NATALIE DO VALLE CAPELLI

ULTRASTRUCTURE AND CHEMICAL ANALYSIS OF OSMOPHORES IN
APOCYNACEAE

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ULTRAESTRUTURA E ANÁLISE QUÍMICA DE OSMÓFOROS EM
APOCYNACEAE

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Abstract

Apocynaceae presents the flowers with the highest degree of synorganization among the eudicots and highly elaborated pollination mechanisms associated with the high diversity of glands. The osmophore stands out as responsible to produce a floral scent which attracts pollinators and, despite its fundamental relevance for pollination, its structure and mechanism of production and release of the perfume is essentially unknown in Apocynaceae. This present work aims to characterize morphologically and ultrastructurally the osmophores of Apocynaceae, besides chemically identifying the compounds that constitute the floral scent. Species from two subfamilies were selected to describe the diversity of osmophores and types of scent in the family. The osmophores were firstly located histochemically and, later, this region was processed for transmission electron microscopy. Micromorphological analysis was performed by scanning electron microscopy, and the identification of volatile oils made by GC-MS. Osmophores are located on the adaxial surface of the free portion of the petals. They varied in the shape of epidermal cells, striation of the cuticle and presence of trichomes. This gland is mostly formed by secretory epidermis and parenchyma, except in *Plumeria*, where the osmophores are exclusively epidermal. The secretory cells presented thick walls in the Asclepiadoideae and secretion produced by plastids and SER in all species. Several vesicles perform the intercellular transport of secretion, as well as its release to the environment. However, *Plumeria* and *Ditassa* transfer the secretion produced to the vacuole before releasing it, and *Tabernaemontana* has a mixed release process. The composition of the scent varied among species, with great production of hydrocarbons, alcohols, ketones or monoterpenes depending on the species. Apocynaceae presents a high morphological and metabolic diversity in the osmophores which cannot be correlated with their morphology, subcellular organization, period of secretion release or pollination syndrome. This is the first comprehensive structural study of osmophores in this family that points out to very distinct evolutionary processes that may be related to multiple emergences in the phylogeny and species-specific associations with pollinators.

Keywords: Apocynaceae, evolution, flower, odour bouquet, osmophore, secretory process, ultrastructure.

Resumo

Apocynaceae apresenta as flores com o maior grau de sinorganização das eudicotiledôneas e mecanismos de polinização altamente elaborados associados à mais alta diversidade de glândulas. O osmóforo destaca-se como responsável pela produção do perfume floral para atração dos polinizadores e, a despeito de sua importância fundamental para a polinização, a sua estrutura e mecanismo de produção e liberação do perfume é praticamente desconhecido nas Apocynaceae. O presente trabalho tem o propósito de caracterizar morfológica e ultraestruturalmente os osmóforos de Apocynaceae, além de identificar quimicamente os compostos que constituem o perfume floral. Espécies de duas subfamílias foram selecionadas, visando descrever a diversidade de osmóforos e tipos de perfume na família. Primeiramente, os osmóforos foram localizados histoquimicamente e, posteriormente, essa região foi processada para microscopia eletrônica de transmissão. Análise micromorfológica foi realizada através de microscopia eletrônica de transmissão. Análise micromorfológica foi realizada através de microscopia eletrônica de varredura e a identificação dos óleos voláteis, através de GC/MS. Os osmóforos estão localizados na superfície adaxial da porção livre das pétalas. Eles variaram quanto ao formato das células epidérmicas, ornamentação da cutícula e presença de tricomas. Esta glândula é, em sua maioria, formada por epiderme e parênquima secretores, exceto em *Plumeria*, onde os osmóforos são exclusivamente epidérmicos. As células secretoras apresentaram paredes espessas nas Asclepiadoideae e secreção produzida pelos plastídeos e REL em todas as espécies. Diversas vesículas realizam o transporte intercelular da secreção, assim como a sua liberação para o meio externo. Contudo, *Plumeria* e *Ditassa* transferem a secreção produzida para o vacúolo antes de liberá-la e *Tabernaemontana* possui processo de liberação misto. A composição do perfume variou entre as espécies, havendo grande produção de hidrocarbonetos, alcoóis, cetonas ou monoterpenos dependendo da espécie. Apocynaceae apresenta uma alta diversidade morfológica e metabólica em seus osmóforos que não pode ser correlacionada à morfologia, organização subcelular, período de liberação da secreção ou síndrome de polinização. Esse é o primeiro estudo estrutural abrangente sobre osmóforos na família e aponta para processos evolutivos muito distintos que podem estar relacionados a múltiplos surgimentos na filogenia e associações espécie-específicas com os polinizadores.

Palavras-chave: Apocynaceae, evolução, flor, odor, osmóforo, processo secretor, ultraestrutura.

Background

Pollination may be such a specialized event that, in some cases, implies not only in the need for a specific pollinator but also for a series of secretory structures which allows the flower to be pollinated (Meve and Liede 1994; Borba and Semir 1998; Demarco 2014, 2017a; Monteiro and Demarco 2017).

In plants that have several secretory structures, mutualistic relationships seem to be more common (Fahn 1979). When the floral glands are analyzed, many of them produce substances that act as attractive to pollinators or produce reward substances for them (Nishida 2014), aid in the dispersal of seeds and act as a protection against herbivores and pathogens (Mithöfer and Boland 2012; Vitarelli *et al.* 2015). The participation of these substances in interactions between plants and animals is undeniable, acting as mediators of a range of ecological responses, and may act as attractive or repellent (Dusenbery 1922; Harborne 1994).

Among the main floral traits the color and the odor are usually the main responsible for pollinator attraction but the smell of the insects is apparently much more accurate than the vision (Kevan and Baker 1983). The presence of scent is related to the production of volatile chemical substances by secretory structures located inside or on the surface of vegetative and/or reproductive organs (Svendsen and Scheffer 1985).

In pollination, the volatile floral substances can play a role as a signal for visitors or even act as the sole available reward of the flower. In addition, some flowers can mimic pheromones of female bees and wasps, attracting the respective males that establish a pseudocopula with flowers, performing a mistake pollination (Silva 1992).

The release of scent by flowers can be highly complex, exhibiting dynamic emission patterns and chemical composition of the volatile compounds. The petals are the main producers of odors and the gland responsible for their production is called osmophore (Vogel

1990). This gland has a restricted occurrence and are often located at the distal portion of the perianth and usually have secretory epidermis (Vogel 1990).

Plants with floral scent may present different pollination syndromes within the same group or may be related to a major type of syndrome. Studies that address these relationships are very informative providing excellent opportunities to determine if pollinator changes are correlated with parallel variations in floral bouquet chemistry (Dobson 2006), since similarities between visual attractant plus the chemical composition of the floral scent, independently of the phylogenetic relationship between the species compared, may suggest that they have the same group of pollinators (Knudsen *et al.* 1993). These similarities have been found, for example, in plants pollinated by butterflies (Knudsen and Tollsten 1995; Raguso and Pichersky 1995; Jürgens *et al.* 2002, 2003), bats (Knudsen and Tollsten 1995; Bestmann *et al.* 1997), beetles (Thien *et al.* 1975; Yasukawa *et al.* 1992; Jürgens *et al.* 2000) and female euglossine bees (Williams and Whitten 1983; Sazima *et al.* 1993).

Increase in floral temperature up to 22 °C higher than the environmental temperature may also be related to osmophore activity, forming an intrafloral microclimate that is attractive to insects both as a shelter and as a mating site (Meeuse 1975). In addition, flowers that produce scent as a resource for the pollinator have their oils collected and transferred to a dilatation of the tibia of posterior leg in Euglossini tribe (Apidae), whose internal structure is glandular. There are speculations that the collected oils are used since as a territorial marker, also as defensive kairomones, or even transferred to the respective females at the moment of the copula (Williams 1983, Williams & Whitten 1983; Roubik 1989).

The diversity of volatile compounds emitted by flowers can be grouped as derivatives of fatty acids, terpenoids and benzenoids, as well as compounds containing nitrogen and sulfur. Some compounds are present in almost all floral scents, others are found only at certain species (Dobson 2006). Despite the greater or lesser resemblance between the bouquet

of each flower, there must be a synchronization between the moment of insect activity and the emission of the fragrance, as well as other parameters of the floral development. Phenomena such as the anthesis occurring at a certain period of the day, changes in the presentation of nectar and pollen, thermogenesis, and changes in position of moving organs are essential to ensure pollination in several groups (Bünning 1967; Hess 1983; Proctor *et al.* 1996).

Floral scents are particularly important in flowers opening at night, where olfactory signals attract pollinators over long distances in deceit pollination systems where odors mimic sex hormones, the scent of flowers that supply nectar, or even the smell of decaying organic matter in sapromiophilic systems (Proctor *et al.* 1996).

All these factors demonstrate the great diversity of osmophore activity that can be reflected in a great morphological diversity, since it is possible to observe flowers emitting distinct scents at different times of the day related to various syndromes of pollination within a single group of plants. Therefore, this dissertation presents a study of the Apocynaceae with aromatic flowers, aiming to analyze, for the first time, the diversity of scent glands in this family. This present work is structured according to the format in which it will be submitted to the Annals of Botany.

Conclusions

The immense diversity found in the Apocynaceae osmophores for all analyzed aspects prevents the correlation of their characteristics with any structural characteristics of the secretory process or with the pollination biology of the species analyzed up to the present moment. New studies are essential to try to determine which evolutionary factors have influenced the appearance of such distinct glands in subfamilies with relatively constant floral morphology and similar pollination syndromes.

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