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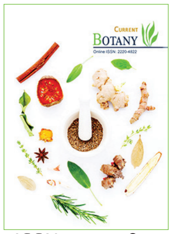

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A concise study on the floral biology of *Thevetia peruviana* morphovariants

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ABSTRACT

Thevetia peruviana, an ornamental medicinal plant of the family Apocynaceae blooms in different colour shades of yellow, orange and white, and is considered morphological replicas of its original parental type. A concise morpho-anatomical study has been carried out on the floral biology of these three morphovariants emphasizing biometrics of various floral parts, the architecture of corolla tube and coralline corona, adnation of anther stigmatic head complex and ovule to embryo formation, using the hand as well as microtome sections, to analyze the similarities and differences among these specimens. Appendiculate and smooth walled hairs in corolla tube, germination of pollen grains in a lateral stigmatic notch, appression of anther lobes on 10-lobed incompletely fused stigma, triaperturate pollen grains, ovule developmental stages, entry of pollen tube into ovule, attachment of funicle on placenta etc are some of the enchanting valuable scientific observations under light microscopy. Gross morphological variations noted in the overall size of various floral parts are regarded as negligible ones, which may happen due to environmental factors. Floral morphology alone is insufficient to give distinct varietal status in the hierarchy of classification, but corolla shades, of course, are of prime importance for phenotypic differentiation, and a multidisciplinary approach will help to expose untapped characters, useful for future studies below the rank at the subspecies level.

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INTRODUCTION

Many ornamental plants of Apocynaceae with attractive colour blossoms have been cultivated elsewhere since centuries back. One such variety *Thevetia peruviana*, has grown in Indian gardens as an ornamental since 1801 for its elegant shiny foliage, sweetly fragrant flowers, adaptability as a hedge, shrub or tree in dry sites, sandy beaches, and toughness to diseases and pathogens (Joshi, 2000; Pullaiah, 2006; Jacobson, 2014). Its bushy nature with dark green, linear, dense foliage; and bright coloured, large, showy flowers give them a unique beauty to the premises where it grows. The taxon blooms in yellow, orange (apricot or peach), and white colour shades (Everett, 1982; Knight, 2007; Odugbemi, 2008). The genus is distributed throughout Kerala, grown wild in some parts of the northern region; and in the rest of the state, planted at homes and near temple sites to worship Hindu idols. Studies regarding yellow flowered plants are frequent in scientific literature, while two other forms remain rather scarce, possibly considered as replicas with similar morphological characteristics. As a result, the three colour forms are usually regarded as morphoforms or morphovariants of *T. peruviana*, thus making their differentiation difficult at the binomial level.

Morphology has been traditionally the most important source of information in plant taxonomy; and still the majority of taxonomic groups recognized today are mainly from floral morphology (Sennblad *et al.*, 1998; Singh, 2010). The easily observable and obtainable morphological data provide the basic language for characterization, identification, classification and relationships (Radford, 1986). Even though, both vegetative and floral morphology provides the majority of characters for key identification, the latter is more reliable and practiced widely.

Floral biology is one of the thorough and extensively explored areas in various levels of classification, which include types of inflorescence and flower; perianth, bract, bracteole, pedicel characters, floral symmetry, cohesion and adhesion of floral parts, types of androecium, gynoecium, fruits and seeds (Sharma, 2009). Embryology, certainly has no claim of supremacy with regard to the ranking of taxa at different levels of hierarchy, but, it has been used to solve several disputes in generic and higher levels of classification (Stuessy, 2008). Many morphovariants below the species level, having only binomial names are considered as replicas of the original, and need to be evaluated at multidisciplinary levels for the systematic ranking in the hierarchy. Presently, very few attempts have been reported to tackle these complexities of infraspecific taxa. Hence an elaborated study has been carried out to understand the floral biology of *T. peruviana* yellow, orange and white variant flowers for elucidating the taxonomic relationship at the subspecies level.

MATERIALS AND METHODS

Materials

Fresh twigs of *Thevetia peruviana* were collected from different districts of Kerala that produce yellow (YFP), orange (OFP) and white blooms (WFP). The taxon (Figure 1) was identified with the help of various regional flora (Matthew, 1983; Cooke, 1905). The morphological identification was further confirmed with the help of keys, descriptions, illustrations and herbarium specimens. The voucher specimens were preserved in the Herbarium cabinet of the Botany Department, St. Teresa's College, Ernakulam for future reference.

Methods

Studies on floral characters were carried out systematically with flower buds of varying stages of development. Micro-preparations (hand sections as well as microtome sections) of anther, ovary, stigmatic head, and embryo were made (Prasad & Prasad, 1975) and images were captured using OLYMPUS MAGNA Trinocular Research Microscope (Germany) equipped with Nikon photographic unit. Physical measurements of various samples were done using graduated scales, graph paper imprints and vernier calipers.

RESULTS

Morpho-anatomy: Physical appearance and anatomical details are described separately.

Yellow Flowered Plant (YFP)

A large, evergreen shrub or a small tree, growing up to 12 - 15 ft in height, cultivated in gardens as an ornamental plant. Inflorescence: few flowered terminal cymes, 4 - 8 blooms of varying stages at a time, later turns extra axillary due to development of axillary buds; peduncle - light green, 1.2 - 1.4 cm long and narrow. Flower: pedicellate, bracteate, large, showy, bright yellow, sweetly fragrant, regular, bisexual, actinomorphic, pentamerous, gamopetalous, hypogynous; bracteole - persistent; pedicel - 2.4 - 2.5 cm long, narrow and light green. Calyx: sepals 5, unequal, free, glandular, deeply lobed, quincuncial imbricate, base broad, apex pointed, 0.8 - 1.05 cm long, persistent. Corolla: petals 5, gamopetalous, lobes sinistrally twisted, corolla tube narrow at the base, 1.4 - 1.5 cm long, broadened above as a throat (0.5 - 0.6 cm) and expanded into 5 lobes (4.5 - 4.8 cm), coronal outgrowths 5, with a tuft of hairs at the corolla throat, these 5 appendages give a star shaped appearance from above, corolla tube internally pubescent; two types of hairs, hairs on corona are longer, flexible, appendiculate, oriented horizontally; hairs in between the basal region of staminal ridges are straight, smooth, in 2 vertical rows on either side of each outgrowth, inwardly directed, smaller than the upper ones; corolla base green peripherally, colour extends along the margin of petals from base to tip (Figure 2).

Orange Flowered Plant (OFP)

Cultivated as an ornamental and less common in gardens. Inflorescence: terminal peduncled cyme, later turns axillary (sub-terminal) and leaf opposed, 4 - 8 flowered; peduncle - narrow, 1.2 - 1.5 cm long, pale green. Flower: bracteate, pedicellate, regular, bisexual, pentamerous, large, showy, peachy orange with mild fragrance, rarely non-fragrant, flower 8.0 - 8.6 cm long; pedicel - pale yellow and 1.8 - 2.2 cm long. Calyx: sepals 5, deeply lobed, glandular, acute, spreading, unequal, lanceolate, light green, 0.7 - 0.9 cm long, 0.3 - 0.4 cm broad base, quincuncial imbricate and persistent. Corolla: petals 5, gamopetalous, bell shaped, lobes obovate, basal tube cylindrical and narrow, 1.3 - 1.5 cm long, tube widened as a throat (0.4 - 0.5 cm), 5 sinistrally twisted and overlapping lobes above (4.4 - 4.7 cm), throat villous, 5 coronal appendages (corolline corona) present, densely pubescent coronary lobes above the throat opposing with stamens; hairs 2 types - transversely oriented appendiculate ones on coronary lobes, and numerous inwardly directed simple smooth hairs in two vertical rows on either side of projecting base of stamens, light green or purple tinge on the outer surface of corolla extending along the margins of each petal from base to tip.

White Flowered Plant (WFP)

Grown as an ornamental, less common in gardens. Inflorescence: terminal, or axillary, leaf opposed, peduncled, cyme many

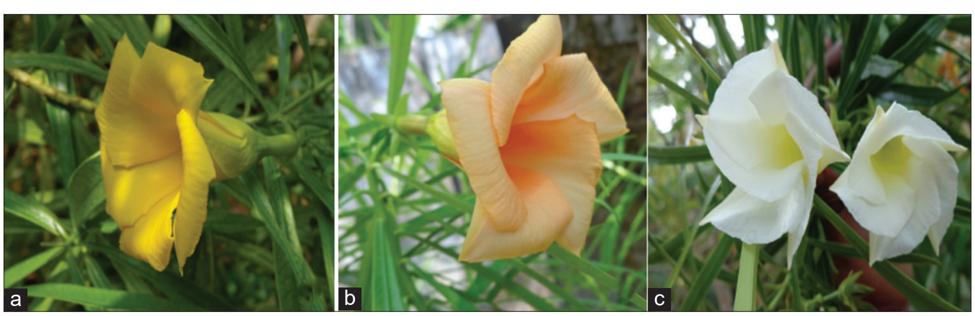

Figure 1: a) Yellow Flowered Plant (YFP) b) Orange Flowered Plant (OFP) c) White Flowered Plant (WFP)

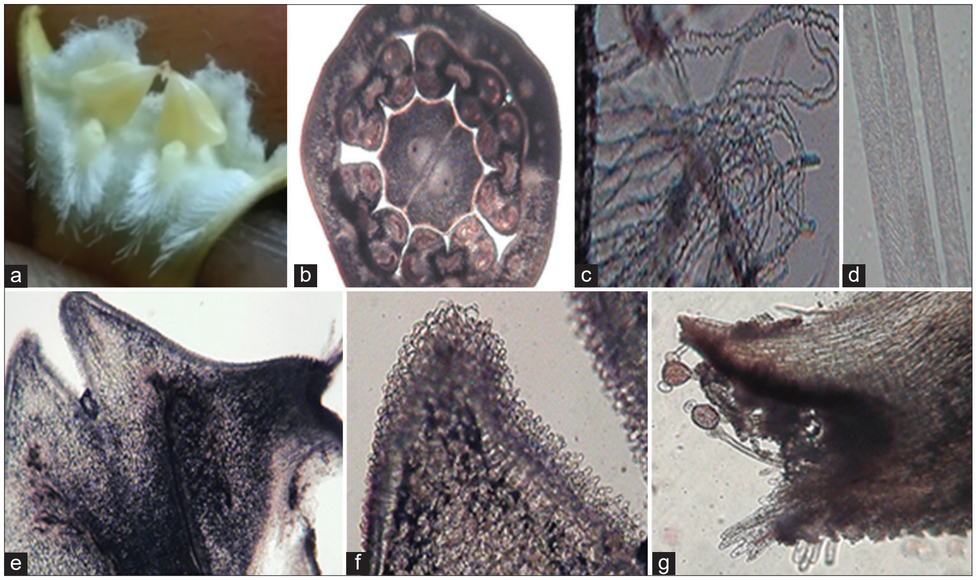

Figure 2: Flower anatomy: a) A portion of corolla split view showing corolline corona, two mature anthers connected by apical hood, broad anther base, downwardly directed hairs (YFP) b) C.S of flower passing through stigmatic region showing five epipetalous anthers with 10 anther lobes, each lobe appressed on 10-lobed and incompletely fused stigma (YFP-5X) c) Appendiculate hairs on corolline corona (OFP-10X) d) Smooth hairs on either side of anther base (OFP-40X) e) 2-cleft apical part and marginal receptive region of stigma (WFP-5X) f) Unicellular glands at sterile apical dome region (WFP-10X) g) Receptive stigmatic notch situated laterally, with germinating pollen grains and unicellular hairs (WFP-10X).

flowered (8 - 10); bracteole - green, persistent, 0.2 - 0.4 cm long; peduncle - light green, 1.5 - 2.0 cm. Flower: bracteate, pedicellate, regular, bisexual, actinomorphic, pentamerous, large, showy, non-fragrant, 8.7 - 9.1 cm long; pedicel - light green, 2.5 - 2.6 cm. Calyx: sepals 5, green, deeply lobed, glandular, acute, spreading, unequal, lanceolate, 0.7 - 0.9 cm long, 0.32 - 0.4 cm broad base, apex acute, persistent, light green, quincuncial. Corolla: petals 5, gamopetalous, bell shaped, lobes obovate, basal tube cylindrical, 1.5 - 1.6 cm long, proximal end slightly widened and pentagonal due to nectar disc, the inner region of corolla tube is silky smooth, tube widening above the middle into corolla throat (0.5 cm), corolla white, with an outer light green or purple shade starting from base to tip, densely pubescent corona lobes near the throat opposing the stamens, give a star shaped look from the periphery, 2 types of unicellular hairs directed transversely and downwardly on coronal appendages and near the connective base, upper expanded corolla lobes 4.2 - 4.9 cm long, sinistrally twisted.

Embryology

Embryological features of androecium and gynoecium were evaluated and represented in separate headings.

Androecium of YFP

Stamens 5, epipetalous, inserted at the corolla throat, opposite to petals and situated just below each lobe of densely pubescent corolline corona; filament - short and broad; anther 2-lobed, narrowly oblong, base broad, apex conical, each lobe is 2-loculed; connective - broad at the base, tapers above, prolonged beyond the lobes as an elongated leathery spur, longer than filament, all five extended spur united together in anticlockwise direction, breaks when the flower opens; stamens free from pistil head, but rest on its surface, anther bisporangiate, 4-loculed, locules longitudinally elongated; connective - massive, broad, parenchymatous, protruded into locule, so locules appeared 'C' shaped in cross section, vasculature in the center; epidermis - single layered, cells columnar; wall layers 3 - 4, stomium present, tapetum - uniseriate, glandular; microspore formation - in tetrads; dehiscence longitudinal and latrose, pollen grains - numerous, immature anther contains two types of pollen grains, majority normal, regular grains, and few smaller, polar view with three germ pores, equatorial view with transversely elongated ectoaperture (Figure 3).

Androecium of OFP

Stamens 5, small, oblong to cordate, mucronate, completely inserted; filaments - extremely short, nearly sub-sessile; lobes - 2, base broad, apex narrow, each lobe 2-loculed; connective - projects beyond the anther lobes as a short, membranous hairy appendage, all 5 projections jointed together and twisted in an anticlockwise direction in buds across the top of the pistil head, appressed, but not adnate to it; Anther bisporangiate, young anther 4-loculed, when mature it becomes 2-chambered, locules longitudinally elongated; connectives - broad, parenchymatous, protruded into the locules, so appeared 'C' shaped in cross section, vasculature in the center; epidermis - single layered, narrow, thin walled and radially elongated; 3 - 4 wall layers, stomium present, endothecium fibrous, tapetum - uniseriate, glandular; dehiscence - longitudinal, latrose; microspores - numerous tetrads and appear as monads; tricolpate, two types, larger ones abundant, smaller ones less frequent.

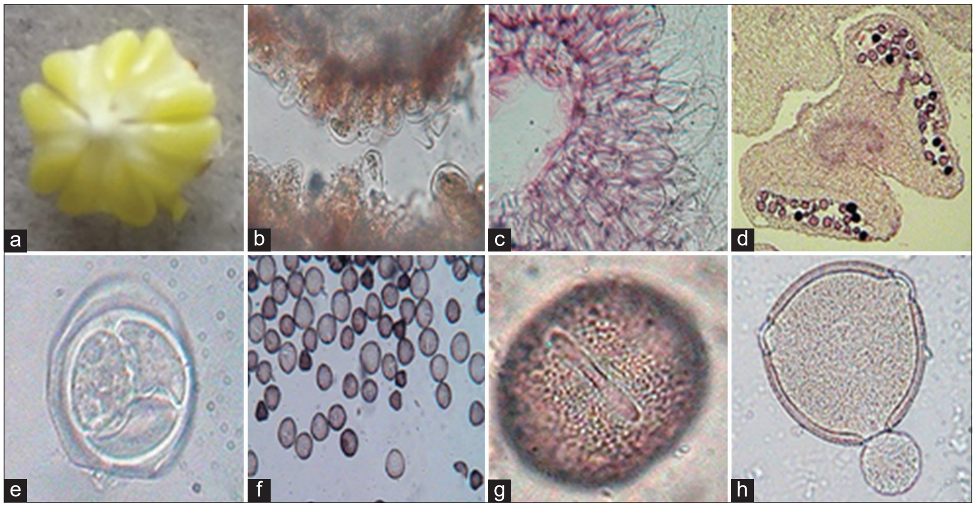

Figure 3: Embryology of anther YFP showing a) Androecium showing 5 stamens Joined together with apical hood b) Inner anther locule with glandular tapetum (40 X) c) inner wall of anther locule showing fibrous columnar endothecial cells (40 X) d) T.S of mature anther (10 X) e) microspore mother cell at tetrad stage (40 X) f) dimorphic grains during development (10 X) g) equatorial view showing transversely elongated ectoaperture and surface ornamentation under LM (60 X) h) polar view showing three germ pores (60 X).

Androecium of WFP

Stamens 5, completely inserted, epipetalous, 2-lobed; filaments - short; lobes - small, 2-loculed, oblong to cordate, mucronate; connective - broad below, narrow above, projects beyond the anther lobes and terminating into short membraneous appendages, united together and twisted in anti-clockwise direction in buds above the pistil head, all 5 anthers cohering as a cone around the stigma, appressed but not adnate to it; anther - bisporangiate, 4-loculed, connective - parenchymatous, broad and protruded into locule, locules 'C' shaped, vasculature in the center; epidermis - single layered; wall layers - multilayered (2 - 3), stomium present, tapetum – uniseriate, glandular; dehiscence - longitudinal; pollen grains - numerous, uniform and in monads, tricolpate.

Gynoecium of YFP

Bicarpellary, basal 3/4th part fused and upper 1/4th portion free; ovary - superior, each carpel is 2-chambered, one ovule in each chamber, hemianatropous ovules with axile placentation; style - slender, dilated near the apex and the base, completely fused in middle part; stigma - massively thickened and bilobed, level with anthers, dome shaped, apex shortly 2-cleft with numerous single celled glands, middle region contracted, receptive surface 10-lobed and laterally placed with a ring of unicellular hairs; nectar disc - prominent, 5-lobed, light yellow, encircle ovary at the base. At midday, the receptive stigmatic marginal cleft was observed with numerous germinating pollen grains, having three activated germ pores with pollen tubes of varying lengths. Bicarpellary ovary 2-chambered initially, placenta enlarged and become 4-chambered during development, single ovule on each locule, funicle attached to the placenta on axial position; integument - unitegmic; chalaza broad, archesporial cells are deeply seated, ovules hemianatropous (Figure 4).

Gynoecium of OFP

Bicarpellary, partially syncarpous, superior; nectar disc - 5-lobed, encircles the ovary, pale yellow; ovary - pale green, 4-chambered due to the enlargement of the placenta, single hemianatropous ovule in each locule, placentation axile; style - narrow and filiform, 1.3 - 1.4 cm long, with a jointed appearance, longitudinally free just below the stigmatic head and above the ovary; stigma - large, broad and massive, apex conical and 2-cleft with numerous unicellular glands, receptive surface directed sideways, 10-lobed, intermingled with numerous unicellular hairs, 10 anther lobes appressed to 10 lobes of the stigmatic head in bud condition; 2 ovules in each carpel, placenta enlarged to false septa, so 4-chambered, ovule attached to the placenta by means of funicle, the position of funicle is midway in the chamber, placentation axile, ovules hemianatropous, single per chamber; integument - unitegmic.

Gynoecium of WFP

Bicarpellary, partially syncarpous, distal 1/4th portion of ovary free; nectar disc - around the ovary with 5 prominent lobes, each lobe again 2 - 3 divided; ovary - pale green, 4-chambered, single hemianatropous ovule inside each locule with axile placentation; style - smooth, narrow, 1.3 - 1.4 cm long, with a jointed appearance, basal region incompletely fused, fusion complete in the middle part, upper portion bifurcated just below the style head; stigma - large, broad and massive, apex dome shaped and 2-cleft, with numerous unicellular glands, margin 10-lobed, receptive surface marginal, with ridges, furrows and numerous small unicellular hairs. Flowers at noon were observed with germinating pollen grains carrying > 50 μm long pollen tube; ovary with 2 ovules in each carpel; ovule - hemianatropous, placentation axile; integument - unitegmic; embryo sac deep seated.

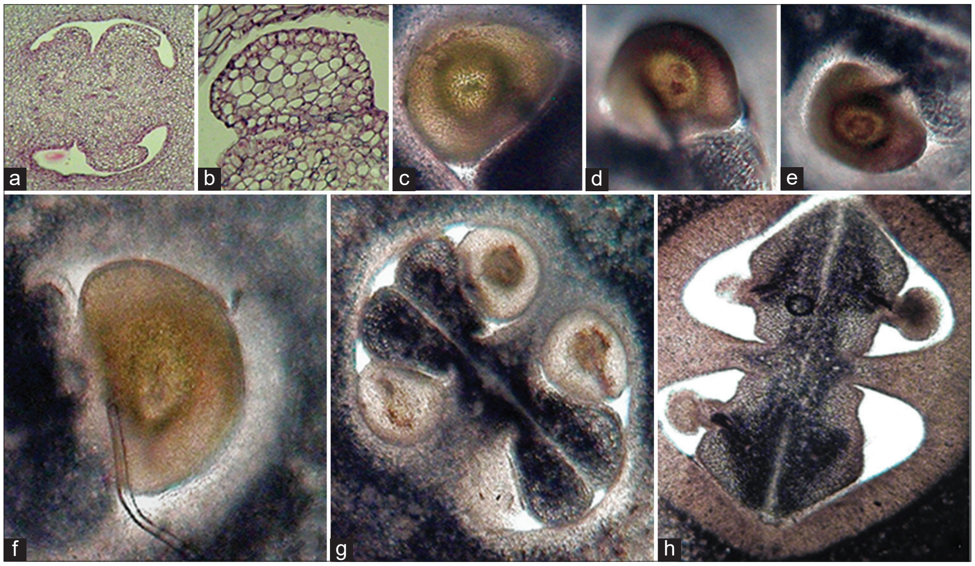

Figure 4: Embryology of ovary: Cross section of ovary (YFP) showing a) three developing ovules (5X) b) outer integument and inner archesporial cells (10X) c-e) nuclear divisions during different stages of ovule development (40X) f) mature ovule with an approaching pollen tube (60X) g) 2-chambered ovary with one ovule in each chamber on massive placenta (10X) h) developing and abortive embryos, position of attachment of funicle to the placenta (10X).

DISCUSSION

The Genus *Thevetia* was placed under the 'dogbane family' after its discovery, as it possesses all major distinguishing features of Apocynaceae. The present study focuses on the biosystematics' analyses of three plants with similar morphological features, for their resemblances and differences. Vegetatively, all three specimens are identical with simple, alternate, dorsiventral lanceolate leaves. The internodes are very short, so inflorescence appears extra-axillary, born in the axils of sub-sessile leaves.

Thorough investigations have been carried out regarding the morphology of yellow flowers (Subrahmanyan, 1995; Sambamurthy, 2005; Sharma, 2009; Gupta, 2012), but the reports of the other two related specimens are found limited. The present study revealed that the floral anatomy of yellow flowers is identical to the orange and white flowers, and showed nearly 100 % resemblances in the majority of characters. The specimens are bicarpellate, bisexual, hypogynous, actinomorphic and gamopetalous with epipetalous stamens, features similar to the other 21 genera of the family Apocynaceae studied by Sennblad *et al.* (1998). Apart from the regular descriptions of distinguishing features, thorough systematics of yellow flowers were provided by many taxonomists during their classification and cladistic analysis (Endress *et al.*, 1996, 2007, 2014; Simoes *et al.*, 2007).

Usually the flowers are blessed with fragrance, but *Thevetia* flowers vary differently in having this amazing peculiarity. The yellow flowers have a serene, sweet scent, but the orange flowers with a lesser intensity. Another variant, the white flowers grown in the same environmental conditions had no fragrance at all, which may be considered as an insignificant taxonomic variation.

The most important character to be discussed is the structure and adnation of essential whorls rather than the non-essential calyx and corolla. The main part of the proper corolla tube is post-genitally fused, which starts at an adnation point of two adjacent petals, some distance away from the base of the petals, and then proceeds basipetally. Using the broad anther lobe, the staminal unit adnate to the angular style head, and the prolonged connectives joined together post-genitally in an anticlockwise direction above the stigmatic head, up to the phase of a flower opening. Just above each anther, 5 large coronary lobes protruding into the center with numerous coarse white marginal hairs, created 5 openings above the style head, through which pollinators could enter. Because of the latrose dehiscence and lobes of the same anther were separated by broad connectives, pollen from adjacent anther lobes mixed and appeared as 5 masses, with a foamy adhesive (Simoes *et al.*, 2007).

Even though, the gynoecium of most taxa of Apocynaceae are usually referred as apocarpous (Sennblad *et al.*, 1998), the two carpels often congenitally fuse for about 3/4th distance from the base, and the upper part of the ovary remains apocarpous. The post-genital fusion of two parallel styles and the style head also remains incomplete. The style-head is the enlarged specialized product of the apical part of the carpel, with zones covered by secretory epithelium (Fallen, 1986); and the body of the style-head with epidermis clearly differentiated into distinct regions specialized in the production of pollen transport adhesive or for receptive function (Simoes *et al.*, 2007). In all three flowers, both style ends are incompletely fused; and the uppermost parts of the stigmatic head remain free giving the appearance of a cleft at its distal end. This sterile apical part is conical with its two lobes parallel and independent, not touching each other, and all the stigmata cells uniformly arranged on it.

The pistil head is 10-lobed and the receptive surface is directed obliquely downwards from the margin, with a ring of unicellular elongated hairs distributed regularly in the upper part of clefts, in small tufts. The stigmatic area is located below the region of anther adnation and is sometimes situated in a 'stigmatic hollow', an annular invagination of the style head; the resulting collar, equipped with a lower wreath of hairs, functions as a 'pollen scraper' (Fallen, 1986). But, Schick (1982) demonstrated that the receptive region of the style-head is typically at the base, often beneath a membranous collar or wreath of longer hairs. The present study clearly demonstrated that the receptive surface is located marginally, within the 2-cleft region.

The infrastaminal appendages are post-genitally fused to the style head according to Endress *et al.* (1996), which is identical in all studied flowers. Infrastaminal and suprastaminal appendages are the outgrowths of the lower corolla tube below and above the staminal sector, which are homologous to the corolline corona (Nilsson *et al.*, 1993). At the base of the gynoecium, nectar lobes are sometimes fused into an annulus encircling the ovary.

In the studied samples, the stigmatic complex and related characters are identical, except for a slight variation in the longitudinal fusion of two styles at its proximal and distal ends. The stigmatic head complex has prime significance because it is considered as one of the most important criteria in the Apocynaceae-Asclepiadaceae demarcation (Endress & Bruyns, 2000). Apart from the above discussed discipline, embryological features of both anther and pistil are useful to tackle many disputed situations, but only a few contributions to the family are available, and even today related studies remains scanty. In the early 19th century, noteworthy contributions in this field were made on various family members (Meyer, 1938; Rau, 1940).

The major resemblances noticed during embryological studies are tetrasporangiate anther with broad connective, single layered glandular tapetum, tetrahedral pollen arrangement, few disintegrating microspores and appearance of mature grains in monads. Among the pistil characters, the most important structures are: the 4-loculed ovary with a single ovule per locule, the axile position of the hemianatropous ovule with unitegmic integument. No differences were observed among the studied samples, both in the structure of mature anther and ovary. A related study was quoted among the cultivars of *Nerium indicum* Mull. with few embryological differences (Devi & Narayana, 1975).

In several genera of Apocynaceae *viz T. neriifolia* Juss., *Alstonia scholaris* R. Br., *Cerbera odollam*, *Trachelospermum fragrance* Hook. f., uniseriate parietal tapetum has been reported (Meyer, 1938; Rau, 1940; Sud, 1984). Orbicules, a general character of Apocynaceae, located in the inner layer of anther locules were small, irregular, angular and folded in *Thevetia bicornuta* Mull. Arg., revealed in SEM analysis (Vinckier & Smets, 2002). Studies reported by Cousin (1979) in *Vinca rosea* L. emphasized that the tapetal cells are of secretary type and are bounded by an acetolysis-resistant 'pellicule' sprinkled with ubisch bodies. The observed degeneration of a smaller number of undersized microspores, was presumably due to the failure of the secretory tapetum to provide full nourishment to all the developing spores in the sporangium. In most Apocynacean members, the tapetum observed is glandular type (Sud, 1984).

In *T. peruviana*, according to Indian taxonomists (Subrahmanyan, 1995; Verma, 2011), the ovules were axile in position; but the development of funicles on the parietal position was reported by Endress *et al.* (1996). Presently, the funicle is attached to the placenta on the midway of the extended false partition, and at the right angle to the placental axis, clearly visible in cross sections. This type of hemianatropous and unitegmic ovules was present in *Trachelospermum fragrans* Hook. According to Sud (1984), the chalazal megaspore develops into an 8-nucleated embryo sac of polygonum type, a usual character shown by the family members.

In an extensive study of microsporogenesis of *Rauwolfia serpentina* (L.) Benth ex Kurz., Ghimire *et al.* (2011) noticed tetrasporangiate anther, dicotyledonous type of anther wall formation, the occurrence of both successive and simultaneous cytokinesis during the meiosis of microspore mother cells, uninucleate and highly vacuolated glandular tapetum, tetragonal and decussate pollen tetrad and three-celled mature pollen grain during shedding time. Even though morphologically plants are similar, marked variations were observed in pollen aperture characters and ornamentation (Nesy & Mathew, 2020). Hence, multidisciplinary analyses will expose untapped characters useful for systematic hierarchy and revision of upcoming classification to include functional trinomials for specimen differentiation.

CONCLUSION

Micro-morphology of floral parts were found identical for all three studied colour variants. Minor quantitative variations scaled on flower characters were considered as negligible ones, usually occurring in the natural flora even in the same geographical, environmental and nutritional grounds. These scientific contributions helped to expose some hidden natural phenomena occurring during pollination and fertilization which will be an added advantage to the subspecies level of plant classification.

CONFLICT OF INTEREST

Authors declare that we have no conflict of interest

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