streptocarpus* or *Monophyllaea*, just after germination, both cotyledons are identical and equally capable of growth. However subsequently, one cotyledon continues to grow by means of the basal meristem to become the macrocotyledon, while the other cotyledon, the microcotyledon, stops developing and withers away (Imaichi, et al., 2000, 2001; Jong, 1970; Mantegazza et al., 2007; Nishii and Nagata, 2007; Tsukaya, 1997). Since the determination of which cotyledon becomes the macrocotyledon occurs after germination (Oehlkers, 1923; Tsukaya, 1997), environmental factors may play an important role in the process. In previous studies, the effect of gravity and light was tested in several Gesneriaceae species. It was found that the direction of light may determine the macrocotyledon in *Chirita lavandulacea* Stapf and *Streptocarpus rexii* Lindl.; i.e. with the cotyledon opposite the light source, that may receive more light, becoming the macrocotyledon (Saueregger and Weber, 2004). It was further shown that the macrocotyledon aligns along the gradient of gravity in *C. lavandulacea*, but not in *Monophyllaea horsfieldii* R.Br. or *S. rexii* (Saueregger and Weber, 2004; Tsukaya, 1997). It was also reported that exposure to red light of 590–750 nm (using plastic filters), resulted in weakly anisocotylous seedlings in *S. rexii*, while it induced 'distinctly larger basal lobes' of the macrocotyledon in *C. lavandulacea* (Saueregger and Weber, 2004). These results suggest that light can affect the growth of Gesneriaceae seedlings.

We therefore investigated the effects of different wavelengths of light, in particular in the red (660±20 nm) and blue (470±30 nm) spectra with LED lights that provide more narrow spectra than plastic filters used previously in Saueregger and Weber (2004). We exposed material at different stages of seedling development in *Streptocarpus*, starting from seed germination. *S. rexii* was selected as research material, since this species has been extensively investigated in previous physiological and developmental genetic studies (e.g. Mantegazza et al., 2007, 2009; Nishii and Nagata, 2007; Nishii et al., 2010).

2. Materials and methods

2.1. Plant material

Seeds of *S. rexii* were kindly provided by the Kyoto Prefectural Botanic Garden, Prof J. Van Staden (University of KwaZulu-Natal, SA), and the Horticulture Division of the Royal Botanic Garden Edinburgh (RBGE; Edinburgh, UK). Seeds were sterilized with a 0.2% sodium hypochlorite solution that contained 0.02% Nonidet P-40 (Sigma-Aldrich, MO, USA) and washed with distilled water. Subsequently, they were sown in 9 cm plastic Petri dishes on a culture medium consisting of 30% strength Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) and 1% sucrose, solidified with 0.8% agarose (Nishii et al., 2004). The seeds were imbibed and the seedlings were cultured in a growth chamber at 23 °C under the different light conditions described below.

2.2. Different length of light exposure during germination

Just after sowing, dishes were moved to the dark, or exposed to continuous light for different lengths of time, 1, 5, and 10 days and then returned to the dark, until their evaluation 13 or 20 days after imbibition (DAI). Control seeds were cultured under continuous light for the same periods of time. Light was provided by white fluorescent tubes (NEOLUMISUER FLR40S, Mitsubishi Electric Osram Ltd., Yokohama, Japan). The seedlings were fixed for further analyses (see Anatomical analyses section).

To assess the seed germination, 20 seeds were sown under different light conditions and germination observed at 13 DAI, with two replicates per treatment. Germination was defined as hypocotyl emergence. To evaluate the morphology under different light treatments, 8 seedlings were examined 20 DAI.

2.3. Different wavelength of light

The above described fluorescent light was used for the control light exposure. For red and blue light illumination, red (SLP-838A-37 S1, SANYO Consumer Electronics Co., Ltd., Tottori, Japan; 660±20 nm) and blue (SLP-0137A-51; 470±30 nm) LED lights were used at 80 W m^{-2}. Seeds were continuously exposed before evaluation. 15 seedlings cultured under different light wavelength were observed 30DAI, and 16 to 26 seedlings after 40DAI. Seedlings were prepared for analysis as below.

2.4. Anatomical analyses

Anatomical analyses were carried out as described in Nishii et al. (2004). Briefly, to measure the cotyledon area, samples were fixed in FAA (5% acetic acid, 45% ethanol, 5% formaldehyde, 45% distilled water) and preserved in 70% Et-OH. Images of cotyledons were captured under an Olympus SZX9 dissecting microscope (Olympus Optical Industries Co., Tokyo, Japan). The images were analyzed by a graphics program, NIH image (Scion Co., Maryland, USA), and the macro- and microcotyledon area and their ratios calculated. For observation of the leaf surface and vascular pattern, samples were fixed in ethanol and acetic acid (4 : 1). The fixed samples were dehydrated in an ethanol series then cleared in chloral hydrate. The cleared samples were observed under an optical microscope (BX51, Olympus).

2.5. Statistical analyses

The data were analyzed in Microsoft Office Excel (Microsoft Corporation, WA, USA); cotyledon area, ratio, length and width, hypocotyl length, petiole length were analyzed by a one-way ANOVA and *P* values between treatments calculated. The values given are treatment means and their standard error of the means.

3. Results

3.1. Definition of germination and anisocotyly formation in S. rexii

The process of germination and consecutive seedling development can be divided into distinct developmental stages (Fig. 1). Seeds start germination 10 to 15 DAI, which was defined by hypocotyl emergence from the seed coat (hypocotyl emergence stage; Fig. 1B). Seedlings of *Streptocarpus* and most Gesneriaceae have a delayed root formation (Weber, 1978, 2004), thus the elongating hypocotyl ruptures the seed coat (Fig. 1B, arrow). Rhizoids (root hair like trichomes in Imaichi et al., 2000) anchor the seedling to the substrate (Weber, 1978, 2004). Later, the cotyledons emerge from the seed coat but are still folded (folded-cotyledon stage; Fig. 1C). The cotyledons are soon unfolded (unfolded-cotyledon stage; Fig. 1D). At 20 to 30 DAI, unequal cotyledons can be observed (anisocotyly stage; Fig. 1E, F). *S. rexii* is a rosulate species, and new leaf primordia are formed from the groove meristem at the base of the macrocotyledon. This leaf is termed a phyllomorph (Jong, 1970; Jong and Burtt, 1975) because of the unique meristem activities. This process is described in detail in previous studies (Mantegazza et al., 2007; Nishii and Nagata, 2007). Adventitious roots are endogenously formed at the base of the hypocotyl (Fig. 1F; see Fig. 5g in Mantegazza et al., 2007).

3.2. Photoblastic seed germination in S. rexii

No seeds had germinated after 1 or 5 days of light treatment. After 10 days of light 84.5% of the seeds had already germinated. At the end of the germination experiments at 13 DAI, for the seeds that had been exposed to light for 1 day (1 dayL), very few had germinated (6.5%). Seeds treated 5 days under light (5 dayL) germinated to 94%. Control seeds exposed continuously to light and those exposed to light for 10 days (10 dayL) all germinated, whereas no seed germinated in continuous darkness. Thus, *S. rexii* seeds require light exposure for at least 5 days to achieve significant germination levels (Fig. 2).

3.3. Effects of length of light exposure on macrocotyledon formation

Control seedlings grown under continuous light showed the initiation of anisocotylous development after 20 days (Figs. 3A, 4A). The size of both cotyledons was only slightly unequal, the area of the larger cotyledon being $0.92 \pm 0.19\ mm^2$, and of the smaller cotyledon $0.74 \pm 0.15\ mm^2$ ($P = 0.481$). One to two branched lateral veins were observed only in the larger cotyledon, the developing macrocotyledon (Fig. 4B). Seedlings developing in the dark from seeds exposed to light for 5 days (5 dayL) and 10 days (10 dayL) did not show anisocotyly (5 dayL larger cotyledon: $0.20 \pm 0.01\ mm^2$, smaller cotyledon $0.19 \pm 0.01\ mm^2$, $P = 0.532$; 10 dayL larger cotyledon $0.29 \pm 0.02\ mm^2$, smaller cotyledon $0.27 \pm 0.01\ mm^2$, $P = 0.389$) (Fig. 3), and had cotyledons smaller than the microcotyledon of the control ($P < 0.001$***; Fig. 4A). Lateral veins were never observed (Fig. 4B). The length and width of epidermal cells were not significantly different between the distal half of the macrocotyledon, and the microcotyledon of any treatment ($P = 0.569$; Table 1). Thus, growth of the cotyledon from cell division but not cell expansion was inhibited in the dark.

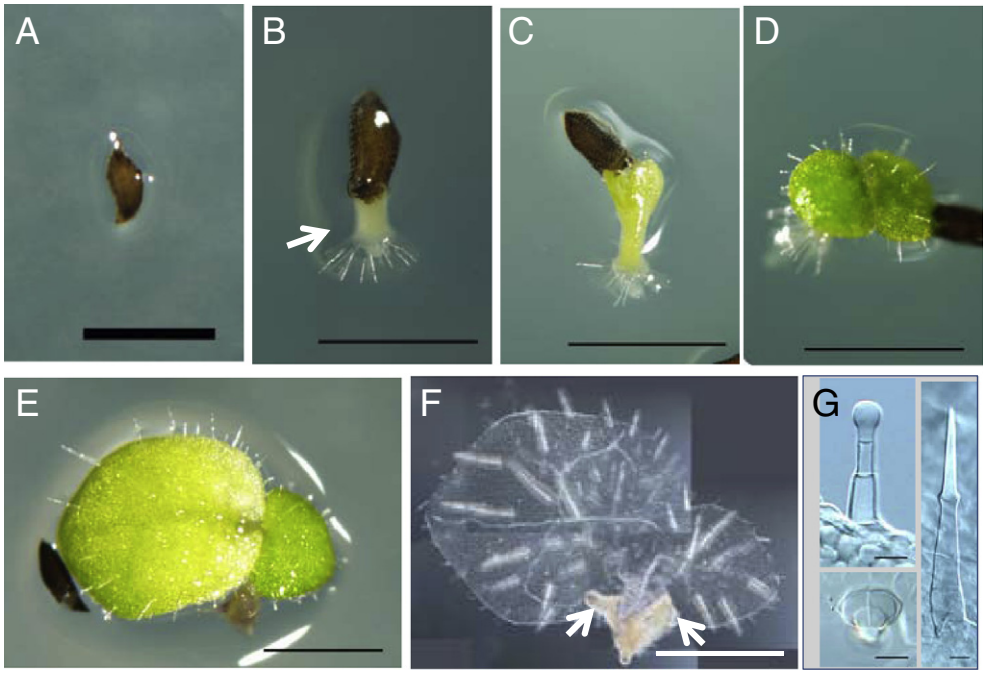

Fig. 1. Germination and seedling developmental stages in *S. rexii*. Seed germination was divided into stages. A, Ungerminated. B, Hypocotyl emergence stage. An arrow indicates the emerged hypocotyl. C, Folded-cotyledon stage. D, Unfolded-cotyledon stage. E, F, Anisocotyly stage. After germination, cotyledons grow unequally. F, Cleared seedling. Lateral veins observed only in the macrocotyledon. Arrows indicate adventitious roots at the base of the hypocotyl. G, Trichomes observed on the cotyledon. Glandular trichomes (top left), short glandular trichomes (bottom left), eglandular trichomes (right). Scale bars = 0.5 mm (A–D), 1 mm (E, F), 0.2 mm (G).

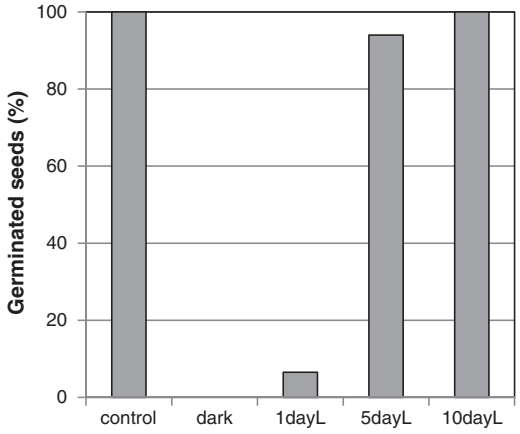

Fig. 2. The effect of light exposure on seedling germination. Seeds were imbibed in continuous darkness (dark), or exposed to light for 1 day (1 dayL), 5 days (5 dayL), and 10 days (10 dayL) before being placed in the dark. Control seeds were exposed continuously under white light. Germination was assessed 13 days after imbibition. Mean values of two replicate experiments for each treatment. $n=20$.

The hypocotyls in control seedlings under light were short (0.81±0.11 mm) with squarish cells (Fig. 3D). The hypocotyls were more than three times longer in seedling grown in the dark ($P<0.001$***). The hypocotyls of 5 dayL seedlings (5.57±0.44 mm) were longer than those of 10 dayL seedlings (2.61±0.29 mm; $P<0.001$***; Fig. 3B, C). The elongation of hypocotyls was in correlation with the elongation of the hypocotyl cells (Fig. 3; control: 15.44×22.11 μm; 5 dayL: 209.48×17.87 μm; 10 dayL: 138.35×23.66 μm, $P<0.001$***). The hypocotyl cell width was similar between control (22.11±0.84 μm) and 10 dayL seedlings (23.66±1.35 μm; $P=0.377$), but slightly narrower in 5 dayL seedlings (17.87±0.62 μm; $P<0.01$**; Fig. 3D–F).

3.4. Effect of red and blue light on macrocotyledon development

At 30 DAI, anisocotyly was fully established in control seedlings (area of larger cotyledon 2.24±0.18 mm^2, smaller cotyledon 0.88±0.06 mm^2, $P<0.001$***), but also in seedlings grown under blue light, whereas equally sized cotyledons were observed in seedlings under red light (area of larger cotyledon 0.58±0.04 mm^2, smaller cotyledon 0.50±0.03 mm^2, $P=0.118$; Figs. 5, 6). The size of cotyledons under red light was smaller than those of the microcotyledons of control and blue light treated seedlings ($P<0.001$***; Fig. 6A). In control seedlings, three to four lateral veins were formed in the macrocotyledon, and one to two in the microcotyledon (Fig. 6B). The macrocotyledon of seedlings grown under blue light showed a similar venation pattern to that of the control ($P=0.385$), but a decreased number of lateral veins were observed in the microcotyledon compared to the macrocotyledon ($P<0.001$***). Under red light, lateral veins rarely formed in either cotyledon (Fig. 6B).

On the adaxial cotyledon surface, three types of trichomes were observed; glandular trichomes, short glandular trichomes, and eglandular trichomes (Fig. 1G). In control seedlings 30 DAI, glandular trichomes and eglandular trichomes were observed across the surface of both the macrocotyledon and microcotyledon.

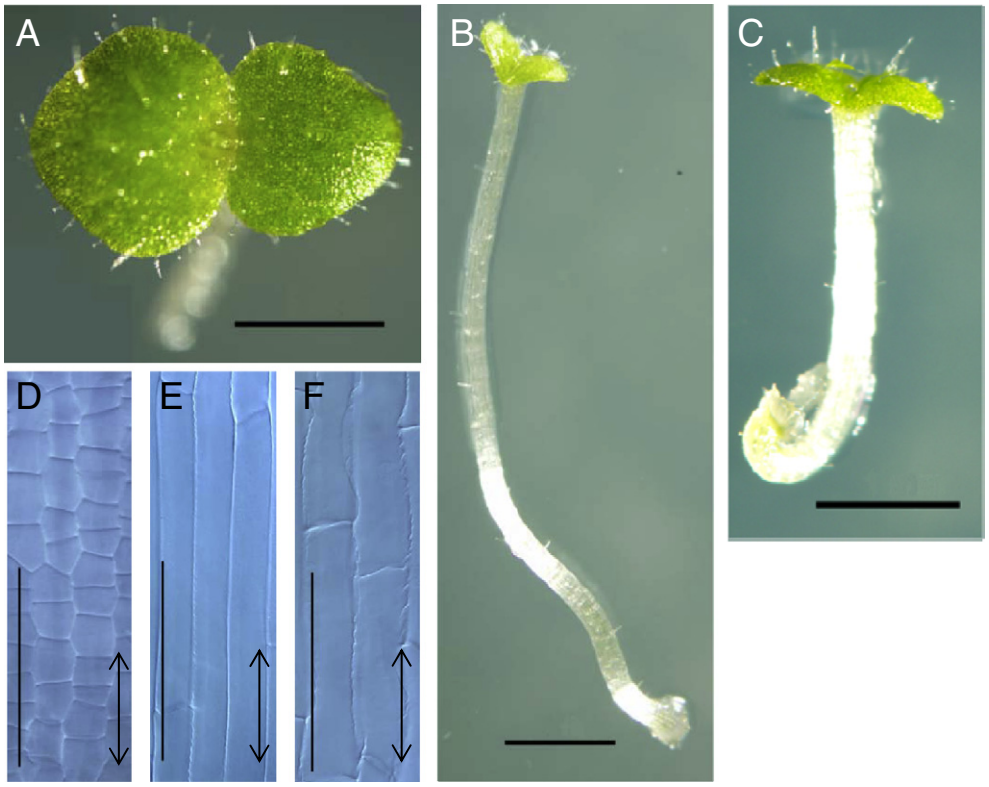

Fig. 3. The effect of light exposure on seedling growth 20 DAI. A, Control seedling showing slight anisocotyly. B, 5 dayL seedling. C, 10 dayL seedling. 5 dayL and 10 dayL seedlings had elongated hypocotyls and smaller equal cotyledons. D–F, Images of hypocotyl epidermal cells. 5 dayL and 10 dayL seedlings had elongated hypocotyl epidermal cells. D, Control. E, 5 dayL. F, 10 dayL. Arrows indicate the cotyledon — root axis of the seedlings. Scale bars=1 mm (A–C), 0.1 mm (D–F).

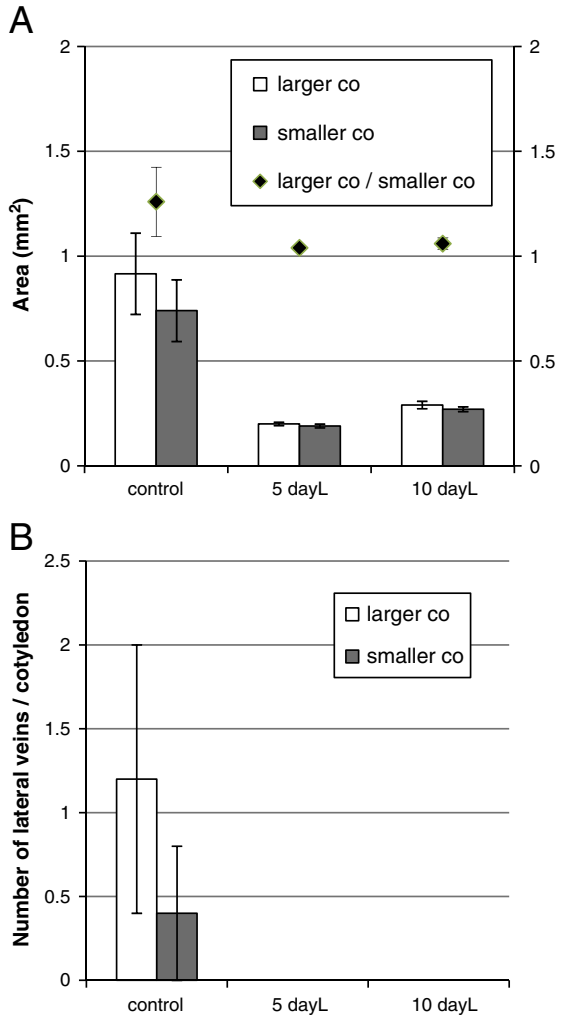

Fig. 4. The effect of light exposure on macrocotyledon development. A, Cotyledon area was measured 20 DAI. Control seedlings showing beginning anisocotylous growth, whereas the 5 dayL and 10 dayL seedlings had smaller size and more equal cotyledon sizes than control seedlings. Cotyledons of 5 dayL seedlings were smaller than in 10 dayL seedlings. B, Lateral vein formation in cotyledons 20 DAI. Control seedlings showed lateral vein formation in cotyledons. In larger cotyledons, more lateral veins had formed compared to the smaller cotyledons. In cotyledons of 5 dayL and 10 dayL seedlings, lateral veins were not observed. $n=8$. Error bars represent the standard error of the mean.

Only a few short glandular trichomes were observed at the base of the microcotyledon, but these covered the proximal half of the macrocotyledon in the region that was newly formed by the activity of the basal meristem (Fig. 7A–D). Seedlings grown under blue light had a similar trichome distribution pattern as the control (Fig. 7H–K). In seedlings under red light, short glandular trichomes were only observed in the basal region (of both cotyledons) (Fig. 7E–G), similar to the microcotyledon in the control (Fig. 7D).

In anisocotylous seedlings of the control and under blue light, smaller cells were observed in the proximal region of the macrocotyledon compared to the distal region of macro- or microcotyledons ($P<0.001$***, Table 2), suggesting cell division and basal meristem activity. However, in seedlings under red light, the epidermal cells in the proximal region of the cotyledons were only slightly smaller than those in the distal region of the same seedlings ($P<0.01$**). The epidermal cells of the proximal region of those cotyledons were about four times larger than those of the proximal region of control macrocotyledons ($P<0.001$***). In the distal region of the larger cotyledon, the epidermal cell size under red light was somewhat smaller than in the control macrocotyledon ($P<0.01$**; Table 2).

The lengths of hypocotyls of control seedlings were 0.70±0.04 mm, while those under red light were 6.69±0.36 mm, about seven times longer ($P<0.001$***). The hypocotyl cell length under red light was 191.92±16.69 μm, about 11 times longer than the control (16.72±1.12 μm; $P<0.001$***; Fig. 5). In seedlings under blue light, both hypocotyls (1.08±0.06 mm) and hypocotyl cells (23.50±1.56 μm) were only slightly longer than those in control seedlings ($P<0.01$*). This suggests that hypocotyl elongation was mainly due to cell elongation.

In control seedlings 30 DAI, the first root initiated from the lower end of the hypocotyl tip, and adventitious roots formed endogenously within the hypocotyl (Fig. 1F). In control seedlings 30 DAI, the number of adventitious roots per seedling was 2.93±0.29, under blue light 2.54±0.14, while under red light hardly any root was formed (0.13±0.09, $n=15$).

In *S. rexii*, 40 DAI, leaf initiation occurred in 25% of control seedlings, and in 27% of seedlings grown under blue light (Fig. 8; Table 3). Although seedlings under red light did not form a macrocotyledon, leaf initiation occurred between the two microcotyledons (Fig. 8E). The proportion of leaf initiation under red light (32%) was comparable to that of the control (25%) and under blue light (27%). Thus, leaf initiation was not inhibited under red light. Unlike the leaf developing in control seedlings, the leaf produced under red light showed an extremely elongated petiole at 60 DAI (Fig. 8G).

4. Discussion

4.1. Photoblastic seed germination in S. rexii

Photoblastic seed germination has been suggested previously for Gesneriaceae seeds (Gardner, 1921), but without providing

Table 1
Length and width of epidermal cells in the distal half of the larger (macro-) and smaller (micro-) cotyledon (control), and cotyledons of 5 dayL, 10 dayL seedlings of *Streptocarpus rexii* 20 DAI. Values are means±standard errors. $n=10$.

	Larger cotyledon		Smaller cotyledon	
	Cell length (μm)	Cell width (μm)	Cell length (μm)	Cell width (μm)
Control	33.16±1.79	21.80±1.26	32.98±2.38	20.48±0.84
5 dayL	35.45±1.48	22.20±0.68	33.02±0.83	20.85±0.31
10 dayL	37.13±2.38	21.35±0.53	36.18±1.96	22.72±1.35

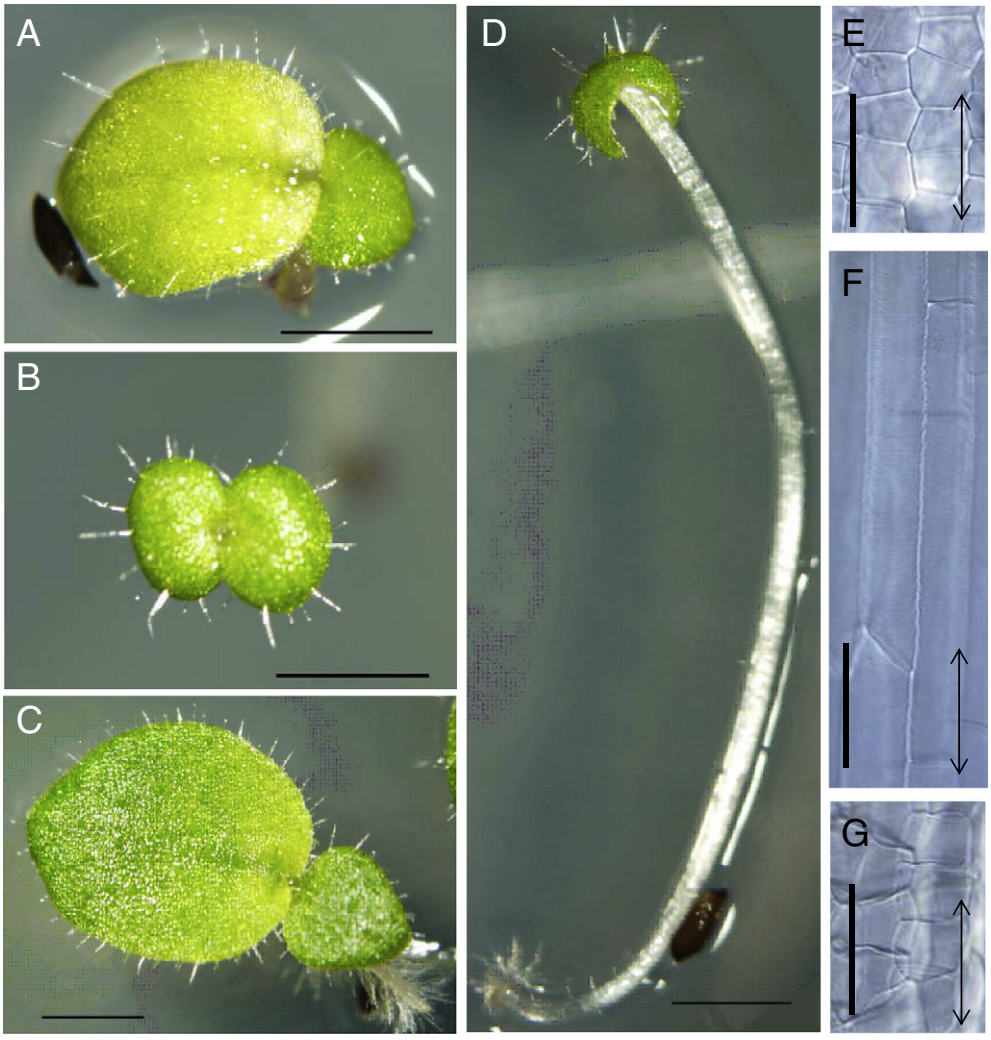

Fig. 5. Effects of light wavelengths on seedling development in *S. rexii* 30 DAI. A, Control seedling under continuous light showed anisocotyly. B, Seedling under red light. C, Seedling under blue light. D, Side view of seedling under red light. Seedling under blue light (C) showed anisocotyly whereas in red light (B, D) smaller equal cotyledons with elongated hypocotyls were formed. E–G, Images of hypocotyl epidermal cells. Seedlings under red light had greatly elongated hypocotyl epidermal cells. E, Control. F, Seedling under red light. G, Seedling under blue light. Arrows indicate the cotyledon — root axis of the seedlings. Scale bars = 1 mm (A–D), 50 μm (E–G).

experimental data. In the present study, we have shown the inability of *S. rexii* seeds to germinate in continuous darkness. We were primarily interested in studying the anisocotylous seedling development of *S. rexii* in the dark. For this to be achieved, we required the seeds to germinate and thus exposed imbibed seeds to various durations of light prior to the dark treatment. In the present study, one day of light was not enough to induce full germination and longer light exposure of five days was required for the majority of seeds of *S. rexii* to germinate. This is clear evidence for photoblastic germination in this species. The light perception must have occurred and triggered processes leading to germination, since the seeds germinated in the dark after light exposure. It remains to be examined whether the increase in germination rate between 1 and 5 day light is caused by the longer imbibition period or longer light exposure. For instance, in lettuce seeds, a longer imbibition time prior to red light irradiation results in a higher germination rate (Borthwick et al., 1954).

The photoblastic germination observed for *S. rexii* seeds may be an adaptation of the plants to their habitat in dense forests and their small seed size (Burtt, 1970; Hilliard and Burtt, 1971). The seeds of *S. rexii* are minute (ca. 0.25 × 0.5 mm) and germinate on the surface of substrates. The seedlings are too small to grow out of crevices or from underground and thus dark germination would not be an advantage. In the field, it was observed that *Streptocarpus itremensis* B.L. Burtt positioned their fruits in moss and the seed germinates in the capsules (M. Möller, personal observations), which might be one way to maximize seedling establishment by avoiding the seeds dropping into deep cracks.

Like all seeds of Old World didymocarpoid Gesneriaceae, *Streptocarpus* seeds lack endosperm (Weber, 2004). In the embryo of *Streptocarpus grandis* N.E.Br., closely related to *S. rexii* (Möller and Cronk, 2001), 'starch grains' are observed in all cotyledon cells in the embryo, which disappear soon after germination. At the folded cotyledon stage, 2 or 3 days after

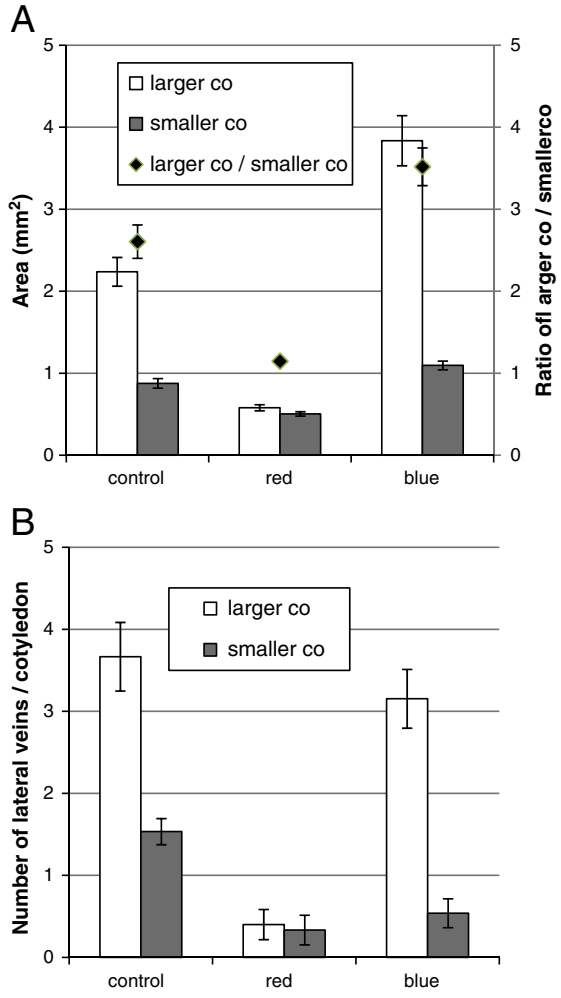

Fig. 6. Effects of light wavelengths on anisocotylous development. A, Cotyledon area was measured 30 DAI. Under white and blue light, macrocotyledons were much larger than microcotyledons. Under red light, both cotyledons were almost equal and smaller than in the control. B, Lateral vein formation in cotyledons 30 DAI. In control or seedlings under blue light, more lateral veins formed in larger cotyledons than in smaller cotyledons. $n=15$. Error bars represent the standard error of the mean.

germination, storage proteins had already disappeared from epidermal cells of cotyledons and chlorophyll has developed in parenchyma cells (Imaichi et al., 2000). It is known that small seeds with low levels of storage reserves often require light signals for germination, and quickly progress to photosynthesis for growth (Smith, 2000).

4.2. Light is essential for normal seedling growth in S. rexii

The growth of *S. rexii* seedlings was very different in the light and dark. Normal seedling development of *S. rexii* in diurnal day/night regimes has previously been reported in detail (Mantegazza et al., 2007; Nishii and Nagata, 2007). Just after germination, the cotyledons grow equally only for a short period by cell division at the base of both cotyledons. Soon, only one cotyledon continues to grow by means of the basal meristem and the other cotyledon ceases to develop further and withers away. In the present study, we found that the cotyledon size in the dark was smaller than that of light-grown macro- or microcotyledons. Since the epidermal cell sizes of the cotyledons were not significantly different between dark and light grown seedlings, the cell division rate seems to have been reduced in the dark, which caused the smaller cotyledon area in the dark. It is not known whether this reduced lamina growth of the cotyledons is due to the lack of energy for photosynthesis or the lack of a light signal working as an environmental cue. Cultured apices of tomato seedlings stop leaf primordia initiation in the dark, but treatments with a paste containing auxin and cytokinin on the apex induced leaf primordia formation without light exposure and thus without photosynthesis (Yoshida et al., 2011). Therefore, light was suggested to work as an environmental cue, rather than an energy source, in the case of leaf initiation from a shoot apical meristem. Further analyses would be necessary to reveal the role of light on the basal meristem activity in *Streptocarpus* here.

Seedlings from five day light treated (5 dayL) seeds showed more severe phenotypes than those 10 dayL treated seeds. Even though germination of these seeds must have occurred in the dark, the seedlings showed unfolded cotyledons of pale green color, suggesting some development of chloroplasts. The hypocotyl length and hypocotyl cell length were longer in 5 dayL seedlings than in 10 dayL seedlings. The extended length of hypocotyls in the *S. rexii* seedlings in the dark was due to extreme cell elongation in the hypocotyl. In the model plant *Arabidopsis thaliana*, seedlings grown in the dark also show elongated hypocotyls, though still folded cotyledons with an apical hook, and an arrested shoot apical meristem (Von Arnim and Deng, 1996).

Our observations suggest that imbibed seeds of *S. rexii* can receive light signals, with a longer light exposure to seeds resulting in stronger effects allowing germination and seedling development even in the dark (see Figs. 2–4). It is known that the reversal of P_{fr} and P_r forms of phytochrome is regulated by light. In *A. thaliana*, functional P_{fr} of phytochrome B can be stored in the seed and can control seed germination once they are imbibed (Smith, 2000; Von Arnim and Deng, 1996). It is possible that imbibed seeds of *S. rexii* can also perceive and accumulate light signals prior to germination, which may then induce photomorphogenesis and prevent skotomorphogenesis after subsequent germination in dark conditions.

4.3. Red and blue light have different effects on seedling growth in S. rexii

Seedling growth was very different under red (660±20 nm) and blue (470±30 nm) light. While seedling morphology under blue light was very similar to that of control seedlings, under red light no macrocotyledon developed, the hypocotyls and hypocotyl cells were extremely elongated and no adventitious root had been initiated.

In *Streptocarpus* species, the macrocotyledon grows by means of the basal meristem located in the proximal region of the cotyledon, which is characterized by smaller, dividing cells (Imaichi et al., 2000; Mantegazza et al., 2007; Nishii et al., 2004; Nishii and Nagata, 2007). The venation pattern is also more complex in the macrocotyledon than in the microcotyledon

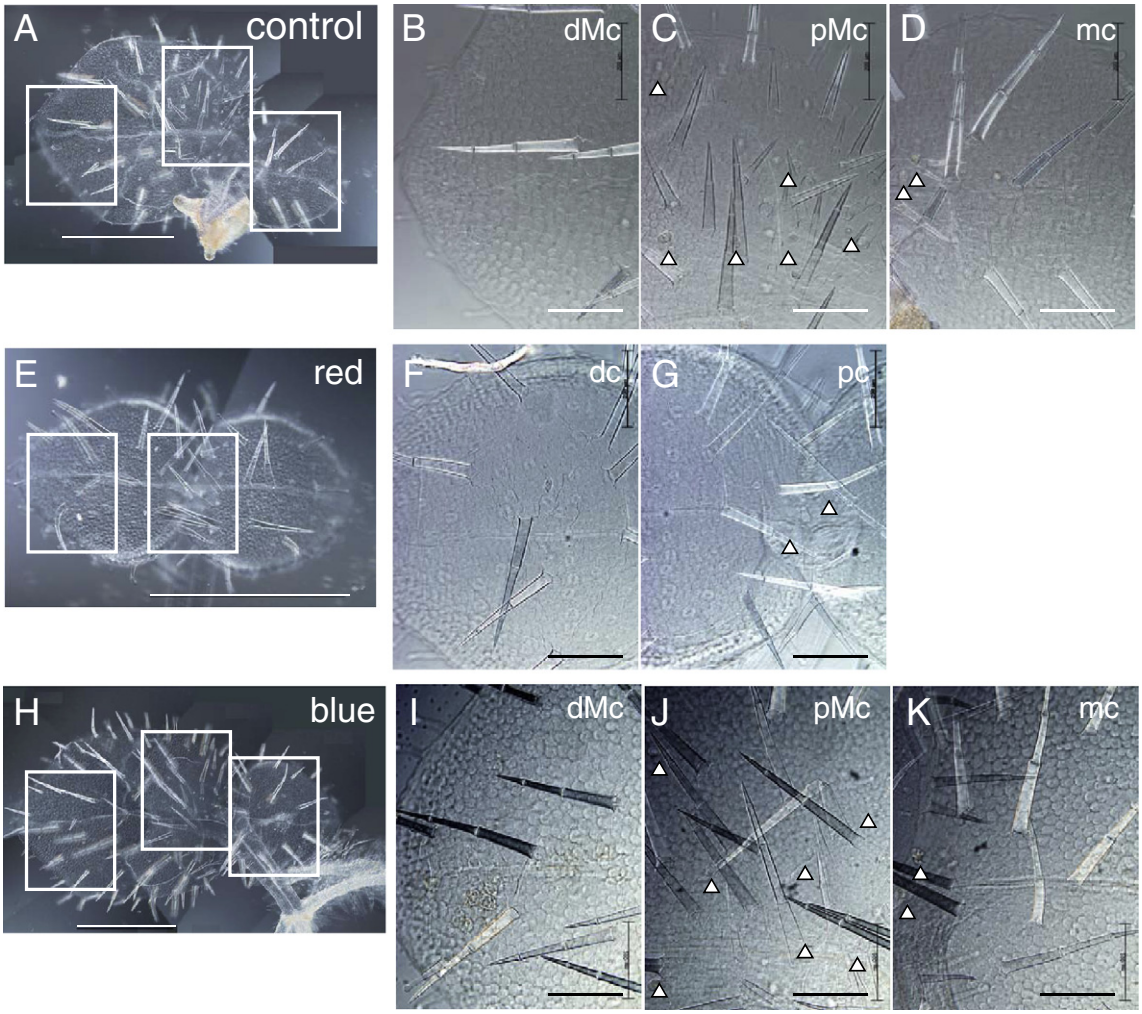

Fig. 7. Distribution of short grandular trichomes (arrowheads) on the surface of cotyledons. A–D, Control. B–D, Magnified views of A. E–G, Seedling under red light. F–G, Magnified views of E. H–K, Seedling under blue light. I–K, Magnified views of H. dMc; distal region of the macrocotyledon, pMc; proximal region of the macrocotyledon, mc; microcotyledon, dC; distal region of cotyledon, pC; proximal region of cotyledon. Scale bars = 1 mm (A, E, H), 0.2 mm (B–D, F, G, I–K).

(Mantegazza et al., 2007; Nishii et al., 2004), and the trichome distribution is different between the macro- and microcotyledon (Nishii et al., 2004). In the present study, we observed that no basal meristem, nor lateral vein formation, or differential trichome distribution was observed in seedlings grown under red light. Conversely, macrocotyledon formation was observed in seedlings under blue light. This indicates that blue light may be more efficient for inducing the macrocotyledon in *S. rexii*. Blue light exposure was also more efficient in suppressing hypocotyl elongation in *S. rexii*, while red light, on the other hand, induced hypocotyl and hypocotyl cell elongation, like dark treatments. Wavelength specific photoreceptors may play some roles in macrocotyledon growth and the suppression of hypocotyl elongation.

In *A. thaliana*, several blue light receptors have been identified, such as cryptochrome and phototropin (Briggs and Huala, 1999), and wavelength specific functions for regulating plant growth have been reported. In pea, for instance, blue light more efficiently inhibits hypocotyl elongation of etiolated seedlings than red light (Kigel and Cosgrove, 1991). In cucumber or sunflower, blue light irradiation induces a very rapid suppression of stem growth in a variety of species, while

Table 2
Epidermal cell size of cotyledons (μm^2) of *Streptocarpus rexii* 30 DAI. Values are mean ± standard errors. $n = 10$.

	Larger cotyledon		Smaller cotyledon	
	Distal	Proximal	Distal	Proximal
Control	2510.16±243.08	284.53±15.85	1532.66±85.29	1655.94±74.19
Red light	1586.72±134.13	1117.66±76.13	839.53±75.38	1176.72±81.35
Blue light	2019.22±220.08	280.47±23.37	1759.84±84.00	1749.53±139.21

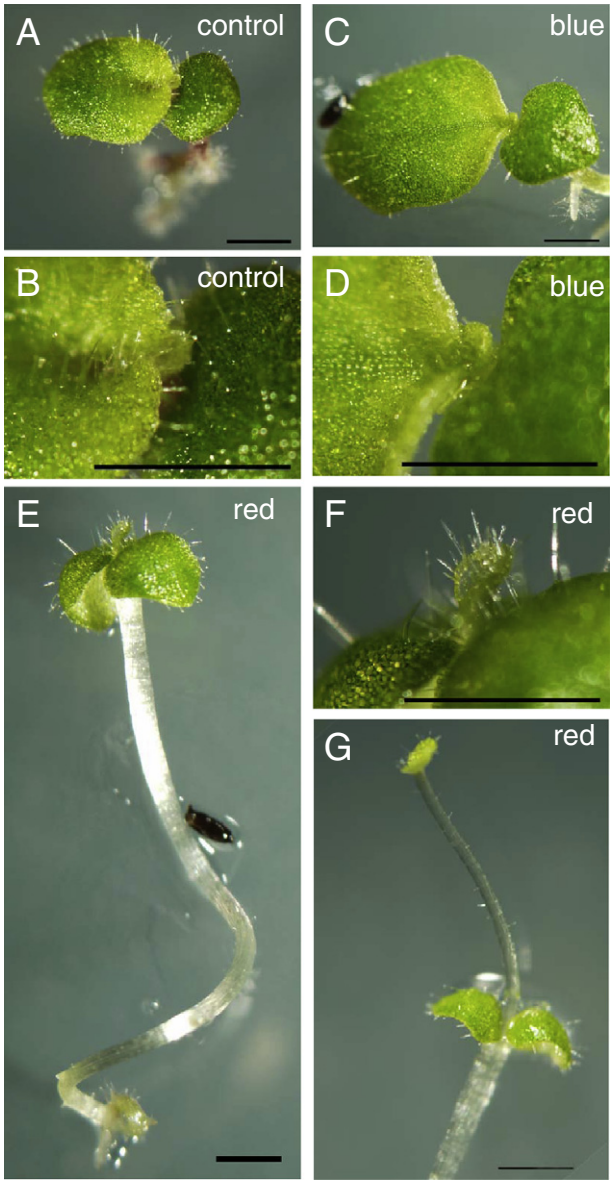

Fig. 8. Leaf initiation in *S. rexii* seedlings under different light wavelengths. A–F, 40 DAI. A, B, Control. C, D, Seedling under blue light. E–G, Seedling under red light. B, D, F, Magnified views of leaf primordia in A, C, and E. 40 DAI, leaf initiation was observed in the control (A, B), and seedlings under red light (E, F), and blue light (C, D). G, Seedling under red light 60 DAI. Developing leaf with elongated petiole. Scale bars = 1 mm.

red-light shows much slower kinetics (Cosgrove and Green, 1981). In the suppression of hypocotyl elongation in *A. thaliana*, both blue and red light exposures are effective, but several mutants of photomorphogenic players in *A. thaliana*, such as *cr88* (*c*hlorate-*r*esistant) and *pef1* (*p*hytochrome-signaling *e*arly *f*lowering) showed the phenotype of insensitiveness to red light dependent hypocotyl elongation (Ahmad and Cashmore, 1996; Cao et al., 2000; Lin and Cheng, 1997), as observed in *S. rexii*.

Since roots in many plants grow underground without light exposure, light has generally more inhibitory effects on root development (Torrey, 1952). For instance, in *A. thaliana* or pea, root growth is inhibited by both red and blue light (Correll and Kiss, 2005; Furuya and Torrey, 1964; Molas et al., 2006; Zeng et al., 2010). In *S. rexii*, on the other hand, white light did not affect the root development during early seedling growth, which may be linked to their habitat (see above). Exposure to blue light induced adventitious root formation similarly to the white light control, while red light did not. Together with the blue light effects on anisocotylous development, this result also suggested that blue light is more important for post-germination seedling development in *S. rexii*.

4.4. Light and plant hormone signals in the seedling morphogenesis of Streptocarpus

In the present study, *S. rexii* seedlings grown under red light showed two microcotyledons, elongated hypocotyls with cell elongation, and inhibitory effects on adventitious root formation. These are similar to the effects of gibberellin treatments. Gibberellin-treated seedlings of *S. rexii*, or *Streptocarpus wendlandii* Sprenger also show two microcotyledons, elongated hypocotyl with cell elongation, and inhibitory effects on adventitious root formation (Mantegazza et al., 2009; Nishii et al., 2004, 2012; K. Nishii unpublished data). Thus, it is possible that light is linked to or regulates the gibberellin signaling pathway in *Streptocarpus*, though further physiological studies are needed here.

Additionally, it is not clear whether high levels of red light or low levels of blue light induced this gibberellin-like effect in *S. rexii*. One hypothesis could be that a high level of red light is integrated into the gibberellin signaling cascade, as shown in *A. thaliana*, in which red light perception by phytochromes regulates phytochrome interactive factors (PIFs). These in turn regulate DELLA proteins that work downstream of the gibberellin signal transduction pathways (De Lucas et al., 2008; Feng et al., 2008). Another hypothesis could be that *S. rexii* mainly senses blue light signals for seedling morphogenesis, such as anisocotyly or adventitious root formation. Thus, a low level of blue light (=red light alone irradiation) caused the gibberellin effect on seedlings. Further physiological studies would be needed to unravel the

Table 3
Ratio of *Streptocarpus rexii* seedlings forming leaf primordia under different light wavelengths 40 DAI.

Effects	Treatment		
	Control	Red light	Blue light
Number of germinated seedlings	16	25	26
Number of seedlings forming a leaf primordium	4	8	7
Proportion of germinated seedlings forming a leaf primordium (%)	25%	32%	27%
Number of seeds sown	16	26	27

interactions between light and hormone signals in the regulation of anisocotyly in *Streptocarpus*.

In conclusion, *Streptocarpus* seeds require light for germination (photoblastic) and subsequent seedling development. Anisocotyly was only observed under white and blue light, thus anisocotyly is a photomorphogenetic effect in *S. rexii*. Red-light-only exposure resulted in a seedling development highly reminiscent of a gibberellin phenotype, while blue-light-only exposure induced normal seedling development. Further studies are needed to unravel the interactions between light and hormones to reveal the detailed signaling cascade and regulatory pathways underlying the extraordinary seedling development in *Streptocarpus*.

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