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Hormonal crosstalk in the regulation of meristem activity and the phyllomorph architecture in *Streptocarpus* (Gesneriaceae): a review

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Abstract: Plants belonging to the family Gesneriaceae exhibit great diversity in shoot architecture. One genus within the family, *Streptocarpus*, encompasses species with different body plans that do not conform to the standard *bauplan* of angiosperms. These include features such as 'anisocotyly', the unequal cotyledon morphogenesis, and the 'phyllomorph' a leaf/shoot construct of which the development is governed by three meristems (groove meristem, petiolode meristem, basal meristem). In the extreme case, the plants only consist of one hugely enlarged cotyledon ('unifoliate' habit). Modification in the position and activities of the meristem are responsible for the morphological flexibility of the genus. This review summarises the interactions between hormones and developmental genes and compares these to model plants. Some mechanisms controlled by class 1 *KNOX* (*KNOX1*) genes appear to be conserved between plants with ordinary shoots and the *Streptocarpus* phyllomorph, while others have diverged. In particular, cytokinins and gibberellins appear to be important for meristem regulation to establish anisocotyly and the development of phyllomorphs in *Streptocarpus* through *KNOX1* regulation. This is supported by expression patterns from hormone metabolism genes. The establishment of anisocotyly is based on an imbalance between cytokinins and gibberellins, causing a shift from apical to lateral dominance that involves the suppression of the microcotyledon and groove meristem and promotion of the basal meristem. We point out future perspectives in the study of *Streptocarpus* organogenesis. *Streptocarpus* may provide a system to study the functional evolution of plant form in relation to adaptation to diverse environmental conditions.

Keywords: Basal meristem, Class I *KNOX* genes, Cytokinin, Gibberellin, Groove meristem, Plant growth form regulation.

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Introduction

Members of the family Gesneriaceae exhibit a large diversity in shoot architecture (Weber, 2004), far greater that in any other plant family. Underlying this diversity, particularly found in the genus *Streptocarpus* Lindl., is the flexibility in the positioning and timing of meristem activity that appears almost randomly spread across the plant, but is in fact precisely placed and has very distinct functions (Burtt, 1970; Nishii *et al*., 2017). *Streptocarpus* is an African, Madagascar and Comoro Islands Gesneriad named after the uniquely twisted development of its fruits (Lindley, 1828; Hilliard & Burtt, 1971). This is not the only unusual feature in this genus, but the plants also show major variation in their vegetative morphogenesis such as anisocotyly, phyllomorphic leaf development, unifoliate growth and leaf abscission zones (Burtt, 1963; Figs. 1, 2; Appendix 1).

In this review, we focus on the interactions of hormones in meristem formation and function involved in the unique vegetative morphogenesis in *Streptocarpus*. Specifically, we summarise the

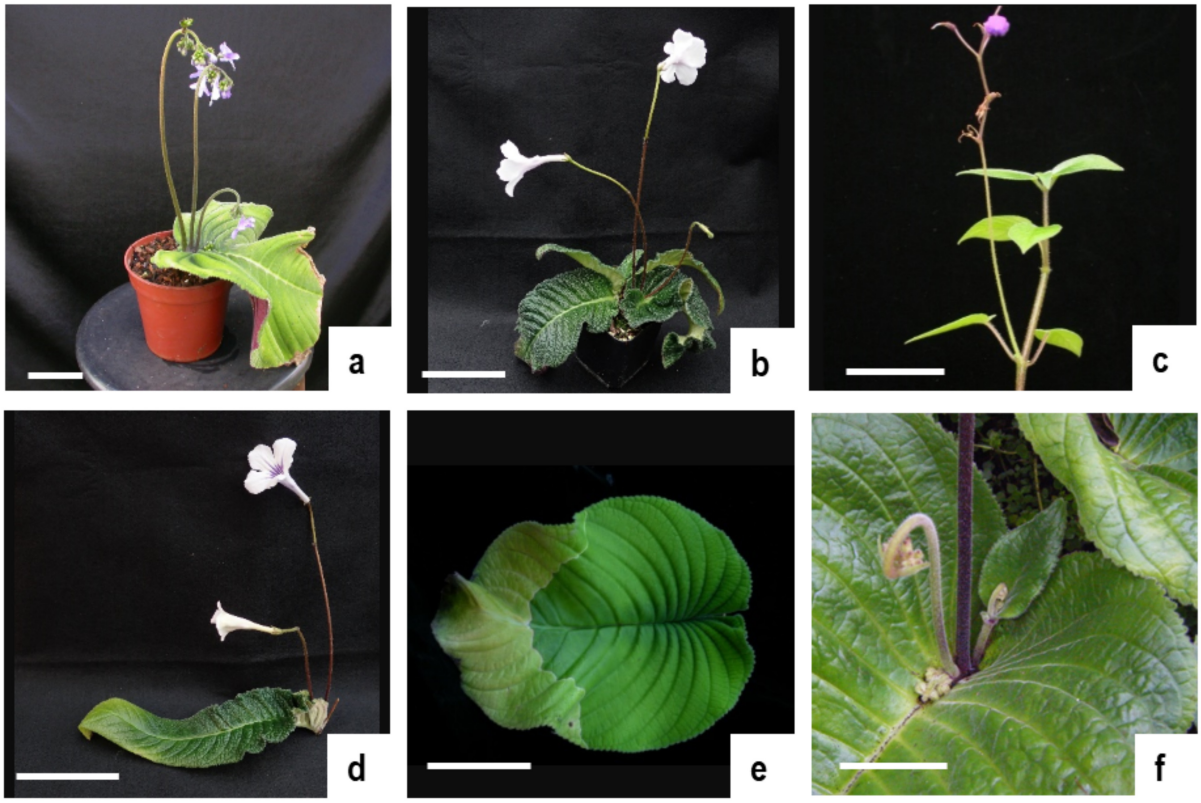

Fig. 1. Diverse morphologies and features present in *Streptocarpus* Lindl.: **a.** Unifoliate: *S. wendlandii* Spreng.; **b.** Rosulate: *S. rexii* (Bowie ex Hook.) Lindl. **c.** Caulescent: *S. glandulosissimus* Engl.; **d.** An excised flowering phyllomorph of the rosulate *S. rexii* resembling a unifoliate; **e.** Abscission zone formed in the lamina of *S. wendlandii*; **f.** Subtending phyllomorph formed in front of the series of inflorescences at the proximal region of the lamina in the unifoliate *S. wendlandii*. Scale bars: 10 cm (from plants cultivated at the Royal Botanic Garden Edinburgh).

results from exogenous applications of plant hormones and their effects in the plant's development, and the putative roles of hormone metabolism genes. The regeneration from leaf explants is also included briefly here because it may shed light on the available physiological machinery in the organogenesis in *Streptocarpus*. Finally, we propose our current model of phyllomorph development and maintenance as governed by plant hormones, and an outlook on future studies.

Anisocotyly, the phyllomorph concept and growth forms in *Streptocarpus*

Anisocotyly, the uneven development of cotyledons has been reported since studies on *Streptocarpus* were published (Crocker in 1861[1860"]), which allows the seedling to acquire photosynthetic tissue rapidly (Burtt, 1970). As in other dicots, two cotyledons are formed during embryogenesis in *Streptocarpus*, and just after germination, both cotyledons grow equally. However, at around 10 days after germination, one cotyledon ceases its development while the other continues to grow (Fig. 2). This results in unequally-sized cotyledons, where the larger cotyledon is termed the macrocotyledon, and the smaller one the microcotyledon (Jong, 1970; Imaichi *et al.,* 2000; Nishii *et al*., 2004). The direction and quality of light may be involved in the macrocotyledon determination (Saueregger & Weber, 2004; Nishii *et al.,* 2012b). Anisocotyly is a shared feature among Old World Gesneriaceae (*i.e.,* subfamily Didymocarpoideae) with some exceptions (Huang *et al*., 2019). New World Gesneriaceae (*i.e.,* subfamily Gesnerioideae), on the other hand, exhibit ordinary (equal) cotyledon development resulting in two microcotyledons (Burtt, 1963; Weber, 2004; Nishii *et al*., 2017).

In their monograph on *Streptocarpus*, Hilliard and Burtt (1971) described the significant variation in

Fig. 2. Seedling development in *Streptocarpus rexii* (Bowie ex Hook.) Lindl.: **a.** Isocotylous seedling just after germination; **b.** Anisocotylous seedling; **c.** Anisocotylous seedling with additional phyllomorph formed from the proximal region of the macrocotyledon (arrow). Scale bars:1 mm.

the vegetative morphs in the genus, and roughly group these into caulescent species in subgenus *Streptocarpella* Fritsch bearing shoots with opposite simple leaves, and acaulescent in subgenus *Streptocarpus* forming a false rosette (rosulate) or remaining unifoliate (Fig. 1; Appendix I). Some acaulescent species are plurifoliate bearing only a few leaves throughout their lifetime. Phylogenetic studies on the evolution of the different morphologies show developmental plasticity that is reflected by repeated switches between the three basic growth forms (Möller & Cronk, 2001).

Initially, it was attempted to compare the variations in the development of the diverse shoot architectures found in *Streptocarpus* with ordinary growth forms (Esau, 1977; Jong, 1970; Jong & Burtt, 1975). However, some features did not fit the model. Of the three main morphs, caulescent, rosulate, and unifoliate, it was found that while caulescents retain an ordinary shoot with a central shoot apical meristem (SAM), the acaulescent, *i.e.,* rosulates and unifoliates lacked a typical shoot and SAM (Jong, 1970). The leaf development of acaulescent *Streptocarpus* shows some further unusual features. In ordinary plants, such as *Arabidopsis thaliana* L. or *Nicotiana tabacum* L., and in caulescent *Streptocarpus*, the leaf consists of a lamina and a petiole, and the proximal end of the petiole is attached to the stem. The SAM is located at the shoot apex and leaf primordia are formed at the peripheral zone of the SAM. Moreover, a phytomer, a structural unit consisting of leaf and stem, is repeatedly formed from the SAM to form an ordinary shoot (Esau, 1977; Barthélémy & Caraglio, 2007; Imaichi *et al.,* 2007; Fig. 3). From his studies on acaulescent rosulate *Streptocarpus*, Jong (1970) concluded that the macrocotyledon and foliage leaf are "exceptional" in that they represent an integrated entity with characteristics of both shoot and leaf. In these *Streptocarpus* lacking a SAM, new leaves are initiated from primordia on an existing leaf. Therefore, he proposed the term phyllomorph for this foliar unit (Jong, 1970; Fig. 3).

To distinguish the developmental origin of phyllomorphs, the first is termed a cotyledonary phyllomorph and represents the developed macrocotyledon, while consecutively formed phyllomorphs are termed additional phyllomorphs (Jong, 1970). Thus, a rosulate possesses a cotyledonary phyllomorph and additional phyllomorphs, and both have similar abilities for bearing inflorescences (Fig. 1). Typical unifoliate species represent the most reduced form and possess only a cotyledonary phyllomorph but occasionally can bear an additional phyllomorph after inflorescence initiation: the subtending phyllomorph (Jong, 1970; Jong & Burtt, 1975) or accessory phyllomorph (Jong, 1970), although Dubuc-Lebreux (1978) included those from *de novo* origin, rather than from a long-dormant groove meristem (see below) (Nishii *et al*., 2012a). Unlike other phyllomorphs, subtending phyllomorphs do not bear roots (Jong & Burtt, 1975).

Each phyllomorph, starting with the cotyledonary phyllomorph, consists of a lamina and a petiole-

stem unit termed a petiolode. Lateral adventitious roots are formed from the lower parts of the petiolode and each phyllomorph is monocarpic, *i.e.,* dies after fruiting (Jong, 1970; Hilliard & Burtt, 1971). The development from seedlings to mature plants in *Streptocarpus* is governed by several meristematic regions observed at the juxtaposition of the lamina and the petiolode. Three meristems work in synchrony: the basal meristem at the proximal region of the lamina is responsible for lamina expansion, the petiolode meristem for petiolode elongation and thickening, and the groove meristem located on the petiolode at the base of the lamina responsible for new organ initiation, such as the inflorescences and new phyllomorph primordia (Jong, 1970; Jong & Burtt, 1975; Imaichi *et al*., 2000; Nishii & Nagata, 2007) (Figs. 3, 4). The macrocotyledon in subgenus *Streptocarpella* has a basal meristem that sustains its enlargement for a short period of time only. It later shows characters of an ordinary leaf, with a lamina and petiole, and a shoot is formed from the SAM (Nishii *et al.,* 2017). Phyllomorphs of species in subgenus *Streptocarpus* section *Streptocarpus* have evolved a further unique characteristic, the ability to form an abscission zone, a transverse abscission line on the lamina dividing it into a proximal and distal part that is cut off during unfavourable conditions (Hilliard & Burtt, 1971; Noel & Van Staden, 1975) (Fig. 1e).

Molecular mechanisms of *Streptocarpus* meristems

Many studies have been carried out over the last two decades to unravel the molecular mechanisms underlying phyllomorph formation and plant development in *Streptocarpus*. Since acaulescent *Streptocarpus* lack a SAM, it was hypothesized that mutations of meristem maintaining genes, such as those of the class 1 *KNOX* (*KNOX1*) homeobox gene family are involved (Cronk & Möller, 1997; Tsukaya, 1997; Imaichi *et al*., 2000). In model plants *KNOX1* genes maintain undifferentiated cells in the SAM, and the *A. thaliana KNOX1* gene mutant *shootmeristemless* (*stm*) lacks a SAM (Long *et al*., 1996; Hake *et al*., 2004). Studies on the expression of homologous *STM1* genes in *Streptocarpus* show different patterns; while it was found in the SAM of the caulescent *S. saxorum* Engl., it was also expressed in the basal meristem and grove meristem of the rosulate *Streptocarpus rexii* (Bowie ex Hook.) Lindl. (Harrison, 2002; Harrison *et al*., 2005). The unifoliate *Streptocarpus dunnii* Hook.f. showed somewhat unstable expression patterns (Harrison 2002; Harrison *et al*., 2005), which might be linked to seasonal changes in growth activities since the

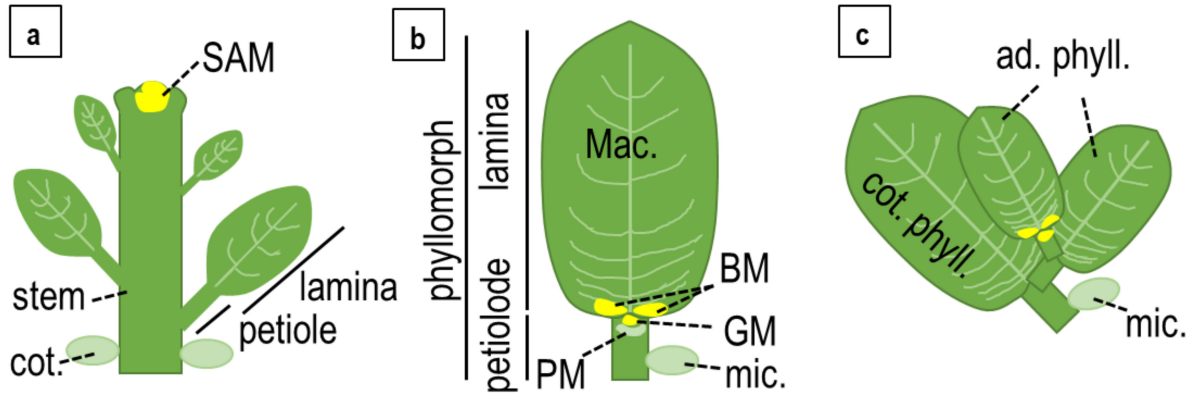

Fig. 3. Schematic illustration of an ordinary shoot (**a**) and acaulescent *Streptocarpus* Lindl. (**b.** unifoliate, **c.** rosulate). Meristem positions are highlighted in yellow. ad. phyll.: additional phyllomorph, BM: basal meristem, cot. phyll.: cotyledonary phyllomorph formed from the macrocotyledon, cot.: cotyledon, GM: groove meristem, Mac.: macrocotyledon, mic.: microcotyledon, PM: petiolode meristem, SAM: shoot apical meristem.

plants require vernalisation (M. Möller, pers. obs.). *STM1* expression is consistently found in the groove meristem and basal meristem in actively growing plants of another unifoliate *Streptocarpus wendlandii* Spreng. (Nishii *et al*., 2017).

Later on, the expression patterns of other developmental genes were investigated, mainly in the rosulate *S. rexii* that has become a model plant. To date, *STM1* and *BREVIPEDICELLUS* (*BP*) from the *KNOX1* gene family, *WUSCHEL* (*WUS*), *ARP*, and *GRAMINIFOLIA* (*GRAM*) from the *YABBY* gene family have been investigated (Harrison *et al*., 2005; Mantegazza *et al*., 2007, 2009; Nishii *et al*., 2010; Tononi *et al*., 2010; Fig. 5). Since these genes are well characterised and known to be the major players in the SAM and lateral organs formation and maintenance in model plants, they can be used to trace organs during *Streptocarpus* seedling development and in phyllomorphs as well. Early on in germination at the stage of cotyledon unfolding, the expression of the meristem marker gene *STM1* is observed in the entire lamina of both cotyledons. Later, but still at the isocotylous stage, it is restricted to the proximal lamina region of both cotyledons. Soon after, *STM1* expression disappears in the microcotyledon but remains in the basal meristem and in the groove meristem of the macrocotyledon. *WUS* has a role for maintaining the SAM in an undifferentiated state (Mayer *et al*., 1998; Schoof *et al*., 2000). *WUS* expression appears to be present in all three meristems of *S. rexii* phyllomorphs (Mantegazza *et al*., 2009) similar to *STM1*, although this ubiquitous expression might require further investigation. A MYB-like transcription factor, *ARP* regulates the dorsoventrality of leaves and is expressed mutually exclusively with *KNOX1* genes in leaf primordia in model plants (Waites *et al*., 1998; Byrne *et al*., 2000; Timmermans *et al*., 1999; Tsiantis *et al*., 1999). Unlike ordinary dicots with simple leaves, co-expression of *ARP* and *KNOX1-BP* genes was observed in the simple-leaved *Streptocarpus*, in the basal and groove meristem in *S. rexii*, and the SAM of the caulescent *Streptocarpus glandulosissimus* Engl. (Nishii *et al*., 2010). This co-expression in the SAM is similar to what is found in compound-leaved plants where *KNOX1* genes regulate leaf meristematic activities required for forming leaflets along with *ARP* genes (Bharathan *et al*., 2002). These findings indicate a breakdown in *Streptocarpus* of the mutual exclusion of *ARP* and *KNOX1* found in model plants with simple leaves. The different gene regulation might contribute to the ability of *Streptocarpus* to expand lamina from a basal meristem involving *KNOX1*. *GRAM* determines the abaxial fate of a leaf (Siegfried *et al*., 1999; Golz *et al*., 2004). *GRAM* is expressed on the abaxial side of *S. rexii* cotyledons similar to *A. thaliana*; and in the phyllomorph it is observed in the basal meristem but not in the groove meristem (Tononi *et al*., 2010). This indicates that *GRAM* retains a specific role in the basal meristem, possibly to determine the abaxial side of lamina while the leaf tissue is formed from the undifferentiated basal meristem.

These findings strongly support Jong's (1970) hypothesis for the developmental organisation of phyllomorphs as the equivalent of shoots and confirm their oddity. Indeed the phyllomorphs show meristem properties in the basal and groove meristem where functional meristem genes usually found in conventional SAMs are expressed. Their localization and interactions in the phyllomorph appear to have been modified during the evolution of the plants. Thus, one could argue functional equivalence between a SAM and at least the groove meristem, whereas the basal meristem has mixed properties of a SAM and a lateral organ (Fig. 5a, b). This may raise the question of whether acaulescent *Streptocarpus* are "hopeful monsters" (Theissen, 2006) by saltational evolution, or the product of step-wise shifts in meristem activity in space and time. Our study on the evolution of *KNOX1* (*STM1*) expression patterns at least, does not follow the "hopeful monsters" route (Nishii *et al*., 2017). The most comprehensive survey of cotyledon development and *KNOX1* expression across Gesneriaceae, to date reveals that the origin of cotyledonary basal meristems expressing *KNOX1*

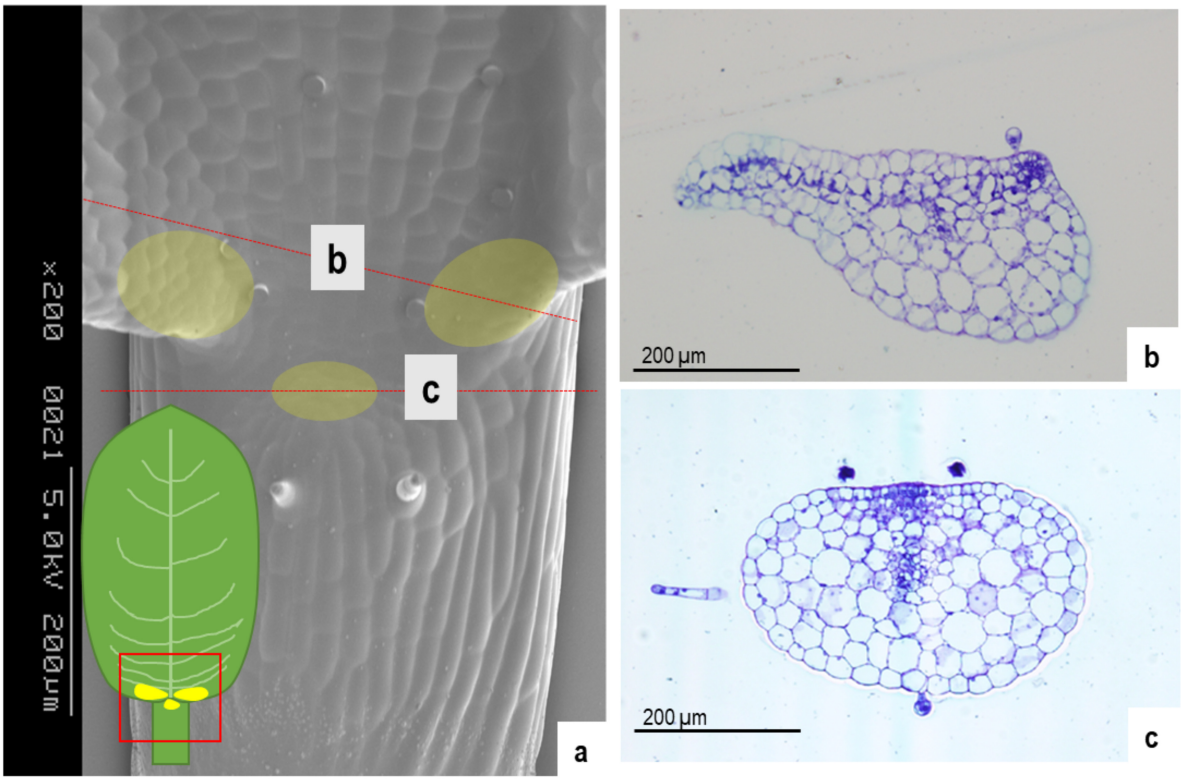

Fig. 4. Micrographs of meristems of a rosulate species of *Streptocarpus* Lindl.: **a.** Image of the junction between lamina of petiolode under the scanning electron microscope. Inset illustration indicates position in the phyllomorph. Dotted lines indicate the positions of sections in **b** and **c** of Toluidine blue stained Technovit mounted sections. **b.** Transverse section through the basal meristem; **c.** Transverse section through the groove meristem.

predates the age of Gesneriaceae as this feature already existed in other relatives of the Gesneriaceae: in the Lamiales, such as in *Antirrhinum majus* L. (Plantaginaceae) or *Jovellana punctata* Ruiz & Pav. (Calceolariaceae) (Nishii *et al*., 2017). Apparently, in the family Gesneriaceae, the evolution of the extremely reduced unifoliate morphs culminated in the Old World lineage. The gradual modification of meristem activity is perhaps the basis for the major morphological diversity in the *Streptocarpus* (Nishii *et al.,* 2017). It is likely that the physiological regulatory mechanisms, such as hormonal regulation, are involved in morphogenesis and play a role in the different *Streptocarpus* plant architectures.

Timeline of plant hormone studies in *Streptocarpus*

The earliest work on the effects of the exogenous application of plant hormones on shoot morphogenesis and leaf explant regeneration in *Streptocarpus* can be traced back to the 1970's. Dubuc-Lebreux and Vieth (Dubuc-Lebreux, 1976, 1978; Dubuc-Lebreux & Vieth, 1975, 1976) reported on the effects of gibberellins (GA) and cytokinins (CK) on the development and regeneration of *Streptocarpus* plants. Appelgren and Heide (1972) comprehensively studied hormone effects on the regeneration from *Streptocarpus* leaf explants. Van Staden (1973) was the first to measure endogenous CK in relation to the formation of abscission zones. Rosenblum and Basile (1984) applied several plant hormones and reported their effects on seedling morphogenesis. Nishii *et al.* (2004, 2012a) examined the effects of exogenous application of CK and GA on unifoliate *Streptocarpus*. Recent studies on the acaulescent rosulate *S. rexii* shed light on the interaction between exogenous CK and GA, and their metabolic genes, and interactions between

exogenous hormones and meristem genes (Mantegazza *et al*., 2007, 2009; Nishii *et al*., 2014; Chen *et al*., 2017). While most of the studies focused on GA and CK, other hormones, such as auxins and abscisic acid have also been investigated (Appelgren & Heide, 1972; Rosenblum & Basile, 1984; Nishii *et al*., 2004).

Exogenous application of GA in *Streptocarpus*

GA regulates the induction of flowering and germination in plants, and has important roles for many other aspects in plant growth (Yamaguchi, 2008), including hypocotyl and stem elongation and leaf enlargement (Cowling & Harberd, 1999; Alabadí *et al.,* 2008; Sun, 2010). In the SAM, GA promotes differentiation of organ primordia (Jasinski *et al*., 2005).

Several studies report on the effects of exogenous GA applications to seedlings or young *Streptocarpus* plants. The results indicate a complex role of the hormone in several developmental aspects including elongated hypocotyls and petiolodes in *S. wendlandii* seedlings through the expansion in cell length rather than cell division (Nishii *et al*., 2012a). GA application to 9-14 months old *S. wendlandii* plants also induced an elongated petiolode and a narrower lamina, though the author did not carriy out microscopic observations (Dubuc-Lebreux, 1976). Thus, GA appears to induce organ etiolation through cell elongation in *Streptocarpus* similar to ordinary plants (Alabadí *et al*., 2008).

Exogenous GA application also has drastic effects on the activity of meristems in *Streptocarpus*. In seedlings, it inhibits the basal meristem of the macrocotyledon which results in the formation of two microcotyledons in the unifoliate *S. wendlandii* (Nishii *et al*., 2012a), the plurifoliate *S. prolixus* C.B.Clarke (Rosenblum & Basile, 1984), and the rosulate *S. rexii* (Nishii *et al*., 2014; Mantegazza *et al*., 2009; Figs. 6, 7). Thus, this response seems to be common in *Streptocarpus*, irrespective of the mode of GA application (soil drench or incorporation into the growing medium).

While exogenously applied GA suppresses the basal meristem activity, it simultaneously promotes the activity of the groove meristem. In one of the earlier hormone experiments, it was reported that exogenous GA application to seedlings induces a "caulescent form", by developing "shoots" in the acaulescent plurifoliate *S. prolixus* (Rosenblum & Basile, 1984). A similar morphology was observed in GA treated rosulate *S. rexii* seedlings. The treated plants did not show enlarged lamina but formed additional phyllomorphs with elongated petiolodes much earlier than control seedling giving the appearance of a shoot (Nishii *et al*., 2014). Thus, the "shoot" reported by Rosenblum and Basile (1984) represents a tandemly arranged assemblage of phyllomorphs with etiolated petiolodes. Surprisingly also in *S. wendlandii*, a unifoliate species that does not bear additional phyllomorphs, exogenous GA induced the formation of an additional phyllomorph from the groove meristem that is usually perpetually dormant in unifoliates (Imaichi *et al*., 2000; Nishii *et al*., 2012a) (Fig. 6d). In turn the additional phyllomorph develops into an ordinary phyllomorph bearing inflorescences (K. Nishii, pers. obs.). Exogenous GA treatments of unifoliate species such as *Streptocarpus michelmorei* B.L.Burtt and *S. wendlandii* 6 ½ to 14 months old showed an increase in additional phyllomorphs originating from the groove meristem (*i.e.,* subtending phyllomorphs). Moreover, adventitious phyllomorphs arose *de novo* on the petiolode (Dubuc-Lebreux, 1976, 1978). Additionally, the subtending phyllomorph showed an increase in additional phyllomorphs similar to the "false shoot" in GA-treated seedlings. Thus, GA appears to negatively regulate the dormancy of the groove meristem and positively regulates the phyllomorph initiation from the groove meristem in *Streptocarpus*.

The opposing effects of GA on the basal meristem and groove meristem allow the hypothesis of an antagonistic balance in a phyllomorph between lamina expansion and new phyllomorph initiation. The most extreme form appears in unifoliate *Streptocarpus*, where the basal meristem is active

from germination onwards until the inflorescence meristem starts developing (Nishii *et al*., 2017). During this time, the groove meristem stays morphologically and developmentally dormant (Jong, 1970; Imaichi *et al*., 2000). In rosulate *S. rexii*, the basal meristem is active for a while but it ceases earlier than in unifoliates, and the meristem balance shifts earlier to the groove meristem to initiate new phyllomorphs before inflorescences are initiated (Nishii *et al*., 2017). Therefore, the basal meristem activity in unifoliate is stronger or more persistent than that in rosulates, and suppresses new phyllomorphs from the groove meristem, and GA may be involved in this pathway (Nishii *et al*., 2012a, 2014)(Figs. 5–7). This phenomenon has been described as "lateral dominance" as opposed to "apical dominance" in ordinary shoots, where the apical shoot suppresses the development of lateral shoot growth (Tsukaya, 1997; Nishii *et al*., 2012a). Considering the role of GA in an ordinary SAM, GA may promote phyllomorph differentiation from the groove meristem. In other words, the groove meristem stays undifferentiated while the existing lamina is expanding, but once the basal meristem activity ceases and GA is upregulated, or *vice-versa*, the dormancy of the groove meristem is broken to form new phyllomorphs, although further studies are needed before conclusive statements can be made here.

Localization of GA metabolism genes in *Streptocarpus*

In model plants, high concentrations of endogenous GA are observed in differentiated leaf primordia that promote cell differentiation (Jasinski *et al*., 2005). The localization of GA in the SAM is finely controlled by its metabolic genes: the GA synthesis gene *GA20-oxidase* is found in leaf primordia and the GA degrading gene *GA2-oxidase* is localized at the base of the SAM that prevents the accumulation of GA in the SAM (Jasinski *et al*., 2005; Bolduc & Hake, 2009) (Fig. 5d).

In *S. rexii*, *SrGA20-oxidase* is expressed in the lamina whereas *SrGA2-oxidase* is found in the basal meristem and in the groove meristem (Nishii *et al*., 2014) (Fig. 5c). Thus, the expression domain for *SrGA2-oxidase* in this species has shifted from SAM to lamina and thus differs from the model plant pattern, even if it retains the same role of meristem maintenance. *SrGA2-oxidase* may prevent GA incorporation in the *Streptocarpus* phyllomorph meristems and maintains cells in an undifferentiated state. On the other hand, and similar to model plants, *SrGA20-oxidase* is found expressed in the differentiated lamina in *Streptocarpus*. Thus, GA may also be required for cell differentiation and growth of lamina tissue in *Streptocarpus*. Intriguingly, during embryogenesis, *SrGA20-oxidase* is expressed between the cotyledons where ordinary plants form the embryonic SAM. This suggests that GA might be involved in the absence of an embryonic shoot apical meristem in *Streptocarpus* (Nishii *et al.,* 2014). Thus, GA appears to be a negative regulator of the SAM in *A. thaliana* or phyllomorph meristems in *Streptocarpus*, but the expression patterns of the GA metabolism genes have shifted, matching their meristem locations.

Exogenous CK promote basal meristem activity in *Streptocarpus*

Cytokinins have important roles for maintaining the SAM, and for cell division and growth of plants (Osugi & Sakakibara, 2015). During the regeneration from leaf explants, CK is known to induce shoot regeneration by interacting with auxin, and also prevents senescence (Gan & Amasino, 1996; Su *et al*., 2011).

In *Streptocarpus*, during the early stages of germination the basal meristem in the microcotyledon appears to be affected most by exogenous CK. Rosenblum and Basile (1984) were the first to report that CK treatment induced "twin phyllomorphs" in the plurifoliate *S. prolixus* as well as unifoliate *S. grandis* N.E.Br., *S. solenanthus* Mansf., *S. erubescens* Hilliard & B.L.Burtt and the caulescents *S. nobilis* C.B.Clarke and *S. muscosus* C.B.Clarke (Figs. 6, 7). This finding has been corroborated for the unifoliate *S. wendlandii* where seedlings grown on medium containing CK, or sprayed with CK when growing in compost show meristem activity

in both cotyledons and induced the formation of two macrocotyledons (Nishii *et al*., 2004; K. Nishii & M. Möller, pers. obs.). Moreover, this effect confirms that in *Streptocarpus,* during the early stages of germination, both cotyledons have basal meristem activity (Nishii & Nagata, 2007). The effect of CK to induce twin macrocotyledons is time limited since once anisocotyly is established the microcotyledon has lost its ability to resume growth (Nishii *et al*., 2004). This suggests that at the isocotylous stage, as long as the basal meristem is active, it is perceptive to exogenous CK to trigger persistent basal meristem activity in both cotyledons, but once the fate of the microcotyledon is determined and the basal meristem activity ceases it is no longer perceptive to CK.

Once two macrocotyledons are established by exogenous CK, both cotyledonary phyllomorphs grow to maturity, and each bears a separate series of inflorescences and subtending phyllomorph (Rosenblum & Basile, 1984) (Fig. 6e, f). In older *S. wendlandii* CK treatment causes only a slight reduction in the formation of subtending phyllomorphs (Dubuc-Lebreux, 1978). This may indicate some effect of CK suppressing the phyllomorph initiation from a usually dormant groove meristem, although the reported effect is small. Rosulate *S. rexii* also develop two macrocotyledons after CK application (Mantegazza *et al.,* 2009).

Not much is known about endogenous CK in *Streptocarpus*. One study reports the distribution of CK in relation to senescence and another the interaction with the phyllomorph meristems (Van Staden, 1973; Chen *et al.,* 2017). In the unifoliate *Streptocarpus molweniensis* Hilliard, the proximal and distal lamina area retain similar levels of cytokinins during early summer. However, these decrease towards the autumn in the distal region of the lamina and may be linked to the formation of an abscission zone (Van Staden, 1973). This shift in distribution towards the end of the growing season might be a pre-requisite and together with the slope of CK gradient, may determine the position of the abscission line on a phyllomorph. This intentional reduction in leaf area is a quite different mechanism compared to ordinary plants in which CK play a role in the prevention of senescence and maintenance of chlorophyll in the lamina (Alberte & Naylor, 1975; Gan & Amasino, 1996), and might best be compared with leaf abscission in which CK is involved in (Xu *et al.,* 2019).

With a view to meristem formation, it is expected that the proximal region of a macrocotyledon or phyllomorph might show higher CK concentrations. However, in the seedling stage of *S. rexii*, the CK concentration is very similar in the proximal and distal region of the macrocotyledon, and the microcotyledon. Only isopentenyladenosine (iPR) shows a slightly higher concentration in the proximal part of the macrocotyledon (Chen *et al*., 2017). It is still possible that the CK localization in the meristematic area of young seedlings was missed because it is very small. Immune-histological methods with CK antibodies might be useful to pinpoint the CK distribution in *Streptocarpus* meristem (*e.g.,* Dewitte *et al*., 1999).

Redundancies and distinctive roles in the *IPT* gene family in *Streptocarpus*

The cytokinin biosynthesis gene family *isopentenyltransferase* (*IPT*) includes genes with redundancies but also with differentiation of functions (Miyawaki *et al*., 2006; Nishii *et al*., 2018). In *A. thaliana*, nine *IPT*s are reported, and among these, *AtIPT7* is upregulated by the *KNOX1* gene *STM* in the SAM and produces CK (Jasinski *et al*., 2005; Yanai *et al*., 2005). CK, in turn, upregulates *STM* in the SAM. In *S. rexii*, five *IPT* genes are known to date, of which two, adenosine phosphate-*IPT SrIPT5* and tRNA-*IPT SrIPT9*, are expressed in the vegetative tissue as well as in floral tissues and roots (Chen *et al*., 2017). Their homologs in *A. thaliana* are *AtIPT5*, expressed in roots and rosette leaves, and *AtIPT9* is found ubiquitously in the whole plant (Miyawaki *et al*., 2004). Other *IPT*s seemed to have acquired specific roles for flower (*SrIPT1*) or root (*SrIPT3*) formation (Chen *et al*.,

2017) (Fig. 5). Of *SrIPT5* and *SrIPT9*, the former in particular shows a strong expression in the groove meristem, and is highly expressed in the proximal part of the lamina. On the other hand, *SrIPT9,* while expressed ubiquitously in the lamina, shows higher expression in the distal part. Both, *SrIPT5* and *SrIPT9* are found in the groove meristem, but have acquired different responses to exogenous hormones: auxin suppresses the expression of both *SrIPT5* and *SrIPT9*, but GA only suppresses *SrIPT9* (Chen *et al*., 2017). Exogenous application of CK does not alter the expression of *SrIPTs*, which might support the notion of sufficient endogenous CK levels in the phyllomorph for its functioning. Therefore, CK itself seems not to control CK biosynthesis, but is maybe regulated by other hormones such as auxin or GA.

The expression of *IPTs* is not exclusive to the distal lamina part, which is consistent with endogenous cytokinin distributions (Van Staden, 1973; Chen *et al*., 2017) (Fig. 5). The expression of *SrIPT9* in particular, was even higher in the distal than the proximal part in a mature lamina. *SrIPT9* is a tRNA-*IPT*, and two tRNA-*IPTs*, *AtIPT2*, *AtIPT9*, exist in *A. thaliana*. Single mutants of those genes do not show clear phenotypes in *A. thaliana*, but the double mutant *atipt 2 9* shows chlorotic effects (Miyawaki *et al*., 2006). In *Streptocarpus*, exogenous GA applications induce chlorotic effects (Nishii *et al.,* 2014), and also downregulate *SrIPT9* (Chen *et al*., 2017). Thus, it can be speculated that *SrIPT9* has some role in the maintenance of chlorophyll in the lamina.

Effects of other hormones on meristem activity in *Streptocarpus*

There are only a few studies available on hormone effects other than CK and GA in *Streptocarpus*. Auxin is one of the major plant hormones involved in various aspects of plant development including shoot formation (*e.g.,* Barton, 2010; Leyser, 2018 (Fig. 5). However, the role of auxin in *Streptocarpus* development is very poorly understood. External application has no clear effect on seedling development in the plurifoliate *S. prolixus*, whereas a treatment with 2,3,5-triiodobenzoic acid (TIBA), a polar auxin transport inhibitor, suppresses cotyledon expansion and induces a "shoot" (*i.e.,* additional phyllomorphs) similar to GA (Rosenblum & Basile, 1984) (Fig. 7). Exogenous auxins suppress *SrIPT*s, and thus it might be involved in controlling the internal CK levels (Chen *et al*., 2017) (Fig. 5) and the regulation of growth in *Streptocarpus*, possibly including anisocotyly. This can be supported by findings that the distribution of auxin affects the symmetric growth of the lamina in tomato and *A. thaliana* (Chitwood *et al*., 2012). It would be interesting to examine the relationship between auxin transport and the regulation of meristems in *Streptocarpus*.

Some effects of abscisic acid (ABA) on *Streptocarpus* seedling development have been reported. ABA inhibits growth, but causes no drastic change in shoot architecture (Rosenblum & Basile, 1984). Similar results are reported in Nishii *et al.* (2004), where ABA application inhibits the cotyledon growth and reduced anisocotyly. These mild effects on anisocotyly and phyllomorph development suggest that ABA only plays a marginal role in *Streptocarpus* ontogeny.

Hormonal effects on the regeneration in *Streptocarpus*

In many plants, the application of auxin and CK induces regeneration from leaf explants and their balance of concentrations regulates regeneration. In general, a high auxin ratio induces the pluripotency of cells, callus, or root regeneration, and a high CK ratio induces shoot regeneration (Ikeuchi *et al*., 2016). While *A. thaliana* requires hormonal application for shoot regeneration, *Streptocarpus* leaf explants can form *de novo* shoots and roots without hormone supplementation (Dubuc-Lebreux & Vieth, 1975; Chaudhury *et al*., 2010). The histological processes of shoot regeneration studied in some species of *Streptocarpus* section *Saintpaulia* (H.Wendl.) Mich.Möller & Haston confirms the direct initiation of shoots (Naylor & Johnson, 1937; Lo *et al*., 1997).

Intriguingly, the ratio of regenerating leaf explants to non-regenerating leaf explants strongly depends on the auxin concentration in the culture media, while the concentration of CK has very little effect (Appelgren & Heide, 1972), although the number of buds per explant is greatly increased with the addition of CK (Chaudhury *et al*., 2010). It may be that the lamina of *Streptocarpus* retains sufficient levels of CK to invoke shoot regeneration (Appelgren & Heide, 1972). However, it seems that the CK concentration in *Streptocarpus* is not high compared to that in *A. thaliana*, although it is difficult to compare interspecifically from different studies (Kiba *et al.,* 2013; Chen *et al.,* 2017). In *A. thaliana*, CK downstream B-type ARR transcription factors regulate *WUS* that in turn initiate shoot regeneration (Zhang *et al*., 2017). Thus, it is possible that genes downstream to CK genes, could be constitutively expressed in *Streptocarpus*, which would contribute to the high regeneration ability in *Streptocarpus*.

GA application inhibits the shoot bud regeneration from callus in *A. thaliana* (Ezura & Harberd, 1995). There are only a few and conflicting results reported of GA effects on the regeneration from leaf explant in *Streptocarpus*. Dubuc-Lebreux and Vieth (1975) reported positive GA effects such as accelerated bud initiation from leaf explants in *S. wendlandii*, but without statistical analyses. On the other hand, exogenous GA negatively affects the regeneration from *Streptocarpus* leaf explant, *i.e.,* reduced regeneration rate, and number of buds or roots, in *S.* x *hybridus* 'Constant Nymph' and *S. prolixus*, with statistical support (Appelgren & Heide, 1972; Rosenblum & Basile, 1984). Such negative effects are also reported in *A. thaliana* (Ezura & Harberd, 1995), and *Solanum lycopersicum* L. (Lombardi-Crestana *et al*., 2012). In *Streptocarpus*, ABA also promotes the regeneration from leaf explant in conjunction with an optimal auxin concentration (Appelgren & Heide, 1972).

The *Streptocarpus* lamina seems to represent a modified physiological entity compared to leaves in ordinary model plants and the tissue responds positively to, but is not depending on, exogenous hormones during regeneration. Understanding the CK signal transduction cascades in *Streptocarpus* might be the key to understand the high regenerative ability in *Streptocarpus*.

Hypothesis of hormone – developmental gene crosstalk in *Streptocarpus* meristems

The SAM is an organized structure with specific zones playing different roles in shoot development. The expression of genes and hormone distributions involved in this process are carefully controlled by *cis* and *trans* acting networks. In the SAM, GA and CK interact via *KNOX1* genes: *KNOX1* genes suppress GA but promote CK biosynthesis. In the model plant *A. thaliana*, endogenous GA degradation increases the expression of *KNOX1* genes (Singh *et al*., 2010). On the other hand, exogenous CK treatment induces an increase in *KNOX1* gene expression (Rupp *et al*., 1999), and *KNOX1* genes positively regulate CK synthesis via *IPT* genes. CK treatments also partially recover *KNOX1* mutant phenotypes (Jasinski *et al*., 2005; Yanai *et al*., 2005). For auxins, polar transport creates physical auxin maxima in locations of incipient leaf primordia initiation in the peripheral zone of the SAM where *KNOX1* is downregulated (*e.g.,* Hay & Tsiantis, 2010; Singh *et al*., 2010) (Fig. 5).

Meristems in *Streptocarpus* phyllomorphs, particularly the groove meristem, retain similar characteristics to a SAM (Fig. 5). This is shown for GA and CK through expression studies of their metabolic genes (see above). Similar to the SAM, the antagonistic roles of GA and CK via *KNOX1* genes are preserved in the meristems of *Streptocarpus* (Mantegazza *et al.,* 2009; Nishii *et al*., 2014; Chen *et al*., 2017) (Fig. 5). Therefore, in *Streptocarpus,* the molecular and physiological units consisting of shoot and SAM function still exist, but have been transferred to the lateral organ, the phyllomorph. Consequently, the expression patterns of meristem genes and related factors are modified for establishing the plant's unique architecture.

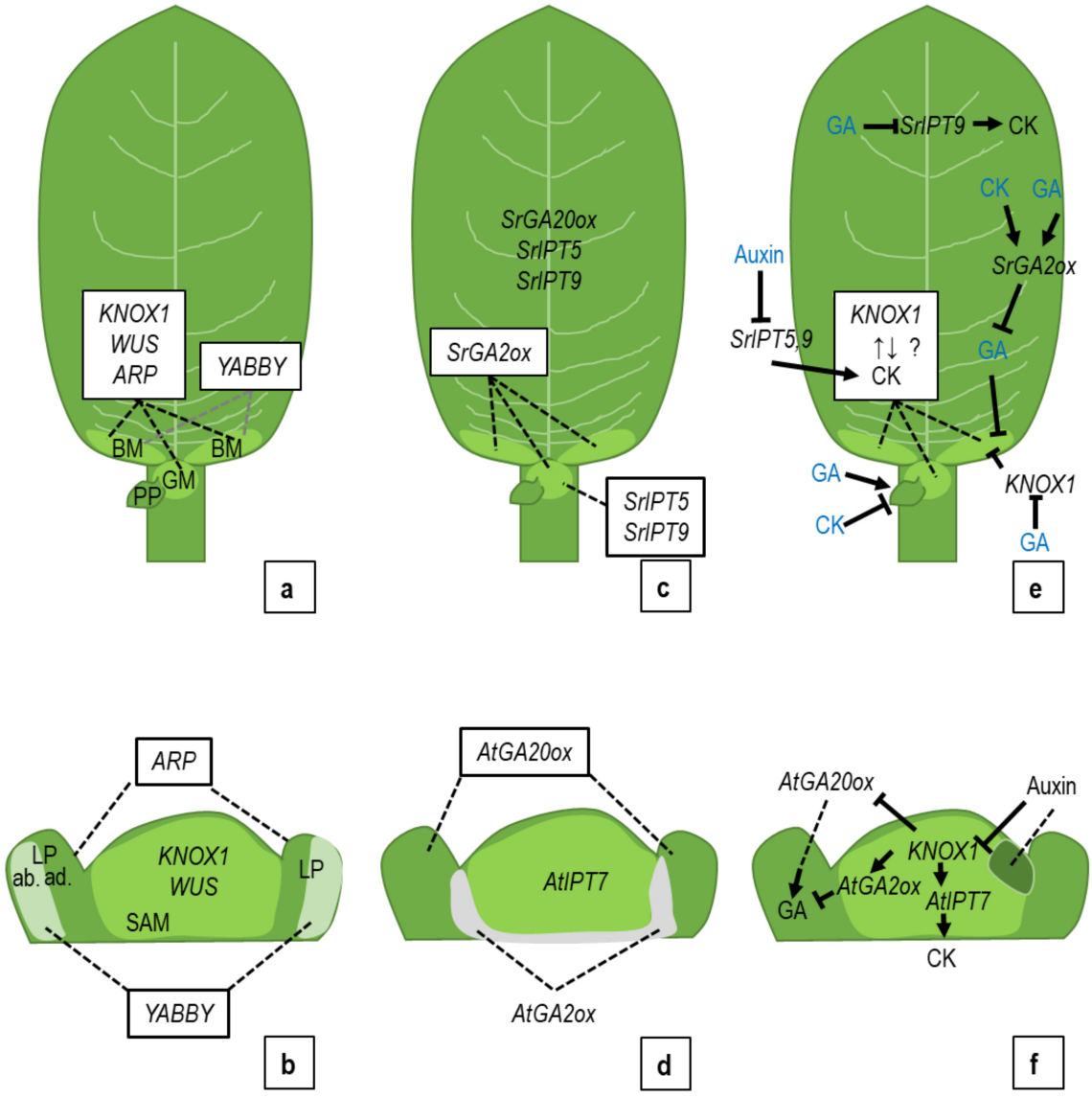

Fig. 5. Schematic summaries of currently reported genetic pathways in the phyllomorph of acaulescent rosulate *Streptocarpus* Lindl. (upper row) and the SAM of the model plant *Arabidopsis thaliana* L. (lower row): **a & b.** Expression patterns of developmental genes. For details and background see also Mantegazza *et al.* (2007, 2009), Nishii *et al.* (2010), and Tononi *et al.* (2010). **c & d.** Expression patterns of GA and CK metabolism genes in *S. rexii* (Bowie ex Hook.) Lindl. (*Sr*) and *A. thaliana* (*At*). *GA20-oxidase* (*GA20ox*) synthesizes and *GA2-oxidase* (*GA2ox*) degrades GA. *Isopentenyltransferase* (*IPT*) synthesizes CK. **e & f.** Current model of hormone-gene interactions in *Streptocarpus* and in *A. thaliana*. In *A. thaliana*, *KNOX1* induces *AtIPT7* and *AtGA2ox* expression, whereas it suppresses *AtGA20ox*, which is usually expressed in the leaf primordia. Auxin transporters create auxin maxima in the position of incipient leaf primordia and suppress *KNOX1* expression (*e.g.,* Barton, 2010; Hay & Tsiantis, 2010). In *Streptocarpus*, *KNOX1* expression is observed in the basal meristem and groove meristem (Mantegazza *et al.*, 2007). Hormone applications in seedlings suggest that CK may maintain basal meristem activity with *KNOX1* expression, whereas GA may suppress basal meristem and *KNOX1* (Nishii *et al.*, 2004, 2012a; Mantegazza *et al.*, 2009). Exogenous GA promotes the initiation of phyllomorph primordia from the groove meristem, whereas exogenous CK suppresses it (*e.g.,* Dubuc-Lebreux, 1978; Rosenblum & Basile, 1984). Either exogenous CK or GA upregulate *SrGA2-oxidase*, reducing GA levels (Nishii *et al.,* 2014). Auxin application suppresses both, *SrIPT5* and *SrIPT9*, while exogenous GA only suppresses *SrIPT9* (Chen *et al.,* 2017). Lettering in black in **e** and **f** is based on evidence from endo- and exogenous hormone studies, and in blue only on exogenous hormone application experiments. ab.: abaxial, and ad.: adaxial leaf surfaces, BM: basal meristem, GM: groove meristem, LP: leaf primordium, PP: phyllomorph primordium, SAM: shoot apical meristem.

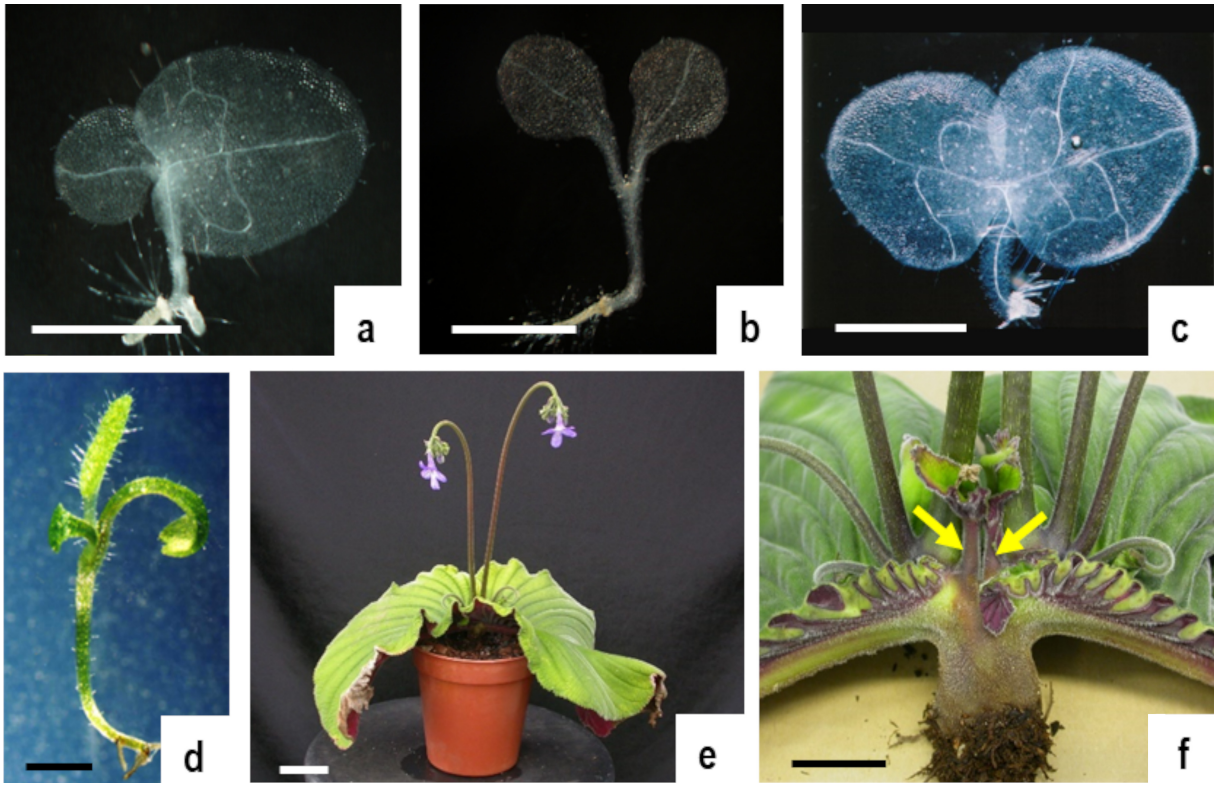

Fig. 6. Effects of exogenous hormone applications on unifoliate *Streptocarpus wendlandii* Spreng.: **a-c.** Seedlings 30 days after sowing. **a.** Control seedling showing anisocotyly. **b.** GA treated seedling showing two microcotyledons and elongated petiolodes. **c.** Benzylaminopurine (BAP; CK) treated seedling showing two macrocotyledons. **d.** GA treated seedling 45 days after sowing, showing an additional phyllomorph between the two microcotyledons. **e & f.** BAP treated *S. wendlandii* seedlings at flowering. **e.** Whole plant with two cotyledonary phyllomorphs developed from two macrocotyledons. **f.** Longitudinally dissected plant showing that each cotyledonary phyllomorph individually retains an acropetal series of inflorescences and each develops a subtending phyllomorph (arrows). Scale bars: 1 mm (**a-d**), 5 cm (**e & f**).

In *Streptocarpus* seedlings, the external application of CK promotes lateral growth whereas GA induces apical growth. This is accompanied by the expression of *KNOX1* in the basal meristems. While CK promotes *KNOX1* in cotyledons, *KNOX1* expression is suppressed there in the rosulate *S. rexii* after GA treatment (Mantegazza *et al*., 2009) (Fig. 5). Thus, GA might be responsible for the inhibition of the basal meristem activity through downregulating *KNOX1*. This might be important in the establishment of anisocotyly by suppressing the basal meristem of the microcotyledon (Figs. 5-7). GA interacts with environmental signals, such as light (Alabadí *et al.,* 2008), and in *Streptocarpus* may regulate the balance between lamina expansion and phyllomorph initiation to suit the environment. GA may promote the organ differentiation from the undifferentiated groove meristem, and thus it induces apical growth in the form of additional phyllomorphs. Thus it seems that *Streptocarpus* possesses regulatory units involving GA, CK via *KNOX1*, similar to ordinary shoots, but it appears that at least in acaulescents an apical dominance model is amended by a lateral dominance model through the relocation of meristems from the shoot apex to lateral organ, or completely replaced as in unifoliates.

Future perspectives

The vegetative organogenesis of *Streptocarpus* has unique features. The underlying developmental mechanisms become slowly understood. However, there are still many challenges to reveal the entire network of interactions between growth forms and genes. The effects of the plant hormones GA and CK on *Streptocarpus* seedlings have long been known and the recent expression studies on their metabolic genes and meristem genes have shed

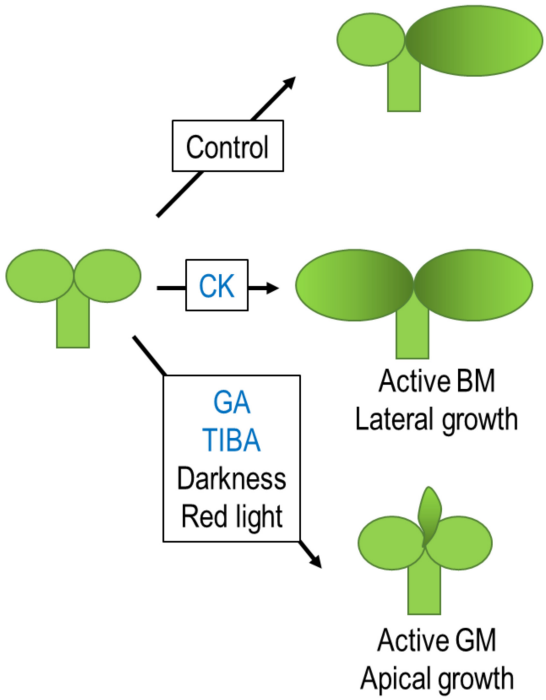

Fig. 7. Schematic illustration of the developmental pathways of *Streptocarpus* Lindl. seedlings as affected by hormones and environmental cues. Exogenous CK induces two macrocotyledons with active basal meristems (lateral growth), whereas exogenous GA and the auxin transport inhibitors (TIBA), darkness, or red light treatments induced two microcotyledons with accelerated phyllomorph initiation (apical growth).

some light on the molecular mechanisms involved in the establishment of anisocotyly and the growth of the unique leafy unit 'phyllomorph'. The role of other plant hormones, particularly auxin, requires to be investigated to understand its involvement in *Streptocarpus* development. Further, most studies were limited to the exogenous application of hormones, but to understand their physiological role more details are needed about the endogenous status of plant hormones. Some environmental factors may also have close links to the hormonal regulation in *Streptocarpus* and these have been very much neglected, particularly with view to the establishment of anisocotyly. There is only one study investigating light where it is shown that the direction of light seems to determine the fate of the macrocotyledon (Saueregger & Weber, 2004). Studies of light signal transduction in *Streptocarpus* are needed to elucidate the first steps in the establishment of a hormone imbalance that seems to be the basis for the unequal development of the cotyledons. The petiolode meristem has likewise received little attention, although its development differs greatly between caulescent and acaulescent *Streptocarpus* species.

The published research regarding the molecular mechanisms of *Streptocarpus* development mainly relies on expression studies of genes characterised in model plants. However, these studies are limited since it is often overlooked that model plants such as *A. thaliana* have accumulated their own uniqueness during its evolutionary trajectory (Francki & Appels, 2007), and not all findings may be relevant for *Streptocarpus*. Continuous advances in next generation sequencing technologies allow an ever growing set of data to be acquired and greatly accelerate the progress of revealing the genetics in non-model plants. In *Streptocarpus*, the chloroplast genome, a transcriptome set, and a genetic map have already been published using

next generation sequencing (Chiara *et al*., 2013; Chen *et al*., 2018; Kyalo *et al*., 2018). With those data available, it may be possible that the next studies may be able to reveal the molecular and physiological regulatory networks underlying the development of *Streptocarpus*. Not only proposing a genetic mechanism, but also carrying out functional confirmatory experiments through genetic modifications are needed to fully understand the molecular programme in *Streptocarpus*. Genetic tools, such as transformation and mutagenesis, have been developed mainly for African Violets, the former *Saintpaulia*, one of most popular ornamentals in the predominantly caulescent subgenus *Streptocarpella* in *Streptocarpus* (Kushikawa *et al*., 2001; Da Silva *et al*., 2017). Virus-iniduced gene silencing was also reported in *S. rexii* (Nishii *et al*., 2020). These techniques may be transferrable to the acaulescent subgenus *Streptocarpus* to examine the functions of the genes involved in its shoot development.

Our understanding of the link between environmental factors and plant hormones in *Streptocarpus* is incomplete, it may be an important driving force for the evolution of anisocotyly and the phyllomorph (Burtt, 1970). These features have allowed the species to occupy vastly divergent habitats such as dark, cool and moist evergreen rainforests on the one hand, and open, hot and dry grasslands on the other. In the latter, anisocotyly allows the plants to even withstand frequent natural fires and regrow from the basal meristem. Moreover, the abscission zone allows it to reduce the lamina to survive the dry winters. This makes the unifoliates not hopeless monsters but highly adapted lineages that have diversified into over 40 species (Hilliard & Burtt, 1971). The genus *Streptocarpus* harbours many intermediates between the three main growth forms and future studies may reveal the genetic cascades that allow a flexibility in development that over evolutionary times added > 170 species to a genus that some would have regarded as "misfits" (Bell, 2008).

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Appendix I. Glossary of specific terms used in *Streptocarpus* studies, based on Jong and Burtt (1975). In the alphabetical order:

Acaulescents: Species without ordinary shoot structure. Each leaf is termed as phyllomorph and a phyllomorph represents the entity of the shoot. Unifoliate and rosulate *Streptocarpus* belong to acaulescent *Streptocarpus*.

Accessory phyllomorph: *Sensu* Jong (1970) equalling additional phyllomorphs; *sensu* Dubuc-Lebreux (1978), combining adventitious and subtending phyllomorphs; *sensu* Nishii *et al*. (2010) equals subtending phyllomorph.

Additional phyllomorph: Phyllomorph formed from the groove meristem of an existing phyllomorph.

Adventitious phyllomorph: Phyllomorph formed *de novo* randomly on the surface of the petiolode and do not originate from the groove meristem.

Anisocotyly: Unequal cotyledon development.

Basal meristem: The meristem located in the proximal region of lamina. It contributes for lamina expansion.

Caulescents: Species with ordinary shoot structure, leaf and stem and the shoot apical meristem.

Cotyledonary phyllomorph: The mature form of the macrocotyledon.

Groove meristem: The meristem located at the juxtaposition between lamina and petiolode. The inflorescences or phyllomorph primordia initiate from the groove meristem.

Isocotyly: Equal cotyledon development.

Macrocotyledon: The larger cotyledon in a pair of cotyledons, showing continuous enlargement ability.

Microcotyledon: The smaller cotyledon in a pair of cotyledons.

Petiolode meristem: The meristem contributes to the elongation and thickening of the petiolode.

Petiolode: The stalk of a phyllomorph. It retains the mixed nature between stem and petiole.

Phyllomorph: The leafy unit of acaulescent *Streptocarpus*. A phyllomorph is consisted with the lamina and petiolode.

Plurifoliates: Intermediates between unifoliate and rosulate. It forms only a few phyllomorph from the existing phyllomorph, or one at a time.

Rosulates: Acaulescent species with additional phyllomorph formed from the groove meristem of existing phyllomorph and form a false rosette.

Subtending phyllomorph: Phyllomorph forming from the groove meristem subtending a series of acropetally developing inflorescences in unifoliate *Streptocarpus*.

Unifoliates: Species only retain the macrocotyledon derived phyllomorph.

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