J. Orchid Soc. India, 38: 77-80, 2024 *ISSN 0971-5371*

OCCURRENCE OF RAPHIDES IN FLORAL PARTS OF WILD ORCHIDS FROM THE WESTERN GHATS OF GOA

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Abstract

The present study reports the occurrence of raphides in the floral parts of wild orchids from the Western Ghats of Goa. A total number of 21 orchid species, including 15 epiphytic and 6 terrestrial species were examined for the presence of raphides in their floral tissues. All the presently studied species exhibited the presence of raphides, which were characterized by their pointed, needle-like shape, with lengths ranging from 15 µm to 90 µm.

Introduction

THE FAMILY Orchidaceae is one of the largest and most diverse families of angiosperms with about 703 genera (POWO, 2024) and 29,481 species (WFO, 2023). These plants are widely distributed and occur mainly in the tropical, subtropical, and temperate regions. Taxonomically, the family Orchidaceae represents the most highly evolved family amongst monocotyledons. The distribution of orchids in Goa is concentrated mainly in the Western Ghats region, which accounts for 86% of the total orchid diversity of the state (Jalal, 2019). Raphides, the needle-like crystals of calcium oxalate (De Candolle, 1827; Dutrochet, 1824), are found in large numbers and can be seen all over the plant (Raman *et al.*, 2014). These crystals are found in a diverse range of plant families, including approximately 74% of angiosperm families (Franceschi and Horner, 1980; Gallaher, 1975; McNair, 1932; Nakata, 2012; Zindler-Frank, 1976). Raphides are easily identifiable by their pointed needle-like shape, with lengths ranging from 16 to 300 µm in various plants (Chu *et al.*, 2014; Cote, 2009). These are typically arranged in parallel, regular cylindrical bundles (Raman *et al.*, 2014). The formation of calcium oxalate (CaOx) often occurs within the vacuoles of specialized cells known as crystal idioblasts (Foster, 1956). Raphides are generally observed in large numbers and are closely packed, with thicker raphides commonly found in leaves and thinner raphides in reproductive organs (Cote, 2009).

Earlier studies by some workers (Dressler, 1993; Margoñska *et al.*, 2021; Ren *et al.*, 2020; Rudall *et al.*, 2013; Swiechzkowska and Kowalkowska, 2015) have documented the presence of raphides in the floral parts of orchids. The present study aims to contribute to the understanding of raphide occurrence by reporting their presence in the floral parts of both epiphytic and terrestrial orchid species from the Western Ghats of Goa.

Material and Methods

In the present study, flowers of twenty one orchid species comprising fifteen epiphytic and six terrestrial species were collected from various parts of Goa and were screened for studying the needle-shaped crystals in floral parts. The epiphytic species comprised *Acampe praemorsa* (Roxb.) Blatt. & McCann, *Aerides crispa* Lindl., *A maculosa* Lindl., *Bulbophyllum sterile* (Lam.) Suresh, *Cymbidium bicolor* Lindl, *Dendrobium barbatulum* Lindl., *D. macrostachyum* Lindl., *D. ovatum* (L.) Kranzl, *D. peguanum* Lindl., *Luisia zeylanica* Lindl., *Porpax filiformis* (Wight) Schuit., Y.P.Ng & H.A.Pedersen; *P. jerdoniana* (Wight) Rolfe, *Rhynchostylis retusa* (L.) Bl., *Smithsonia straminea* C.J.Saldanha, and *Vanda testacea* (Lindl.) Reich.f. While, the terrestrial orchids were *Crepidium versicolor* (Lindl.) Sushil K. Singh, Agrawala & Jalal, *Habenaria crinifera* Lindl.; *H. diphylla* (Nimmo) Dalzell; *H. marginata* Colebr.; *Pecteilis gigantea* (Sm.) Raf., and *Peristylus plantagineus* (Lindl.) Lindl. All the orchid species collected during the present study were identified using flora of Goa (Abraham and Vatsala, 1981; Rao, 1986).

Peelings of fresh flower parts of selected orchid species were examined under microscope for the presence of raphides. A portion of the peel was stained in 1% aqueous solution of saffranin and mounted in glycerine. Slides were viewed under 10× and 40× magnification of light microscope. The size of the calcium oxalate crystals was measured under light microscope with stage and ocular micrometer. The photographs were taken with Samsung camera attached to the microscope under 40× and 10× magnifications.

Received: June 13, 2024; Accepted: August 26, 2024

Results and Discussion

The present study documented the presence of raphides in the floral parts of all 21 selected orchid species. These raphides appeared as bundles within idioblasts and were also dispersed on the surface of floral parts as needle-shaped crystals. The size of these needle-shaped crystals varied, ranging from 15 µm to 20 µm in *Smithsonia straminea* and from 60 µm to 90 µm in *Peristylus plantagineus* (Table 1; Fig. 1). In all the presently studied species, raphides were predominantly organized in parallel and regular cylindrical bundles. These observations are in line with the earlier findings of Cote (2009) and Raman *et al*. (2014).

The present study revealed sporadic occurrence of raphides in spur and lip in comparison to other tepal tissues. Similar studies was made earlier by Kowalkowska *et al.* (2015), who found that raphides in *Bulbophyllum wendlandianum* were concentrated mainly in tepal tissues. Similarly, Swiechzkowska and Kowalkowska (2015) reported that in *Epipogium aphyllum*, raphides were less frequent in the lip and spur as compared to other tepal tissues. Margoñska *et al*. (2021) found idioblasts with raphides in the petals and sepals of *Crepidium versicolor*, which supports the present findings. Additionally, Ren *et al*. (2020) noted that in *Coruna stylis*, raphides were typically confined to auricle lobes, varying in density amongst species and inflorescences, with dense raphide concentrations sometimes turning auricle apices black or brown. The role of calcium oxalate crystals observed in the floral parts varied depending on the plant type, organ, or tissue. These reflective raphides may attract potential insect pollinators by enhancing the visual appeal of the flowers (Franceschi and Horner, 1980; Franceschi and Nakata, 2005; Nakata, 2003). According to Margoñska *et al*., 2021, these raphides may also play a role in guiding insects towards the flowers. Further, these authors added that raphides may contribute to the release of volatile compounds from the cell walls, which, together with nectar-like droplets, may serve as olfactory cues to attract pollinators. Furthermore, the presence of raphides in orchids might indicate slime or mucilage production (Smith, 1923) and could be associated with

Table 1. Occurrence of raphides crystals in floral parts of wild orchids from the Western Ghats of Goa.

Species	Habit	Flowering period	Size of crystals (µm)
Acampe praemorsa (Roxb.) Blatt. & McCann	E	March-June	30-45
Aerides crispa Lindl.	E	May-June	27-60
A. maculosa Lindl.	E	May-June	45-60
Bulbophyllum sterile (Lam.) Suresh	E	December-January	45-60
Crepidium versicolor (Lindl.) Sushil K.Singh, Agrawala & Jalal	T	July-August	30-50
Cymbidium bicolor Lindl.	E	June-July	46-82
Dendrobium barbatulum Lindl.	E	April-May	35-45
D. macrostachyum Lindl.	E	May-June	60-75
D. ovatum (L.) Kranzl.	E	December-March	33-60
D. peguanum Lindl.	E	December-January	33-40
Habenaria crinifera Lindl.	T	August-September	36-48
H. diphylla (Nimmo) Dalzell	T	August-September	36-50
H. marginata Colebr.	T	September-November	36-73
Luisia zeylanica Lindl.	E	June-August	30-49
Pecteilis gigantea (Sm.) Raf.	T	September-October	36-82
Peristylus plantagineus (Lindl.) Lindl.	T	July-September	60-90
Porpax filiformis (Wight) Schuit., Y.P.Ng&H.A.Pedersen	E	July-September	36-45
P. jerdoniana (Wight) Rolfe	E	May-July	33-45
Rhynchostylis retusa (L.) Bl.	E	June-August	30-49
Smithsonia straminea C. J. Saldaha	E	June-August	15-20
Vanda testacea (Lindl.) Reich.f.	E	March-May	33-45

E, Epiphytic; T, Terrestrial.

floral secretions (Bogarin *et al.*, 2018). The varying quantities of calcium oxalate crystals in plant tissues also offer protection against herbivory (Chu *et al.*, 2014; Cote, 2009).

The present study offers valuable insights into the adaptive strategies of orchids in the Western Ghats and enhances our understanding of plant defence mechanisms. It also highlights potential areas for further

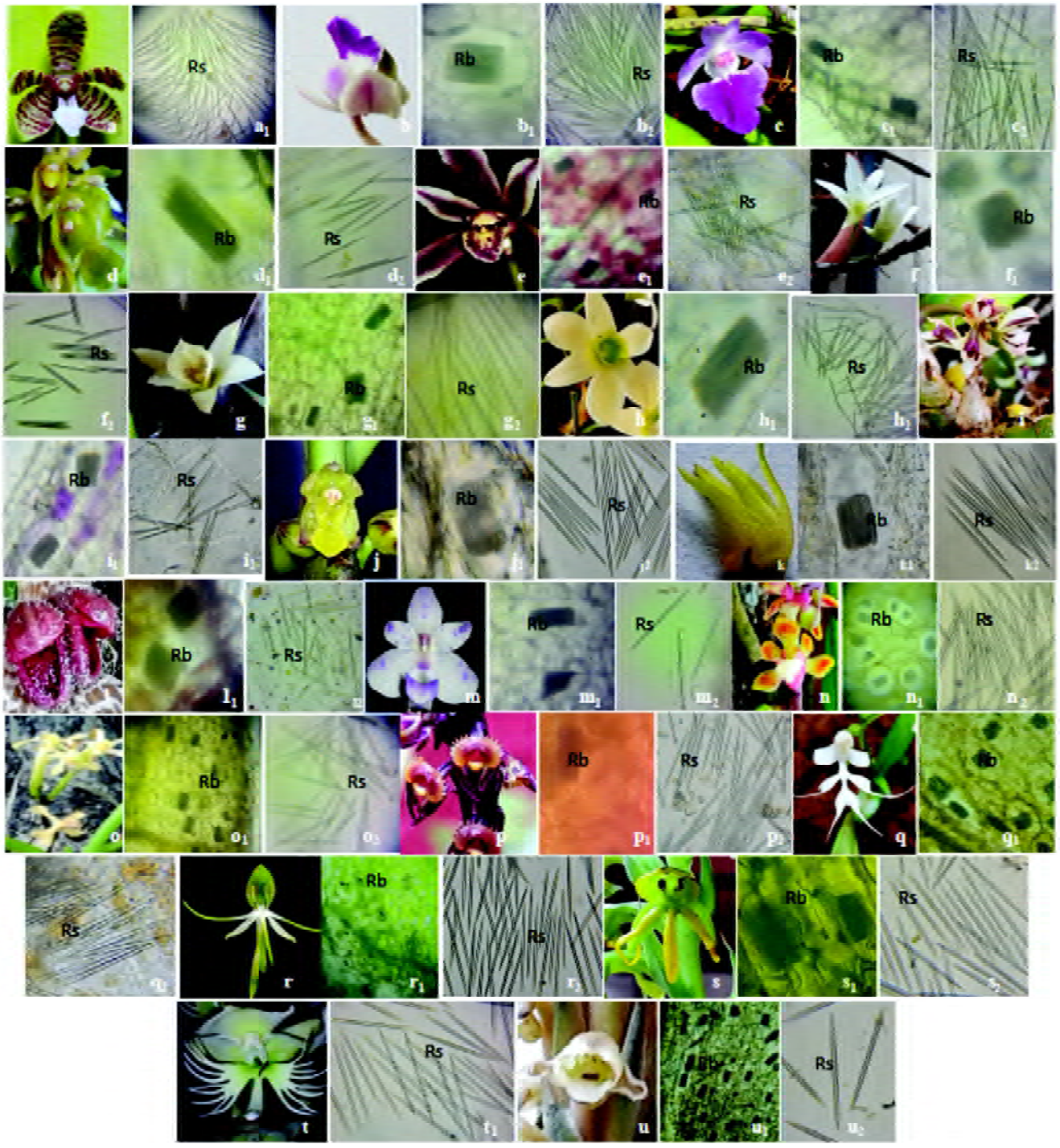

Fig. 1. Raphides in floral parts of wild orchids from Western Ghats of Goa: a-a_1, *Acampe praemorsa*; b-b_2, *Aerides crispa*; c-c_2, *A. maculosa*; d-d_2, *Bulbophyllum sterile*; e-e_2, *Cymbidium bicolor*; f-f_2, *Dendrobium barbatulum*; g-g_2, *D. macrostachyum*; h-h_2, *D. ovatum*; i-i_2, *D. peguanum*; j-j_2, *Luisia zeylanica*; k-k_2, *Porpax filiformis*; l-l_2, *P. jerdoniana*; m-m_2, *Rhynchostylis retusa*; n-n_2, *Smithsonia straminea*; o-o_2, *Vanda testacea*; p-p_2. *Crepidium versicolor*; q-q_2, *Habenaria crinifera*; r-r_2, *H. diphylla*; s-s_2, *H. marginata*; t-t_1, *Pecteilis gigantea*; u-u_2, *Peristylus plantagineus*. (R_b- Raphides in bundle; R_s- Scattered raphides).

research, such as investigating the role of raphides in orchid-pollinator interactions.

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