



Trichomes and naphthoquinones protect *Streptocarpus dunnii* Hook.f. against environmental stresses

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ABSTRACT

Streptocarpus dunnii Hook.f. (Gesneriaceae) grows wild in South Africa in rocky terrain and at high altitude. It possesses unique patterns of growth, is acaulescent, unifoliate and monocarpic and produces unique naphthoquinones. Morphological examinations of *Streptocarpus* species have tended to concentrate on germination, cotyledon anatomy and development of the primary leaf (phyllomorph) while mature phyllomorphs are rarely examined. This study sought to examine the morphology of the mature phyllomorph and to more accurately determine the type and site of naphthoquinone(s) excretion. Plants were developed from invitro plantlets originating from authenticated seeds and allowed to grow in a peat-based potting compost for a year, under ambient room conditions. Mature lamina samples were examined morphologically by light, stereo and scanning electron microscopy. Results showed anisocytic stomata only on the abaxial epidermis, with numerous non-glandular and glandular trichomes on both epidermises, especially at the proximal end of the phyllomorph. Energy-dispersive X-ray analysis indicated both trichome types to be rich in potassium. Glandular trichomes were more abundant on the abaxial surface, especially at non-glandular trichome bases over veins and adjacent to stomata in lamina areas between veins. These trichomes were shown for the first time to be the source of the naphthoquinones in *S.dunnii*. Chromatographic and FT-IR analyses confirmed the glandular secretions as being mainly (±)-dunnione. Results suggest that mature phyllomorphs with densely packed epidermal trichomes and a restricted distribution of stomata are adaptations by this plant to dehydration stress, while dunnione-type naphthoquinones may function in the amelioration of oxidative stress whether of biotic or abiotic origin.

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1. Introduction

Gesneriaceae, the family to which *Streptocarpus* Lindl. (Cape Primrose) belongs, is comprised of over 150 genera, many of which are of economic importance as horticultural ornamentals such as *Saintpaulia* (African Violets) and *Sinningia* (Gloxinias) (Weber and Skog, 2007). The ca. 3540 gesneriad species are distributed throughout the world (Weber, 2004; Christenhusz and Byng, 2016) with many growing in habitats that are water-deficient and nutrient-poor. Some species are epiphytic, e.g., *Codonanthe gracilis* (Pereira-Dias and Santos, 2015) while others are epipetric, e.g. *Sinningia bullata*, or lithophytic, e.g. *Petrocodon* species (Xu et al., 2014; Guo et al., 2016) and some are desiccation tolerant, e.g. *Ramonda* species (Rakić et al., 2015). Because of such diversity and evolutionary novelty, members of the Gesneriaceae are increasingly the subject of phylogenetic analyses (Jong and Burt, 1975; Harrison et al., 1999; Denduangboripant, 2001; Möller and Cronk, 2001; Qiu et al., 2015; Tao et al., 2016; Ling et al., 2017). Although these analyses have suggested a new comprehensive

classification of the entire Gesneriaceae family (Weber et al., 2013), traditionally it has been divided into two sub-families: – the neotropical Gesnerioideae found in South and Central America as well as Caribbean Islands and the paleotropical Didymocarpoideae (syn. Cyrtandroideae) found in South Africa, Southeast Asia and Pacific Islands (Möller et al., 2009). Morphological evaluation of multiple characters appears to mainly support this division (Smith, 1996) but it is still largely based on whether an endosperm develops in the seed (Gesnerioideae) or not (Didymocarpoideae) and how the cotyledons then grow after germination – equally (Gesnerioideae) or unequally (Didymocarpoideae).

The Gesneriaceae (Order Lamiales) display common anatomical characteristics such as dorsiventral leaves with anisocytic stomata situated usually only on the lower surface, petiole and stems and both glandular and non-glandular trichomes (Saharsrabudhe and Stace, 1974; Yuen and Dehgan, 1982). Some glandular trichomes (e.g. in *Monophyllaea*, Didymocarpoideae) secrete calcium carbonate while others (e.g. *Didymocarpus*, Didymocarpoideae) have glands (Palee et al., 2006) that are filled with yellow-brown to dark-red pigments (Adityachaudhury et al., 1976). Few phytochemical reports into gesneriad genera have been published but, to date, the metabolites identified include anthocyanins, flavonoids, carotenoids,

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terpenes, steroids, phenolics, lignans, xanthenes, anthraquinones and naphthoquinones (Harborne, 1966; Stöckigt et al., 1973; Kvist and Pedersen, 1986; Verdán and Stefanello, 2012; Hook et al., 2014; Rakić et al., 2015). Some of these may have taxonomic relevance (Harborne, 1966; Kvist and Pedersen, 1986) while others may be of future value as anticancer agents or biocides (Sheridan et al., 2008; Tong et al., 2011; Widhalm and Rhodes, 2016; Soares et al., 2017).

The Didymocarpoideae to which *Streptocarpus* belongs constitute an important component of the endemic flora of limestone karst regions in China, growing where soils are typically shallow, nitrogen deficient, with low water content and periodic drought induced by heat stress (Kang et al., 2015). Many endemic plants are calciphytes (syn. calcicoles) (Möller et al., 2011) and characterised by higher calcium (Ca^{2+}) and magnesium (Mg^{2+}) contents relative to other plants. *Primulina* species, for example, are able to hyperaccumulate leaf calcium even in soils with a low calcium Ca^{2+} concentration (Hao et al., 2015; Tao et al., 2016). Other morphological, biochemical and molecular adaptations are shown by desiccation-tolerant resurrection plants such as the Chinese *Boea hygrometrica* (Cyrtandroideae) (Mitra et al., 2013) and the rare *Ramonda nathaliae*, a calcicole endemic to the Balkan peninsula which also displays abundant vesicular arbuscular mycorrhiza in its roots (Rakić et al., 2013).

Streptocarpus is an Old World genus made up of over 130 accepted species that are indigenous to mainly Africa and Madagascar (Hilliard and Burt, 1971; de Villiers et al., 2013). Although extensively cultivated commercially and in the wild, some species are found in forests (e.g. *S. primulifolius*) while others prefer shaded rocky outcrops, cliffs and steep earth banks (e.g. *S. montigena*). This genus displays unique patterns of growth and great variation in vegetative structure. Plants of subgenus *Streptocarpella* are caulescent, having a conventional shoot architecture, and produce leaves from a shoot apical meristem (SAM). Acaulescent species (subgenus *Streptocarpus*) lack a true stem and conventional SAM but can still produce a cluster of leaves (rosulate form) from the base of more mature leaves. Other acaulescent species, however, are unifoliolate, producing only a single leafy organ (a phyllomorph) formed by continued growth after germination of only one cotyledon (the macrocotyledon), the other eventually withering away, i.e. showing anisocotylous growth. The phyllomorph has a proximal petiole-like stalk (the petiolode) and an inflorescence that can arise from the phyllomorph midrib, producing bracts and flowers in a conventional manner. Three meristems are involved in this unique type of growth: (i) the basal meristem, producing lamina growth, (ii) the petiolode meristem, contributing to petiolode growth and (iii) the groove meristem, involved in inflorescence development (Jong and Burt, 1975; Möller and Cronk, 2001; Harrison et al., 2005). Because of such developmental novelty, species within the genus *Streptocarpus* are increasingly being examined to unravel the complexity of the genetic mechanisms controlling patterns of cell division and leaf organisation (Mantegazza et al., 2007, 2009; Nishii et al., 2010, 2017; Tononi et al., 2010). Various factors have been found associated with anisocotylous development, including gravity (Saueregger and Weber, 2003), the plant hormones gibberellin and cytokinin (Nishii et al., 2004, 2012a; Mantegazza et al., 2007; Chen et al., 2017) and light which is an absolute requirement for *Streptocarpus* germination (Nishii et al., 2012b).

Streptocarpus dunnii Hook.f. (subgenus *Streptocarpus*) is an infrequently researched plant that has a scattered distribution in the rocky and treeless South African habitat of the Eastern Transvaal (Mpumalanga) and Swaziland (Hilliard and Burt, 1971; Hughes et al., 2007). The plants grow at high-altitude (1700–2200 m) in nutritionally poor damp grasslands. Although able to survive such open areas exposed to full sun light, they usually prefer shaded places under rocks (lithophytic) or wedged in crevices (chasmophytic). These plants, therefore, need to withstand a natural environment of moisture-deficient winters and wet summers, with temperature extremes of 26 °C in December to 3 °C in June. The tubular flowers of an orange-red colour unique to *S. dunnii* are produced from November to February (i.e. warm summer months

in the southern hemisphere) and considered to be pollinated by the malachite sunbird (Hughes et al., 2007). The plant may live for several years before flowering and needs to last through a dry season at least once before flowering. Also, as it is monocarpic it will die down after flowering and setting seed. Large numbers of very small seeds are produced in long capsules that twist when dry, releasing the seeds for wind pollination. Seeds of *S. dunnii* were originally collected in 1884 and sent to Kew Gardens where, in subsequent years, numerous “beautiful hybrids” with attractive red flowers were developed from it (Anon, 1910), such as *S. kewensis* from *S. dunnii* x *S. rexii*. Such hybrids are still being developed today and used to study mechanisms by which the chloroplast (cpDNA) and nuclear ribosomal DNA (nrDNA) can be inherited in plants and how hybridization can be involved in plant evolution (Möller et al., 2004; Denduangboripant et al., 2007).

Streptocarpus dunnii is acaulescent and unifoliolate (<http://pza.sanbi.org/streptocarpus-dunnii>). This single leaf is oblong, ca. 60 cm long and up to 20 cm wide, hairy on both surfaces, wrinkled (rugose) and veined. All parts, except the upper leaf surface, have been described as showing orange-red granules which according to Flora Capensis “it is known in no other *Streptocarpus* and in every dried specimen in the herbarium, the spirit (used in poisoning) has dissolved out of every part of this plant an orange stain” (Clarke, 1904). No identification of these granules was made until 1939 when the isolation of the “yellow stain” from mature plants was reported and identified as the naphthoquinone dunnione (Price and Robinson, 1939). Review of subsequent publications regarding this and other naphthoquinones in *S. dunnii*, has shown that these compounds vary in types and amounts according to age, plant part, nutrients and growth conditions. Mature leaves and flowers were found to contain predominantly (\pm)-dunnione, roots of these mature plants only 1-hydroxy-2-hydroxymethylanthraquinone and immature plantlets mainly alpha-dunnione (Inoue et al., 1983). In vitro cultured plantlets grown in the dark also produced alpha-dunnione as main constituent (Inoue et al., 1983) but light-cultured plantlets yielded (\pm)-dunnione (Sheridan et al., 2011) while undifferentiated cell suspension cultures produced mainly hydroxyanthraquinones (Inoue et al., 1983; Nestor 2006). The sites of synthesis and biological significance to *S. dunnii* of dunnione and related quinones have never been addressed. Most microscopical examinations of *Streptocarpus* species, especially *S. rexii*, have concentrated on cotyledon anatomy and development from germination to the formation of the primary phyllomorph (Viemont, 1980; Mantegazza et al., 2007; Nishii and Nagata, 2007; Nishii et al., 2012b) while the development of mature structures remains under-examined. The objectives of this study, therefore, were to use a combination of light and scanning electron microscopy as well as phytochemical methods to: (i) examine the morphology of mature phyllomorphs of cultivated *Streptocarpus dunnii* and (ii) to more accurately determine the type and site of naphthoquinone(s) excretion and speculate on reasons for their formation.

2. Materials and methods

2.1. Plant material

In vitro plantlets of *Streptocarpus dunnii* were originally developed from authenticated seed material and maintained by continuously sub-culturing at 6-week intervals on to a Phytigel™-solidified, hormone-free, modified Murashige and Skoog medium, as previously described (Sheridan et al., 2011). Some plantlets were transferred to a propagation tray containing a shallow layer of peat-based potting compost. Plantlets began to grow under ambient room conditions, mature after about one year and formed the source material for this research.

2.2. Light and stereo microscopy

Samples from adaxial and abaxial epidermises were taken in early autumn (September–November) from proximal and distal areas of the

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