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ORIGINAL ARTICLE

Anisocotyly and meristem initiation in an unorthodox plant, *Streptocarpus rexii* (Gesneriaceae)

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Abstract In common with most Old World Gesneriaceae; *Streptocarpus* Lindl. shows anisocotylous growth, i.e., the continuous growth of one cotyledon after germination. Linked to this phenomenon is an unorthodox behaviour of the shoot apical meristem (SAM) that determines the growth pattern of acaulescent species (subgenus *Streptocarpus*). In contrast caulescent species develop a conventional central post-embryonic SAM (mainly subgenus *Streptocarpella*). We used *S. rexii* Lindl. as a model to investigate anisocotyly and meristem initiation in *Streptocarpus* by using histological techniques and analyses of the expression pattern of the meristematic marker *SrSTM1* during ontogeny. In contrast to *Arabidopsis thaliana* (L.) Heynh., *S. rexii* does not establish a SAM during embryogenesis, and the first evidence of a SAM-like structure occurs during post-embryonic development on the axis (the petiolode) between the two cotyledons. The expression pattern of *SrSTM1* suggests a function in maintaining cell division activity in the cotyledons before becoming localized in the basal meristem, initially at the proximal ends of both cotyledons, later at the base of the continuously growing macrocotyledon, and the groove meristem on the petiolode. The latter is equivalent to a displaced SAM seemingly originating de novo under the influence of endogenous factors. Applied cytokinin retains *SrSTM1*expression in the small cotyledon, thus promoting isocotyly and re-establishment of a central post-embryonic SAM. Hormone-dependent delocalization of the process of meristem development could underlie anisocotyly and the unorthodox SAM formation in *Streptocarpus*.

Keywords Anisocotyly · Cytokinins · Groove meristem · Shoot apical meristem · *SrSTM1* · *Streptocarpus*

Abbreviations

BAP	6-Benzylaminopurine
CLV	*CLAVATA*
DAP	Days after pollination
DAS	Days after sowing
DIG	Digoxygenin
KNOX	Class 1 knotted1-like homeobox genes
RAM	Root apical meristem
SAM	Shoot apical meristem
SEM	Scanning electron microscopy
SrSTM1	*Streptocarpus rexii SHOOTMERISTEMLESS1* homologue
STM	*SHOOTMERISTEMLESS*
WUS	*WUSCHEL*

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Introduction

Genetic understanding of the diverse plant developmental pathways found in natural species is taught from few model plants conforming to a standard *bauplan* that have been analysed in great detail. They provide the basis of our understanding of how plant diversity is genetically controlled, and serve as templates to investigate "unusual" plant forms. One of these unusual forms is found in certain *Streptocarpus* Lindl. species. They exhibit such an unorthodox *bauplan* to necessitate the introduction of new botanical terms to describe their unusual morphological features, such as *anisocotyly*, *petiolode* and *phyllomorph* (Jong 1970). The unequal development of the two cotyledons after germination (*anisocotyly*) is common to most members of the Old World Gesneriaceae (Jong 1970; Cronk and Möller 1997; Nishii et al. 2004) and likely constitutes the prerequisite condition for the development of the extraordinary growth forms of acaulescent species of *Streptocarpus* (Hilliard and Burtt 1971; Rosenblum and Basile 1984; Möller and Cronk 2001). Anisocotyly is thought to advantage the growth in typically extreme habitats of *Streptocarpus* in deep shade, thus to boost the production of photosynthetic tissue soon after germination from the endospermless seeds (Burtt 1970). The vegetative plant body of acaulescent *Streptocarpus* species consists of *phyllomorphs*. Each phyllomorph is composed of a stem-like petiole, termed *petiolode*, and a foliose component, the *lamina* (Fig. 1). The development of the phyllomorph is controlled by three meristems at the junction of the lamina and petiolode: (a) the *basal meristem* which provides the growth of the lamina; (b) the *petiolode meristem*, involved in the growth of the midrib and elongation of the petiolode; (c) the *groove meristem* which provides the growth of new phyllomorphs and inflorescences (Fig. 1; Jong 1970, 1978; Jong and Burtt 1975).

The unique morphology of *Streptocarpus* is intrinsically linked to the interplay of these three meristems. They interact at the seedling stage already and are responsible for the great vegetative diversity in the genus, resulting in species with a single leaf throughout the individual's life (*unifoliate*), species producing several to numerous leaves arranged in a more or less irregular rosette (*rosulate*), besides "ordinary" caulescent species possessing a normal shoot apical meristem (SAM) and a branched stem system (Hilliard and Burtt 1971).

To date two main pathways have been shown to affect meristem establishment and maintenance in *Arabidopsis* Heynh. and these are the KNOX pathway, and the WUSCHEL/CLAVATA (WUS/CLV) pathway (Vollbrecht et al. 1991, 2000; Brand et al. 2000; Schoof et al. 2000). Previous work has shown that the KNOX pathway is present in *Streptocarpus*, and that different patterns of KNOX expression and localization reflect, but do not cause the differences in form between species (Harrison et al. 2005). This evidence comes from investigations on the expression of *SSTM1*, a *SHOOTMERISTEMLESS* (*STM*) homologue of the *KNOX* gene family (Harrison et al. 2005). *STM-like* genes are expressed in cells of the SAM but down regulated in presumptive organ primordia, and they play a fundamental role among *KNOX* genes responsible for meristem regulation (Long et al. 1996). Thus, *STM-like* genes can be used as meristematic markers.

In model plants the interaction of plant hormones with *KNOX* genes has been shown to represent one of the key factors regulating the expression of genes required for SAM formation and function (Rupp et al. 1999; Hay et al. 2004). In this respect, it is interesting to note Rosenblum and Basile (1984) who examined the effect of

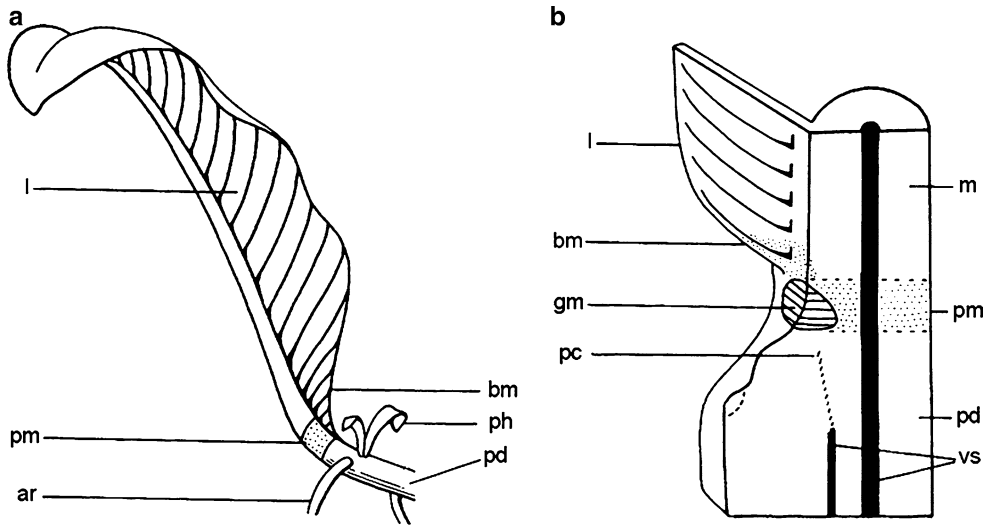

Fig. 1 Diagrammatic representation of phyllomorphic organization in *Streptocarpus*, modified from Jong and Burtt (1975). **a** Cotyledonary phyllomorph. **b** Longitudinal section through a petiolode. *ar* adventitious root, *bm* basal meristem, *gm* groove meristem, *l* lamina, *m* midrib, *pc* procambium, *pd* petiolode, *ph* secondary phyllomorphs, *pm* petiolode meristem, *vs* vasculature

hormone application on the variation of growth forms in *Streptocarpus*. They claimed that exogenous hormones re-establish morphogenetic capacities previously thought to be "lost" in the rosulate and unifoliate species, such as isocotyly and caulescence. In particular, cytokinins were found capable of inducing phyllomorphic development of the normally non-accrescent microcotyledon, i.e., the development of basal meristems in both cotyledons (Rosenblum and Basile 1984; Nishii et al. 2004). It would thus be interesting to investigate the interaction of cytokinin and *SrSTM1* expression in the light of the hormone effect on the fate of the SAM.

Here we aim to determine when and how the unusual growth patterns of *Streptocarpus rexii* during its ontogeny arise. We describe the meristem initiation and development during embryogenesis and seedling development by histological techniques and by analyzing the expression of *SrSTM1*. Coupled with experiments with exogenously applied cytokinin our results will provide insights into the developmental processes leading to anisocotyly and the unconventional SAM formation in *Streptocarpus*.

Materials and methods

Plant material

Plants of *S. rexii* Lindl. (Gesneriaceae; Lindley 1828), accession number RBGE 20030814 (Tsitsikamma, Cape, SA) were grown in glasshouses at the Royal Botanic Garden Edinburgh (E), and voucher specimens deposited at E.

Histological analysis

For the histological analysis of developing embryos fruits were collected at weekly intervals from artificial pollination until maturation (16 weeks). Stages of embryogenesis were identified according to size; shape and cell number present in each stage of development, as described by West and Harada (1993). For analysis of primary phyllomorph development seeds were sown on compost and collected every day until day 15, and then every week until the emergence of the first phyllomorph.

Developing fruits and seedlings were fixed in FAA overnight at 4°C. After dehydration in an ethanol series (70–90–95–100%, 1 h each), samples were embedded in Technovit 7100 (Heraeus Kulzer, Wehrheim, Germany) and cut at 4 μm thickness. The sections were stained with DAPI (1 μg/ml, 5 min) and observed with a fluorescence microscope Dialux 22 (Leitz GmbH, Oberkochen, Germany).

Scanning-electron microscopy (SEM)

Seedlings were fixed in FAA (3.7% formaldehyde and 5% acetic acid in 50% ethanol) overnight at 4°C. The fixed material was subjected to dehydration in an ethanol series (75–90–95–100%), then critical point dried, mounted on stubs and coated with gold before observation with a LEO 1430 scanning electron microscope (LEO Electron Microscopy, Thornwood, NY, USA).

In situ hybridization

Digoxygenin-labeled (DIG) *SrSTM1* RNA was generated using an in vitro transcription kit (Roche Diagnostics GmbH, Mannheim, Germany).

The highly variable 5′ region of the *SrSTM1* sequence of Harrison et al. (2005) spanning from amino acids 32 to 98 was used as a template and was amplified using primers 22NOT7P (CCATGATGATG ATGATGCCTGC) and 22T7P (TAATACGACTC ACTATAGGGGCATCTCCGTCTCCTTCCATG) for the antisense probe and SENSF (TAATACGACTCAC TATAGGGCCATGATGATGATGATGCCTGC) and SENSR (GCATCTCCGTCTCCTTCCATG) for the sense probe.

Hybridization and immunological detection were performed as described by Lopez-Dee et al. (1999) with minor modifications. The hybridization was carried out at 45°C overnight. The detection was performed using the Dig-detection kit (Roche Diagnostics) and the antidigoxigenin antibody was used at 1:1,000 dilution.

Plant-hormone treatment

Seeds were sterilized with 0.2% sodium hypoclorite for 2 min and washed with distilled water, then sown on an MS basal medium (Murashige and Skoog 1962). Seedlings were transferred 12 DAS to a medium containing 10^{-5} M 6-benzylaminopurine (BAP) just after the cotyledons had unfolded. Control plants were grown on MS medium without hormones. For anatomical and in situ analysis seedlings were collected weekly and fixed in FAA as above.

Results

Embryogenesis in *Streptocarpus rexii*

Seven days after pollination (DAP) the polygonum type embryo sac was composed of two slender synergids at the micropyler end, the egg just above these, three

polar nuclei at the centre, and three ephemeral antipodal cells at the chalazal end (Fig. 2a). At fourteen DAP the zygote had undergone a transverse division producing a small apical cell, the embryo proper, and basal cells which gave rise to the suspensor (Fig. 2b). At 21 DAP a two, then four-celled embryo was recognizable deriving from longitudinal divisions of the apical cell (Fig. 2c). A transverse division followed to produce an octant stage embryo proper (Fig. 2d). The eight-celled embryo then underwent periclinal divisions to produce the protoderm (Fig. 2e). At 28 DAP the embryo was in the early globular stage (Fig. 2f). The transition from the globular to the heart stage results from periclinal divisions at specific lateral regions of the embryo (Fig. 2g). At 35 DAP the embryo entered the heart stage, showing two cotyledonary lobes (Fig. 2h). At 42 DAP the cotyledons and axis had elongated as a result of cell division and expansion and the developing procambium became visible (Fig. 2i). At 49 DAP the morphological organization was completed and the embryo composed of two linear cotyledons closely juxtaposed with no SAM evident between these (Fig. 2j). The length of the embryo was examined at different time intervals, showing that *S. rexii* embryos have an initial period of slow growth followed by a rapid increase in embryo length starting at about 30 DAP until 49 DAP (see Suppl. 1). The growth curve of *S. rexii* embryo otherwise resembled the typical sigmoid dicot growth curve (Lersten 2004). Thus embryogenesis in *S. rexii* was histologically comparable that of *Arabidopsis* up to the heart stage but then deviated from a typical trajectory by failing to form a SAM.

Delocalised embryonic expression of *SrSTM1* in *Streptocarpus rexii*

To test whether the absence of an embryonic SAM in *S. rexii* reflected a failure to establish, embryonic *SrSTM1* expression was examined using RNA in situ hybridization in developing embryos. The specificity of the *SrSTM1* probe used was verified by Southern blot analysis, which showed that the probe hybridized to only one genomic fragment at high stringency in samples digested with different restriction enzymes (data not shown). Sense probes were used in in situ hybridizations *to evaluate background signal levels (Suppl. Fig. 2).

SrSTM1 mRNA was first detected in the one celled-embryo (Fig. 3a), and then throughout the developing

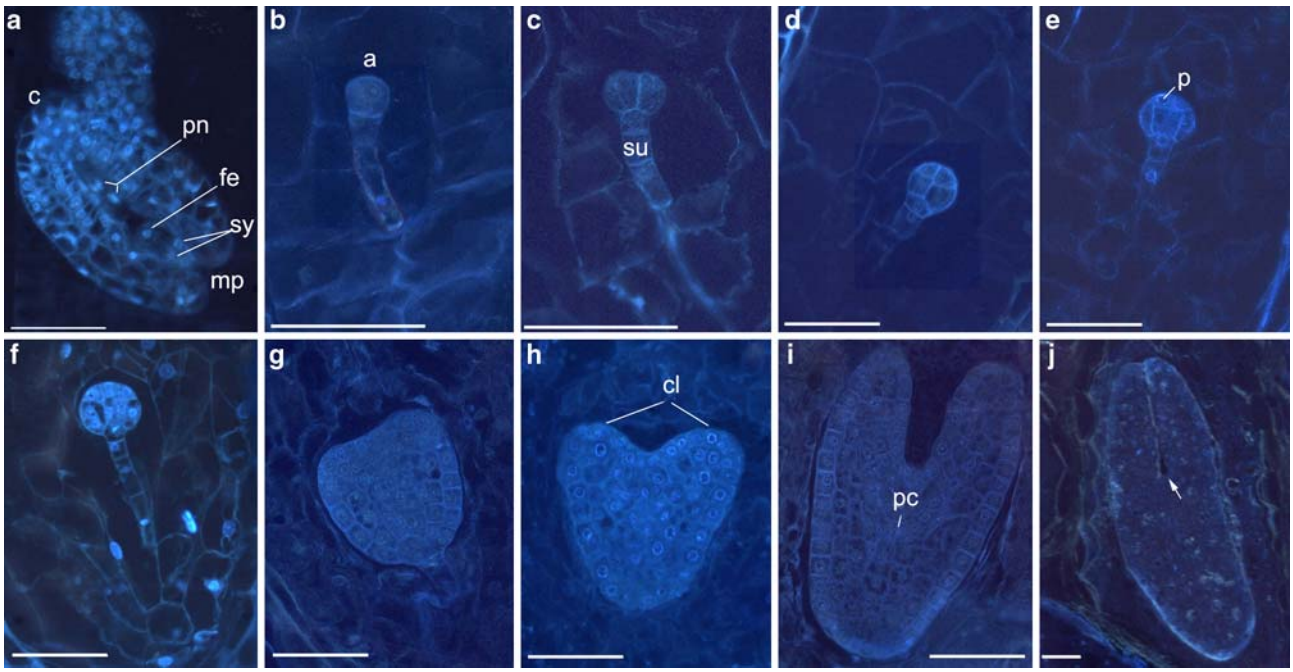

Fig. 2 Embryogenesis of *Streptocarpus rexii*. Developing fruits were collected at weekly intervals, fixed, embedded and DAPI stained after sectioning. DAPI-stained nuclei emit strong bluish fluorescence. **a** Zygote 7 DAP. Embryo sac composed of two synergides (*sy*) at the micropylar (*mp*) end, fertilized egg (*fe*), three polar nuclei (*pn*) in the centre of the embryo sac, and three ephemeral antipodal cells at the chalazal end (*c*). **b** One-celled embryo proper with apical cell (*a*) 14 DAP. **c** Two or four-celled embryo proper with suspensor (*su*) 21 DAP. **d** Octant stage embryo. **e** Transition to globular stage, protoderm (*p*). **f** Early globular stage embryo 28 DAP. **g** Transition stage embryo. **h** Early heart stage embryo 35 DAP, cotyledonary lobes (*cl*). **i** Late heart stage embryo 42 DAP, procambium (*pc*). **j** Linear cotyledon stage embryo 49 DAP. No SAM is evident between the cotyledons. *Arrow* indicates the position where SAMs ordinarily appear. Scale bars 100 μm (**a**–**j**)

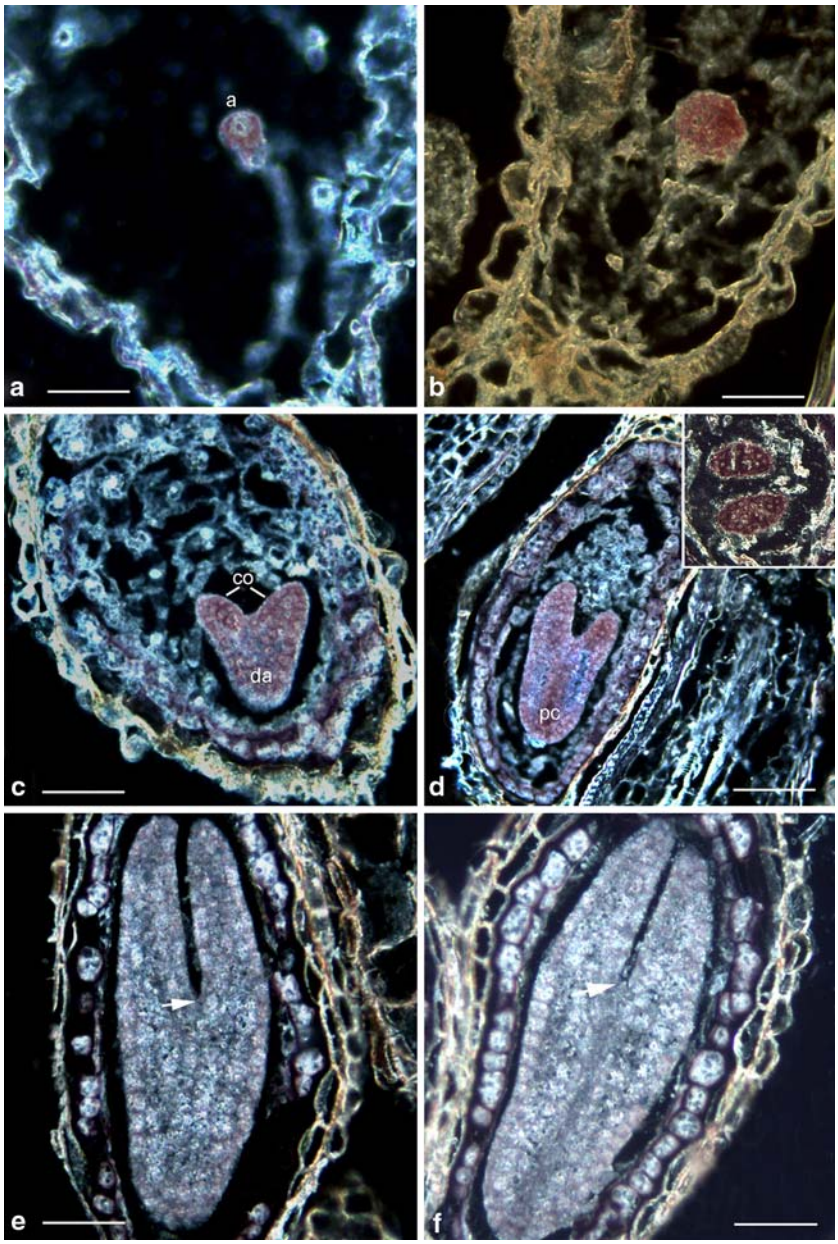

Fig. 3 *SrSTM1*expression during different stages of embryogenesis in *Streptocarpus rexii*. *SrSTM1* messenger hybridization is indicated by *red-purple staining*. **a** One-celled embryo. *SrSTM1* was localized in the apical cell (*a*). Note the basal cell is not visible in this section. **b** Early globular stage. *SrSTM1*was expressed throughout the embryo. Note: the suspensor is not visible in this section. **c** Early heart stage. A higher *SrSTM1* expression was detected at the tip of the cotyledons (*co*) and the distal apex (*da*). **d** Transition stage. Additional in situ signal was present in the procambium (*pc*). The *inset* shows the expression of *SrSTM1* in a transversal section through the cotyledons. **e–f** Linear cotyledon stage. *SrSTM1* was downregulated in the embryo from this stage onwards. *Arrows* indicate the point where *SrSTM1*-like genes are usually detected in plants with a conventional SAM. Scale bars 25 μm (**a, b**), 50 μm (**c–f**)

embryo up to the globular stage (Fig. 3b). At the heart stage *SrSTM1*expression was highest at the distal extremes of the embryo, in the cotyledons and at the putative site of the root apical meristem (RAM; Fig. 3c). The transition from heart to torpedo stage was characterized by additional localization in the procambium (Fig. 3d). Expression of *SrSTM1* was never observed in the fork between the cotyledons (Fig. 3c–f) where *KNOX* gene expression is detected in plants with a conventional SAM (Smith et al. 1995; Long et al. 1996). No *SrSTM1* expression was detected in fully formed embryos examined from 49 DAP onwards (Fig. 3e, f) until maturation (Suppl. Fig. 1). Embryonic *SrSTM1* expression therefore coincided with the growth phase of the embryo.

Thus, although *S. rexii* shows no morphological evidence of an embryonic SAM, expression of one *KNOX* marker for meristem identity was detected in growing embryos but linked to cell division activity rather than at the orthodox SAM position between the cotyledons.

A layered meristem initiates post-embryonically in *Streptocarpus rexii*

To confirm the stage of development at which a layered meristem initiates in *S. rexii*, we followed post-embryonic development using histology and SEM. Seedling development was followed from sowing to the establishment of unequal cotyledon growth (anisocotyly),

which marks the initiation of adult growth patterns (Fig. 4 and Suppl. Fig. 3).

The cotyledons emerged 13–14 DAS, equal in size and form, and anisocotyly became apparent from about 21 DAS onwards. No evidence of a SAM was detected in seedlings with equal cotyledons (Fig. 4a, b), consistent with the lack of an embryonic SAM. No SAM was detected at the onset of anisocotylous growth, about 21 DAS (Fig. 4c, d), although the larger cotyledon showed foci of small dividing cells at its base (Fig. 4d). Later on during anisocotylous growth, the groove meristem initiated on the axis between the two cotyledons, more precisely on the petiolode of the macrocotyledon (Fig. 4e–g). At 35 DAS, this meristem became domed (Fig. 4h–j), and later gave rise to the first true phyllomorph (Fig. 5h, i).

Meristem initiation coincides with de novo expression of a *Streptocarpus KNOX* gene, *SrSTM1* in *Streptocarpus rexii*

To determine whether meristem initiation in *S. rexii* reflects initiation of *KNOX* pathways, localization of *SrSTM1* transcripts was examined during seedling

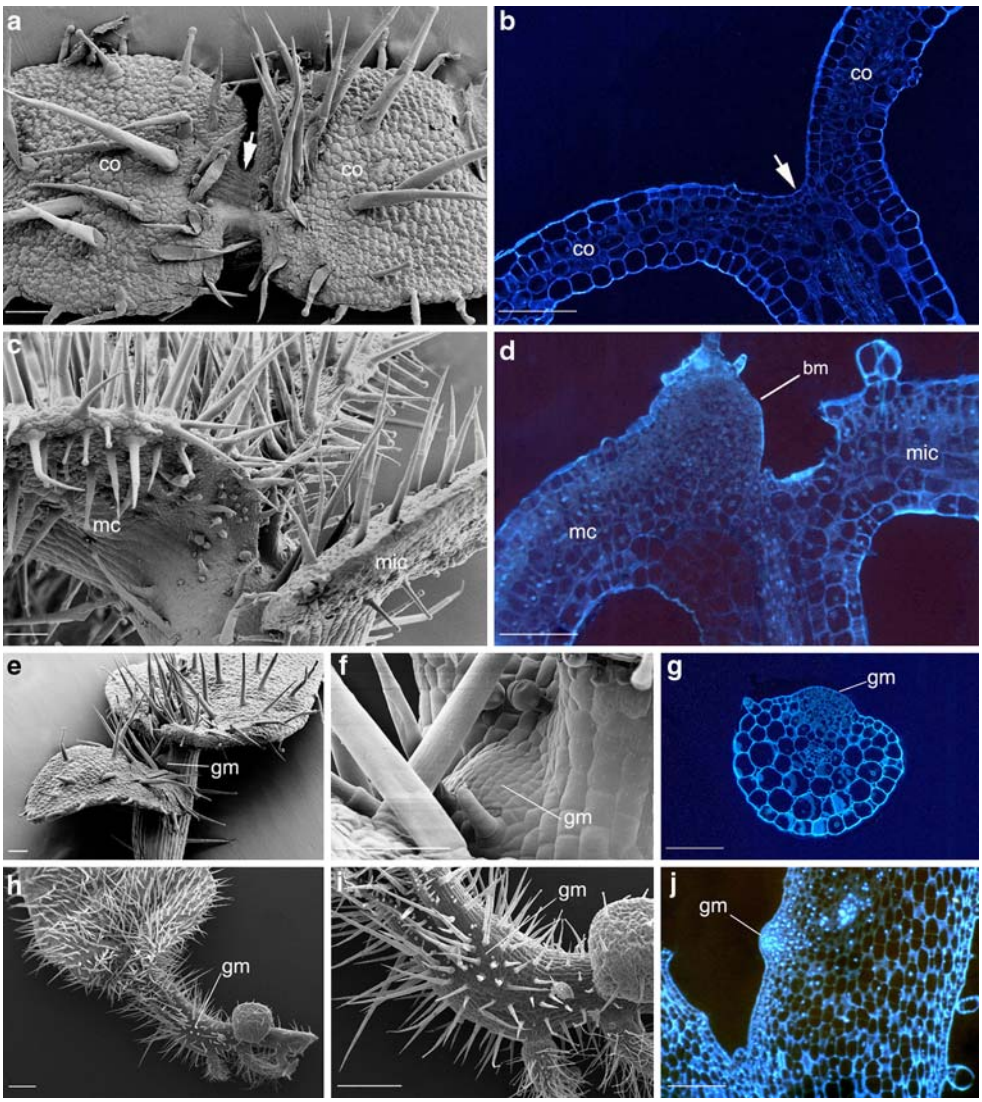

Fig. 4 Development of meristems in *Streptocarpus rexii* seedlings in SEM (**a, c, e, f, h, i**), and DAPI stained histological longitudinal (**b, d, j**) and transverse sections (**g**). DAPI-stained nuclei emit strong bluish fluorescence. **a, b** A seedling 14 DAS showing two equally sized cotyledon (*co*) and no central SAM (*arrow*). **c, d** A seedling 21 DAS exhibiting anisocotyly. The macrocotyledon (*mc*) shows basal dividing cells, the basal meristem (*bm*). *mic*, microcotyledon. **e** A seedling 28 DAS (magnified in **f**) showing a bulge of meristematic cells, the groove meristem (*gm*). **g** Transverse DAPI stained section of a seedling 28 DAS, showing the developing groove meristem (*gm*). **h** A seedling 35 DAS (magnified in **i**) with enlarged groove meristem (*gm*). **j** A seedling 35 DAS showing small meristematic cells in the groove meristem (*gm*). Scale bars 100 μm (**a–g**, **I–j**), 500 μm (**h**)

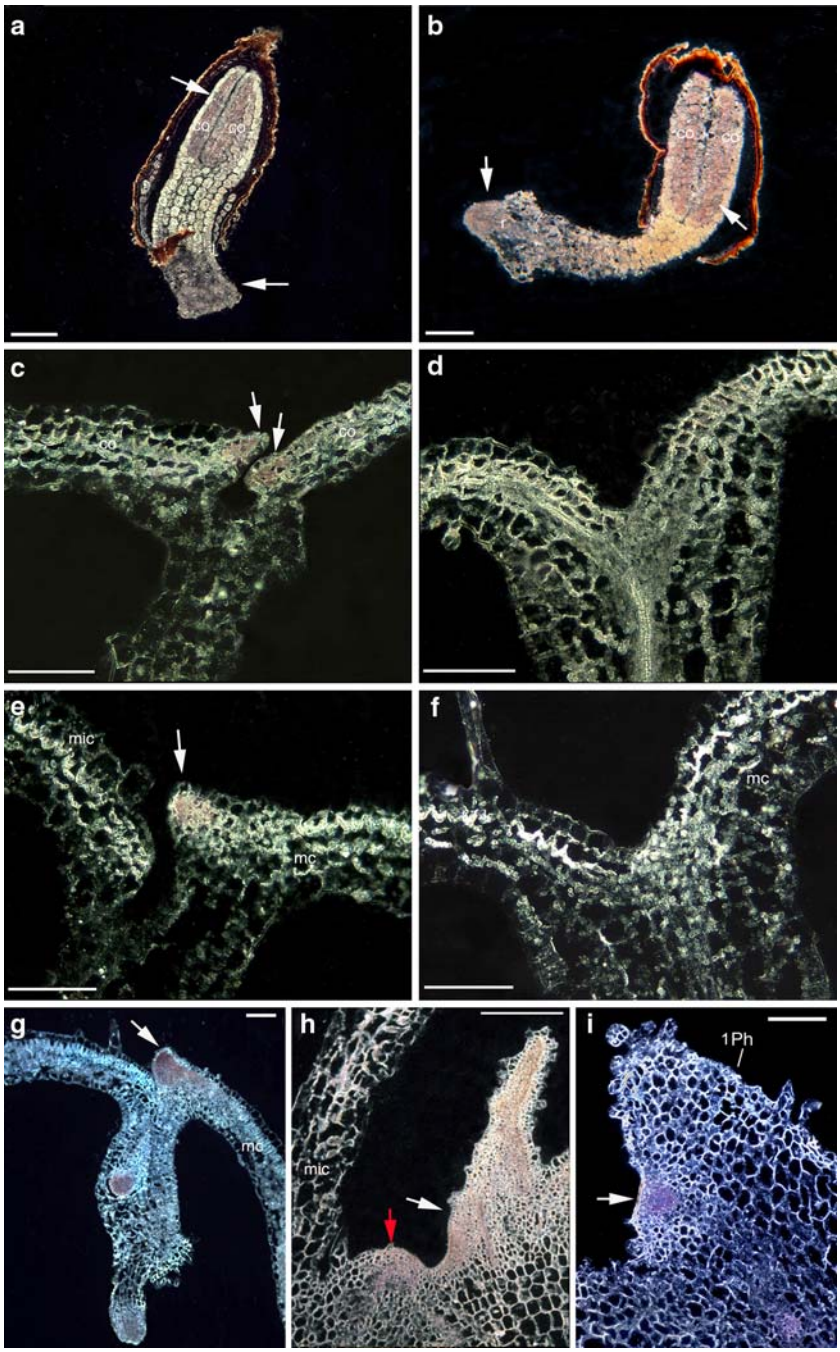

Fig. 5 *SrSTM1* expression during seedling development in *Streptocarpus rexii*. *SrSTM1* messenger hybridization is indicated by *red-purple staining*. **a** Seven DAS. *Arrows* indicated *SrSTM1* expression in the cotyledons and the basal region of the hypocotyls indicating the position of a putative RAM. **b** Eleven DAS. *SrSTM1* expression is observed in both cotyledons and the root tip meristem. **c, d** A seedling 14 DAS. *SrSTM1* expression is evident at the base of both cotyledons in the peripheral section (**c**), but not in the median section of the same seedling (**d**). **e, f** A seedling 21 DAS. *SrSTM1* expression is located at the base of one cotyledon, the macrocotyledon (*mc*), visible in the peripheral section (**e**), with no expression in the median section of the same seedling (**f**). **g** A seedling 28 DAS. *Arrow* indicates *SrSTM1* expression in the basal meristem of the macrocotyledon (*mc*). Expression is also seen in all root tip meristems. **h** A seedling 35 DAS. *Red arrow* indicates *SrSTM1* expression in the emerging groove meristem. Expression is also visible in the basal meristem of the obliquely sectioned macrocotyledon (*white arrow*). **i** Longitudinal section through the first phyllomorph (*1ph*). *Arrow* indicates *SrSTM1* expression in the groove meristem on the upper side of the first true phyllomorph developing from the groove meristem of the cotyledonary phyllomorph. Scale bars 100 μm (**a**–**h**); 1 mm (**i**)

growth. *SrSTM1* expression was seen in root meristems at all stages (Fig. 5a, b, g). From seven (Fig. 5a) to eleven (Fig. 5b) DAS *SrSTM1* expression was localized throughout both cotyledons. No expression was observed in between the two cotyledons. Fourteen DAS expression became restricted to the bases of both cotyledons at the site of foci of dividing cells (Fig. 5c). In seedlings 21 and 28 DAS, when anisocotyly became obvious, *SrSTM1* expression was lost from the microcotyledon, but was retained in the macrocotyledon (Fig. 5e, g).

No *SrSTM1* expression was detected in central median longitudinal sections of seedlings 14 or 21 DAS (Fig. 5d, f). Thirty-five to 42 DAS, and about 21 days after the establishment of anisocotyly, *SrSTM1* expression initiated as a conspicuous layered meristem emerged from the axis between the cotyledons, the petiolode (Fig. 5h). Expression was also maintained to the side and base of the larger cotyledon, indicating the basal meristem (Fig. 5h). *SrSTM1* expression was also retrieved from near the base of the petiolode of the first true phyllomorph indicating its groove meristem

from which the second phyllomorph will arise (Fig. 5i). Thus, *SrSTM1* expression was absent from the conventional position of shoot apex, and initiated apparently de novo when a new axis of growth (first true phyllomorph) arose from the stem-like part of the larger cotyledon (the petiolode).

Cytokinin relocalises *SrSTM1* expression in *Streptocarpus rexii*

To determine whether hormone-induced repositioning of the meristem in *S. rexii* (Rosenblum and Basile 1984; Nishii et al. 2004) coincides with repositioned induction of *KNOX* expression in the seedling, in situ hybridizations using a *SrSTM1* probe were performed following cytokinin treatment. In contrast to untreated seedlings (Fig. 4d), the application of BAP to *S. rexii* seedlings removed the growth inhibition of the microcotyledon, and resulted in the formation of two equal-sized cotyledons (Fig. 6a). Both cotyledons had foci of dividing cells in the basal and lateral region of the lamina, in which *SrSTM1* expression was detected (Fig. 6a–c). Centrally between the cotyledons of BAP treated seedlings, an additional region of small meristematic cells was observed (Fig. 6d–f), which subsequently give rise to the first leaf (Suppl. Fig. 4). In situ hybridization on median longitudinal sections showed that these cells expressed *SrSTM1*. Thus, cytokinin treatment represses anisocotyly and facilitates *SrSTM1* expression in a conventional central apical position.

Discussion

The lack of an embryonic SAM is common to rosulate and unifoliate growth forms

Previous work has examined post-embryonic meristem establishment in *Streptocarpus*, but did not determine how the meristem initiated. We show here that *S. rexii* and *A. thaliana* share embryonic developmental trajectories, though shifted along the time axis especially in

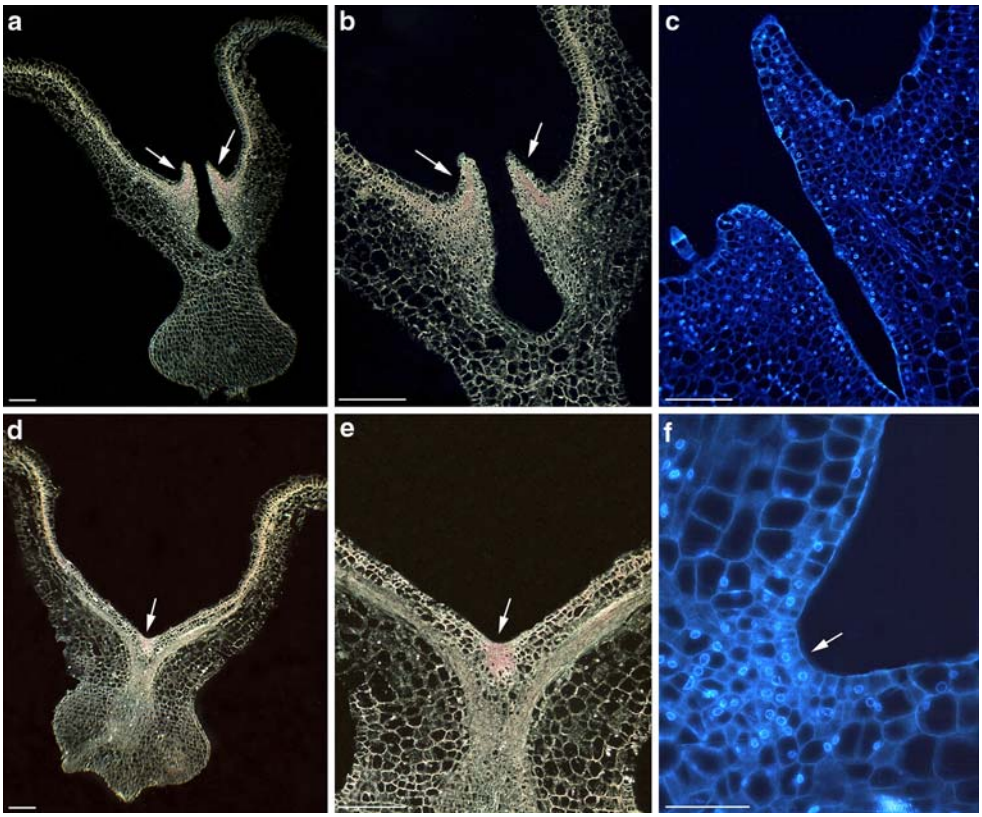

Fig. 6 Longitudinal sections of a seedling 21 DAS of *Streptocarpus rexii* incubated on a medium containing 10^{-5} M 6-benzylaminopurine (BAP) from cotyledons unfolding onwards. **a** *SrSTM1* expression in basal meristems of both macrocotyledons in a peripheral section. *Arrows* indicate position of basal meristems (magnified in **b**). **c** DAPI stained section showing small meristematic cells in the formation of two basal meristems at the base of the macrocotyledons. **d** *SrSTM1* expression in a central meristem in a median section. *Arrow* indicates the single central meristem (magnified in **e**). **f** DAPI stained section of a BAP treated seedling showing the small meristematic cells in the central meristem (*arrow*). Scale bars 200 μm (**a**–**e**), 100 μm (**f**)

the initial stages prior to cell divisions. In *S. rexii*, we recorded a general tendency to longer developmental stages, and ca. 120 days are required for the seeds to reach full maturity instead of the ca. 25 days of the model plant. The embryo shows a typical basal-apical axis and the organs develop according to the stages described by West and Harada (1993) for *A. thaliana* until the torpedo stage, when the lack of an embryonic SAM in *S. rexii* becomes apparent. This is in line with previous investigations on unifoliate representatives of *Streptocarpus* and *Monophyllaea* R.Br. (Jong 1970; Tsukaya 1997; Imaichi et al. 2000; Ayano et al. 2005). We can expand this phenomenon to rosulate *Streptocarpus* species here, and conclude that the lack of a conventional SAM can be considered a common phenomenon in unifoliate and rosulate Old World Gesneriaceae.

SrSTM1 involved in maintaining meristematic function in the embryo

The lack of an embryonic meristem in *S. rexii* is reflected by the absence of *SrSTM1* expression between the two cotyledons at the usual position of a SAM. In *Arabidopsis* the first evidence of the SAM as marked by *STM* expression is apparent in the early-mid globular stage embryo, and expression is confined to a single cell at the centre of the embryo (Long et al. 1996; Long and Barton 1998). Later on expression is confined to a medial stripe across the top of the embryo. In *S. rexii*, *SrSTM1* is absent in these areas. It appears to be diffusely expressed in those areas undergoing intense cell division activity, such as the cotyledons and the distal apex, the putative origin of the RAM, and the procambium, but it is never recovered in the fork between the cotyledons. The expression of *KNOX* genes in regions not strictly related to a histological SAM was previously described in *Oryza sativa*, where *OSH* genes were expressed in the area of subsequent shoot development (Sentoku et al. 1999). Our results corroborate the role of *STM-like* genes in the establishment of positional information in the presumptive shoot region rather than its strict localization uniquely in the SAM. Thus, if *SrSTM1* expression is taken as a marker for the identity of subsequent meristematic regions, results presented here suggest that these initiates embryonically, but are then lost later on during embryogenesis before re-initiating post-embryonically.

Correlation between absent SAM and the expression of *SrSTM1*

Previous studies have shown that the loss of expression of *STM* results in the failure of SAM establishment during *Arabidopsis* embryogenesis (Barton and Poethig 1993). Our results in *S. rexii* similarly show that when no *SrSTM1* expression is seen in between the cotyledons, there is no SAM establishment. As we found *SrSTM1* expression in cotyledonary tissues, and previous work has shown that STM proteins localize in leaf tissue in *Streptocarpus* (Harrison et al. 2005), it is likely that the regulation of *SrSTM1* expression differs between species with acaulescent and caulescent forms. We suggest that this shift in gene function during seedling development triggers the process of unusual development that follows.

SrSTM1 expression accompanies the indeterminate growth phase of cotyledons

Previous work has shown that in acaulescent *Monophyllaea* the removal of one cotyledon before the onset of anisocotyly induces the other to develop, suggesting that the mechanism for indeterminate growth is conserved until suppressed by endogenous or exogenous factors in the small cotyledon (Tsukaya 1997). Our results show that *SrSTM1* is expressed in the basal meristems of both cotyledons until anisocotyly becomes apparent. After the onset of anisocotyly, the pattern of expression of *SrSTM1* becomes restricted to the macrocotyledon, marking the region of subsequent foliar growth. The shift in *SrSTM1* from a wider expression pattern in the whole young cotyledons to the more localized pattern at later seedling stages correlated with the establishment of the basal meristem in the large cotyledon and groove meristem on the petiolode suggests a dual role with temporal and spatial change in function, initially maintaining cell division capabilities in the growing cotyledons, before initiating active meristematic centers in localized regions of growth. This agrees in part with the *KNOX* function to maintain indeterminate cell fate and suppress differentiation (Kerstetter et al. 1997).

Groove meristem versus SAM

For its tunica-corpus configuration at later stages of development and its capability to originate phyllomorphs, the groove meristem in *S. rexii* has been compared to the standard SAM of dicotyledonous plants, albeit being in an unconventional position (Jong and Burtt 1975). In *S. rexii*, we found that expression of *SrSTM1* is restricted to the base and side of the large cotyledon (basal meristem), and is absent from the shoot apex, and apparently initiates de novo when a new axis of growth arose from the stem-like part of the cotyledon (the petiolode). The meristematic bulge

(groove meristem) that originates shares many properties with conventional SAMs in that it is layered and gives rise to foliar organs. In this respect, the groove meristem could be regarded as a displaced SAM resulting from a developmental process spatially and temporally deviating from a conventional *bauplan*. The expression of *SrSTM1* provides evidence of the uncoupling of genetic processes underlying the formation of the meristem. A likely candidate responsible for the displacement of the SAM is the diffuse petiolode meristem whose activity above and below the SAM (thus creating a mesocotyl) shifts it from its central location in young, isocotyledonous seedlings onto the petiolode of the macrocotyledon of older, anisocotyledonous seedlings. However, whether subsequent phyllomorphs originate from the persistent groove meristem or reinitiate de novo (Harrison et al. 2005) is not known at present. The mechanisms employed for SAM formation could be reiteratively initiated in the petiolodes of subsequent foliar organs, from cells of the diffuse petiolode meristem that gain capability of foliar initiation under endogenous or exogenous factors.

Link between cytokinin, morphology and developmental gene

The results of our cytokinin experiment on the rosulate *S. rexii* are fully congruent with those described by Rosenblum and Basile (1984), as the application of exogenously applied BAP induced the same morphological changes in the seedlings; i.e., isocotyly was maintained by both cotyledons developing persistent basal meristems and expanding laminas. The appearance of a central apical meristem between the two macrocotyledons suggests that the genetic pathway underlying a conventional growth pattern is apparently retained in the rosulate *S. rexii*, and can be redeployed under hormonal treatment. The spatial change in the expression of *SrSTM1* under BAP treatment suggests a hormone–gene interaction that could give some insight into the onset of anisocotyly and anomalous SAM development. Recent evidence from *Arabidopsis* (Hay et al. 2002; Jasinski et al. 2005; Yanai et al. 2005) shows that KNOX proteins promote meristem function by repressing gibberellic acid (GA) and activating cytokinin biosynthesis. On the other hand, it has been suggested that *STM*-like genes could act both upstream and downstream of the cytokinin-dependent process of meristem development, thereby subjected to a feedback-loop regulation (Hay et al. 2004). Results shown here suggest that *SrSTM1* expression is normally suppressed in the small cotyledon at the onset of anisocotyly, but can be de-suppressed under cytokinin application. Application of exogenous cytokinins prevents the repression of *SrSTM1* in the small cotyledon, which thus equals the meristem development of the large cotyledon. In this case of equal growth of both cotyledons, the shoot apex is placed in a conventional position. However, if these results provide evidence of the cytokinin-dependent process of *SrSTM1* regulation in *S. rexii*, the correlation of KNOX with GA is still to be tested, and in particular in light of the hypothesis that uncoupling of the KNOX and GA pathway might have occurred in *Streptocarpus* (Harrison et al. 2005).

Aspects of evolution of morphological novelty in *Streptocarpus*

The WUSCHEL/CLAVATA (WUS/CLV) pathway has been shown to affect meristem establishment and maintenance in *Arabidopsis* with gene function complementary but independently expressed from KNOX (Mayer et al. 1998). Further investigations on additional factors involved in the KNOX or WUS/CLV pathway are required to determine gene functions and shed light on the mechanism of regulation of indeterminacy and meristem formation in *Streptocarpus*. Spatial and temporal shift in gene expressions are likely to play a fundamental role in the unusual establishment of the SAM, and a cross talk between hormones and *KNOX* genes emerges as a central factor in the developmental program resulting in the morphological innovation of *Streptocarpus*. If this is the case, and delocalization of embryonic *KNOX* expression is characteristic of the Old World Gesneriaceae it could provide a mechanism for the evolution of anisocotyly that characterizes the sub-family. However, it is important to point out that gene functions and interactions that underlie meristem regulation in *S. rexii* do not necessarily imply an equivalent in the rosulate growth form as a whole, and caution should be taken in generalizing a developmental model. In fact, the evidence that the rosulate condition has apparently multiple origins in the evolutionary history of *Streptocarpus* (Möller and Cronk 2001) indicates that independent events might underlie phenotypic similarity. Furthermore, considering the fact that *SSTM1* itself is not directly responsible for the different growth forms (Harrison et al. 2005) and the large number of factors that interplay in meristem formation and regulation, the possibility of the evolution of different *KNOX*-dependent pathways underlying convergent phenotypes of distantly related species of *Streptocarpus* appears as a plausible hypothesis. Under this model, small independent variation in the regulation of homeotic genes responsible for leaf and shoot formation would allow parallel events affecting

crucial morphological characters such as anisocotyly and vegetative growth patterns.

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