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Studies on reproductive organs and morpho-anatomical features of leaf and stem in three traits of *Vinca rosea* L.

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Periwinkle or *Vinca rosea* L. has medicinal importance with three phenotypes i.e., Pink, Red eye and White. The present investigation distinguishes the three traits i.e., white, pink and red-eyed flower-bearing traits of *V. rosea* based on morphology, palynology, pollen grain germination and study of anatomical features of leaf, stem and variations in parts of the flower. The results revealed that significant differences were recorded in stomatal frequency, stomatal index, size of the petals, pollen viability and pollen germination. The highest per cent viability appears in pink flower-bearing plants (98.8%) followed by red-eyed flower-bearing plants (34.3%) and White flower-bearing plants (34%). Similarly, the percentage germination of pollen grains is high in pink flower-bearing plants (98.1%) followed by red-eyed flower-bearing plants (33.3%) and white flower-bearing plants (33.3%).

Keywords: Anatomy, Germination, Morphology, Red eye, Viability.

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Introduction

Vinca rosea L. syn. *Catharanthus roseus* (L.) Don., commonly called periwinkle flower is an evergreen, perennial herbaceous plant and native of Madagascar. This species belongs to the Magnoliophyta division, under the class of Magnoliopsida, Gentianales order, Apocynaceae family. The sap of most parts of the *V. rosea* is milky latex which is important and useful for medicinal uses. Flory[1] recognized three phenotypes which from his descriptions and his mention of forms in the "light pink range' i.e., pink, red-eye and white. The characters of the various colour forms are summarized by Wanscher[2], who described the flower colour phenotypes of this species. The bloom of a natural wild plant is pale pink with a purple eye in the centre, but the horticulturist has developed over a hundred varieties with colour ranging from white to pink to purple[3]. In Periwinkle flower colours, the most commonly observed are pink, white corolla with red-eye and white[1]. The author attributed these three corolla colours to the epistatic interaction of two genes R and W, with the R_W_ genotype being pink, R_ww being red-eyed and rrW_ and rrww being white-flowered.

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Pollen characteristics have been used considerably in the taxonomy of Angiosperms[4]. The first successful attempt at using pollen characters in the classification of plants was made by Prasad[5] and other workers contributed in the study of pollen architecture in tracing phylogeny in *Vinca* sp.[6]. Erdtman[7] proposed NPC system pollen/ spore classification based on apertures, their number and position. According to him, the spores are ditreme or monotreme in *Vinca* species.

Periwinkle is grown commercially for its medicinal uses in Australia, Africa, India, and Southern Europe. It has medicinal value owing to the presence of alkaloids like ajmalicine, serpentine and reserpine, which are well known for their hypotensive and antispasmodic properties. The anti-cancer principles namely vincristine and vinblastine are also produced from periwinkle. The root bark contains the alkaloid alstonine which has been used traditionally for its calming effect and its ability to reduce blood pressure[8-10]. In India, people used to squeeze the fresh juices out of the leaves to treat wasp stings. Leaves produce more than 70 different alkaloids[11].

The present investigation distinguishes the three floral phenotypes of *V. rosea* in their morphology, anatomy and percentage of pollen germination. Estimation of pollen viability is important for the analysis of gene flow in plants because it may be

useful in taxonomic, ecological, genetic and palynological studies. The aim of the present study to evaluate the pollen grain viability of three traits. The importance of the present study was analyzing the differences in percentage pollen germination in three traits of *V. rosea* to overcome the problems facing in the basic biological laboratory.

Materials and Methods

Plant collection

Specimens of three floral phenotypes of *V. rosea* (White, pink and red-eyed) were sampled in Mysore district and within the city limits of Mysuru. The material for present investigation was collected from the footrest of Chamundi hill, Mysuru and JSS College campus, Saraswathipuram, Mysuru. The collected plant materials were identified by M. M. Swamy, HOD, Department of Botany, JSS College for Women, Saraswathipuram, Mysuru, Karnataka.

Sections of stem and leaf

Ten fine hand sections of leaves and stems from the three phenotypes were taken. Then the sections were stained with safranin and mounted in glycerin. For sectioning, every time fresh material was taken.

Pollen mounting and measurement

Pollen grains from fresh anthers of pink, white and red-eyed traits were collected for further studying the morphology of the pollen grains. Pollen grains from 4-5 different flowers of the same traits were collected. In each case, the measurement of the largest equatorial diameter of pollen grains was taken. Photograph of pollen grains was taken with the help of a camera (Model, Nikon, Coolpix (12.0 Megapixels) S4000, wide 4X Zoom) and microscope under 40 X (Labomed, Model CXL MONO, SL. No. 110438371).

Estimation of pollen viability and germination

The viability was determined directly by the difference in size and by staining capacity of pollen grains using acetocarmine, acetoorcein and safranin. Darker and larger size pollen was considered viable. Six slides per plant were evaluated, two for each stain, using a light microscope (40X × 10X = 400X) and 300 pollen grains were analyzed for each slide. Erdtman[12] procedure followed for pollen mounting. Results were expressed as a per cent of viable grains present in each trait and percentage of pollen germination was evaluated according to the procedure provided by Zeng-Yu, *et al.*[13].

Measurement of leaf length, leaf surface area and stomatal index

Radar shape of leaf characters (Leaf length, leaf width, leaf apex and leaf base angle) in studied plant species was carried out using Microsoft excel (2010). For stomatal index (SI) calculation standard technique was employed i.e., scraping method[14]. The lamina size is determined by measuring the area of the leaf. The standard chart shows the ranges of areas for the different leaf classes[15]. Epidermal peels were taken from leaves of three plants of each trait to study stomatal organization in all the three traits. Mean values of 10 observations showing the size of the leaves, stomatal frequency, SI, size of the petals were taken.

Results

Morphological analysis

Morphological variations between white flower, pink flower and red-eyed flower of *Vinca rosea* (Fig. 1). Leaf length and width of red-eyed flowering plants were observed to be 6.3 and 2.9 cm, respectively and leaf area 18.27 cm^2. The leaf is microphyllous. The base angle of red-eyed flowering plants (87.5°) was an acute and apex angle (105°) was an obtuse type. The leaf length and width of pink flowering plants were 5.8 and 2.5 cm, respectively and leaf area 14.50 cm^2. The base angle of pink flowering plants (93.2°) was obtuse and apex angle (113.7°) was of obtuse type. Leaf length and width of white flowering plants shows 5.5 and 2.7 cm, respectively and leaf area 14.85 cm^2. Hence, the type of leaf was microphyllous. The base angle of white flowering plants (98.7°) is obtuse and apex angle (95°) was an obtuse type. Mean values of 10 observations showing the size of the leaves, stomatal frequency, SI, size of the petals in each trait were taken and projected in Table 1. In the stem section of *V. rosea*, find out the presence of cell inclusions (Fig. 2) it is not present in leaf parts in all the three traits. Radar shape of leaf characters (Leaf length, leaf width, leaf apex and Leaf base angle) studied in three traits revealed that there is a slight difference found between them (Fig. 3). The petal size (L × B) was measured, it showed that the length of the petal varied from 2 to 2.5 cm in white and pink flower-bearing plants and it varied from 1.5 to 1.8 cm in red-eyed flower-bearing plants. The width of petals varied from

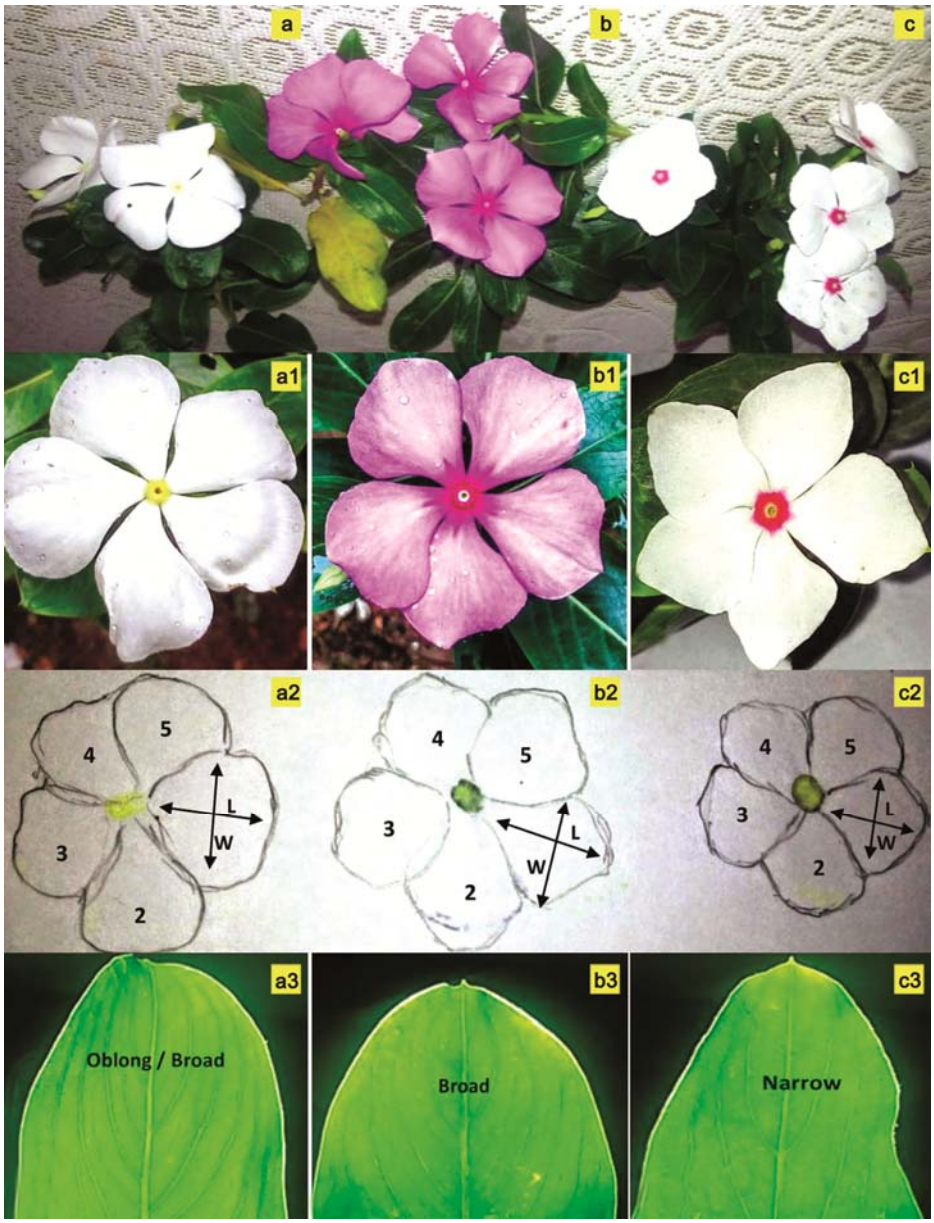

Fig. 1 — Morphological variations between three traits of *Vinca rosea* (a – white flower trait, b – pink flower trait, and c – red-eyed flower trait) a, b, and c – flowering plants, a1, b1 and c1 – frontal view of the petals, a2, b2, and c2 – the measurement of petals by tracing technique and a3, b3 and c3 – leaf apex morphology.

Table 1 — Calculation of stomatal index (SI) in all the three traits of *Vinca rosea*

Sl. No.	No. of epidermal cell			No. of subsidiary cell			Stomatal index (SI)		
	REF	PF	WF	REF	PF	WF	REF	PF	WF
01	108	78	75	36	26	25	69.33	59.33	58.33
02	134	99	95	38	28	27	66.35	56.28	55.42
03	160	120	116	40	30	29	65	55	54
Avg.		-			-		66.89	56.87	55.91

Note: REF – Red-eyed flower, PF – Pink flower, WF – White flower

2 to 2.3 in pink and white flower-bearing plants and red-eyed flower-bearing plants varied from 1.5 to 1.8 cm. Whereas, the size of the corolla tube remained constant in all the three traits of *V. rosea* (2.5 cm).

Morphological characteristics of pollen grains

Morphological features of pollen grains were analyzed (Fig. 4). From the microscopic observation, it is confirmed that the presence of intact pollen (no breakage of exine and membrane not disrupted) and broken pollen (breakage of exine and membrane

disrupted). The parameters used for the characterization were the diameter of pollen grains and the number of spores. The measurements of pollen grains showed variations from species to species in *Vinca*.

Pollen units

In all three traits, the pollen grains do not remain united after microsporogenesis at maturity. The traits present single pollen grains called a monad.

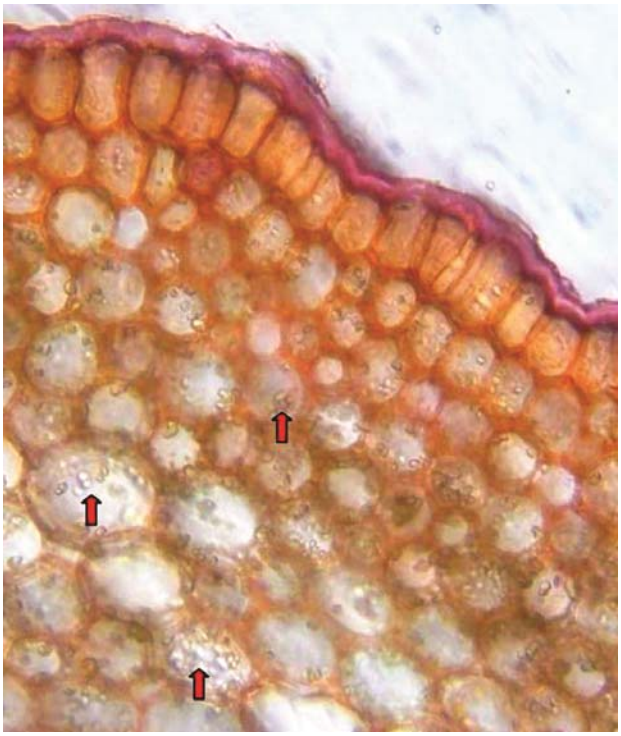

Fig. 2 — Arrows shows presence of cell inclusions occur only in stem regions of all the three traits.

Polarity

In the present investigation, the collected plant pollen grains are monad type so polarity not applicable.

Symmetry

The pollen grains are in the radiosymmetric condition in all three traits.

Shape

In both polar view (PA) and equatorial view (ED), the shape of pollen grains in all the three traits is circular.

Size

According to the data, the smallest microspore was 5 × 2.4 µm in diameter and the largest spores may be (around 200 µm in diameter). It is taken by polar axis and equatorial diameter by micrometry method. Measurement of pollen grains under 10 X reveals that red-eyed, pink, and white flower pollen grains ranged from 250-280, 330-350, and 320-330 µ, respectively.

Aperture

Either monopolar or bipolar.

Percentage of pollen grains germination

Percentage of pollen germination and viability comparison between three traits of *V. rosea* were recorded. The results revealed that the highest per cent viability appeared in pink flower-bearing plants (98.8%) followed by red-eyed flower-bearing plants (34.3%) and white flower-bearing plants (34%). Similarly, the percentage germination of pollen grains is high in pink flower-bearing plants (98.1%) followed by red-eyed flower-bearing plants (33.3%)

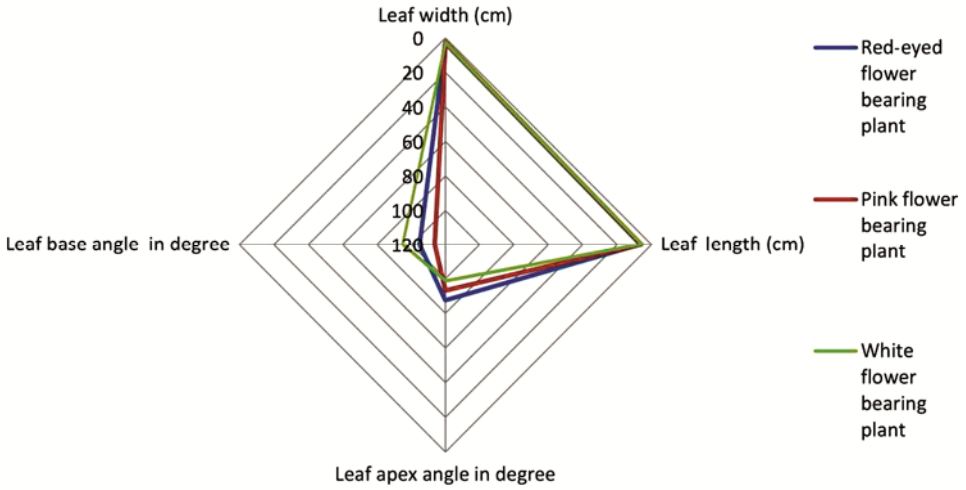

Fig. 3 — Radar shape of leaf characters (Leaf length, leaf width, leaf apex, and leaf base angle) in three traits of *Vinca rosea*.

and white flower-bearing plants (33.3%), observations were taken after 25 min time interval and the final observation was recorded after 55 min (Table 2).

Anatomical features of leaf, stem, and flower

In the present study anatomy of the leaf, stem and flower (Gynoecium) were taken to observe the features. The anatomy of leaf in Fig. 5 and anatomy of the stem in Fig. 6 showed no considerable difference in their anatomy except with slight variation in midrib shape but this feature also occurs within the species. The same results were recorded in the transverse section of the ovary and it is illustrated in Fig. 7.

Discussion

Madagascar periwinkle is a popular ornamental plant found in gardens and the surrounding area of homes across the warmer parts of the world. Their discovery led to one of the most important medical

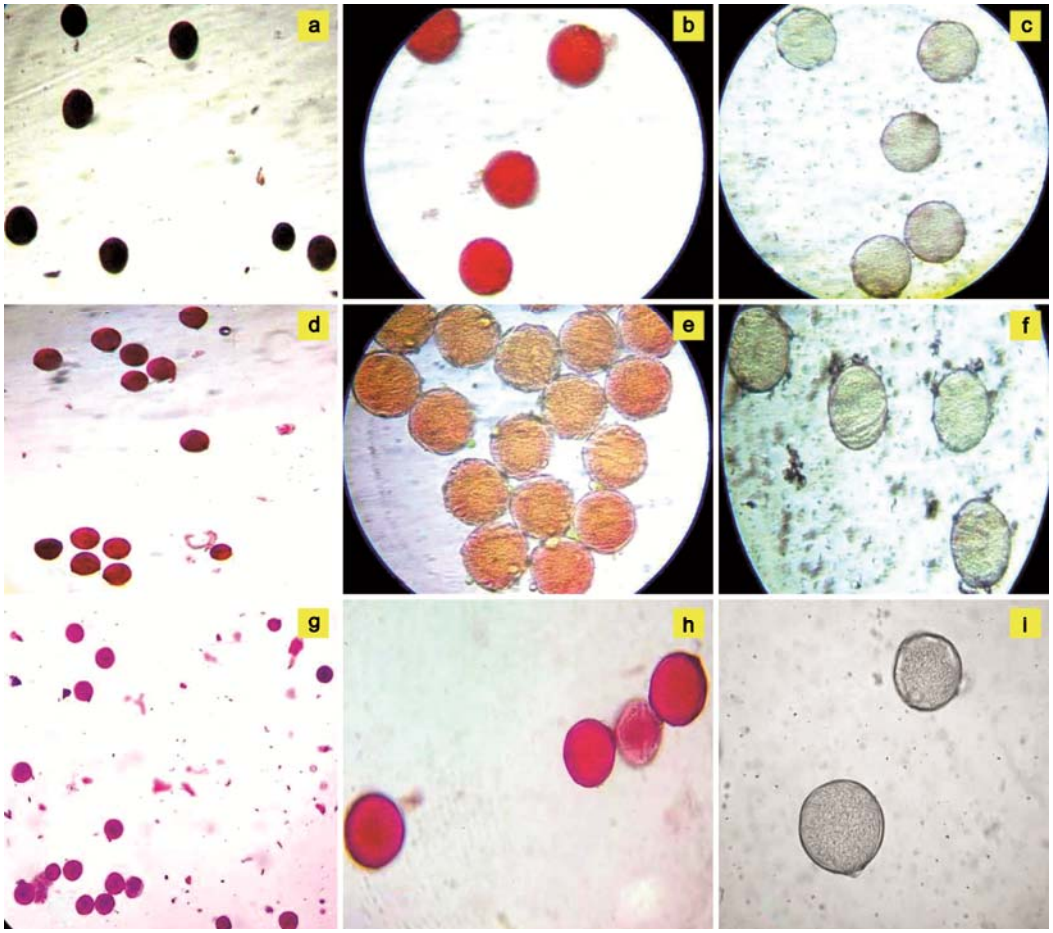

Fig. 4 — Pollen grains of three traits of *Vinca rosea*. a-c, pollen of red-eyed traits stained with acetocarmine and aceto-orcein. d-f, pollen of pink flower traits stained with acetocarmine and aceto-orcein. g-i, pollen of white flower traits stained with acetocarmine and aceto-orcein.

Table 2 — Percentage pollen germination and viability comparison between three traits of *Vinca rosea* along with the control

	% Germination												Control	% Viability		
	REF				PF				WF				REF/PF/WF	REF	PF	WF
	25 m	35 m	45 m	55 m	25 m	35 m	45 m	55 m	25 m	35 m	45 m	55 m	NIL	34	99	33.5
	NIL	NIL	21	22	87	93	97	98	NIL	NIL	22	23	NIL	35.5	98.5	34.5
	NIL	NIL	17	20	89	93.5	98	98.5	NIL	NIL	20	21	NIL	33.5	99	34
	NIL	NIL	53	58	91	96	98.5	98	NIL	NIL	48	56	NIL	34.3	98.8	34
Avg.	NIL	NIL	30.3	33.3	89	94.1	97.8	98.1	NIL	NIL	30	33.3	NIL	34	99	33.5

Note: 'NIL' – Represent No Germination of pollen grains; REF – Red-eyed flower, PF – Pink flower, WF – White flower

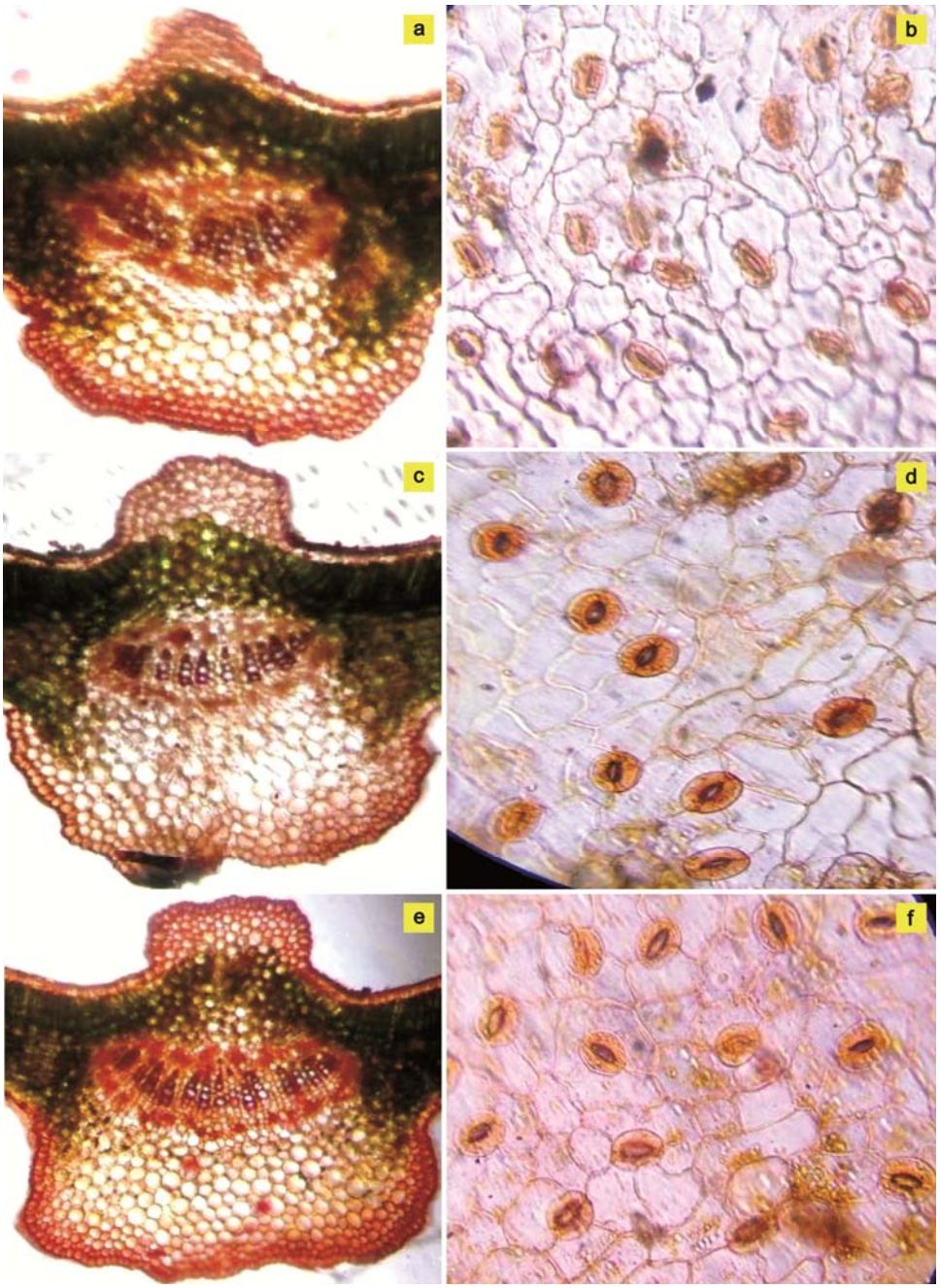

Fig. 5 — Anatomy of leaf in all the three traits of *Vinca rosea*. a1-a2: leaf T.S. of the red-eyed flower-bearing plant (10X) and stomata (40X), b1-b2: leaf T.S. of the pink flower-bearing plant (10X) and stomata (40X), c1-c2: leaf T.S. of white flower-bearing plant (10X) and stomata (40X).

breakthroughs of the 20th century. The flowers are adapted to pollination by the long-tongued insect, such as moth or butterfly. This species is also able to self-pollinate. Its seeds have been seen to be distributed by ants[16]. Based on flower colour, three varieties are known namely – *alba* with white petals, *ocillata* with carmine red eyes, and *roseus* with rose coloured flowers[17]. The commonly found flower colours in *V. rosea* are pink, white, red-eyed and pale pink centre is reported and is governed by the epistatic interaction between four genes namely, A, R, W and I[17]. Hence, in the present work, three traits were selected. Studies done on two populations of *Dianthus carthusianorum* by Wojcik *et al.*[18] were analyzed with respect to their morphological and physiological traits as well as genetic markers.

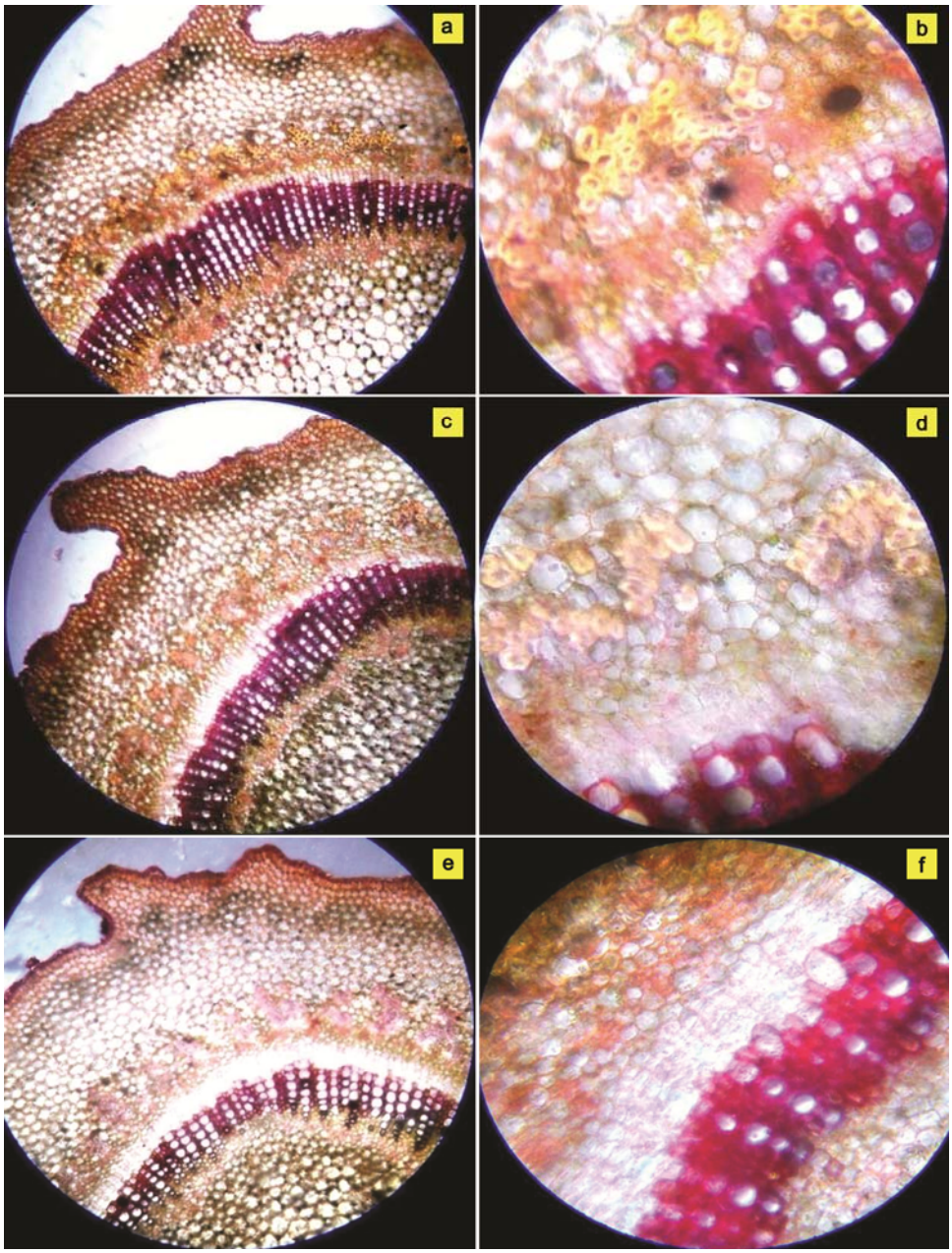

Fig. 6 — Anatomy of the stem in all the three traits of *Vinca rosea*. a1-a2: Red-eyed flower-bearing plant 10X and 40X, b1-b2: pink flower-bearing plant 10X and 40X, c1-c2: white flower-bearing plant 10X and 40X.

Lewis[19] reported that genealogical differentiation of leaf dissection and size in European samples of *Geranium sanguine.* Paula[20] suggested that the acetic orcein and acetic carmine stains can overestimate the viability of the pollen grains because they stain both viable and non-viable pollen the same colour (red). The safranin stains distinction between viable and non-viable grains on the differential staining of the protoplasm. Based on the little literature information the present work was carried out to overcome the problems facing in the laboratory about pollen germination and viability test. The present work introduces novel ideas for differentiation of the traits in the same species of *Vinca* like using different types of stains, anatomical measurements, etc.

Ultrastructure and investigations with a scanning electron microscope of pollens of *Vinca* sp. were carried out by Cousin[21]. Comparative pollen morphological analysis and its systematic implications on three traits of European oak were

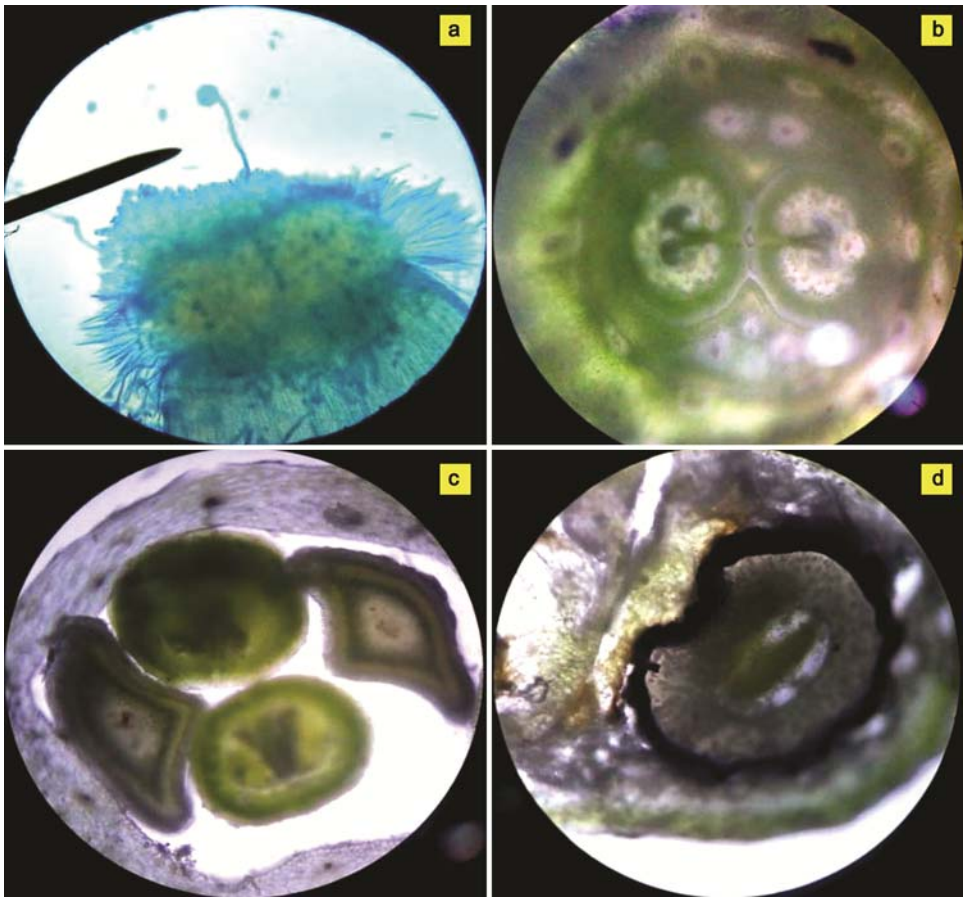

Fig. 7 — Anatomy of female reproductive organs in all the three traits of *Vinca rosea.* a – stigma region under 10X mounted in cotton blue lactophenol, b and c - T.S. of the ovary under 40X, d – T.S. of seed under 40X.

analyzed by Wrońska-Pilarek *et al.*[22]. Different stages of pollen development were examined with optical, scanning and transmission electron microscopy by El-Ghazaly[23]. The male fertility of crop plants is a function of pollen production and viability. This can be evaluated by simple observation, but pollen viability is more difficult to quantify under *in vitro*. The *in vitro* pollen germination assay for sorghum was developed by Tuinstra and Wedel[24]. In their experiments evaluating common germination media, large differences in germination were observed in response to changing concentrations of sucrose, boric acid, and calcium nitrate in agar-based media. However, in the present work, it revealed that the highest per cent viability appeared in pink flower-bearing plants. Red-eyed and White flower-bearing plants showed less per cent of viability. Similarly, the percentage germination of pollen grains is high in pink flower-bearing plants followed by red-eyed flower-bearing plants and White flower-bearing plants at different time intervals. The key feature of this work is that all the experiments were conducted by using basic biological laboratory equipment and hence the present work is not an advanced one.

Comparison between the anatomical and morphological structure of leaf blades and foliar regions in *Hirtella physophora* were studied by Leroy *et al.*[25]. Similar work carried out by Sultana[26]. The stem and leaf anatomical studies of *Euphorbia hirta* L. were conducted for finding identical traits by Sultana[26]. Three species of *Emilia* namely *E. coccinea*, *E. sonchifolia* and *E. preatamissa* Milne-Red head were investigated using their petiole, stem, flower stalk, midrib, and epidermal features[27]. Patil *et al.*[28], studied morpho-anatomy of leaf, stem, and root of *Boerhaavia diffusa* and its adulterant plants. The study focused on comparative SEM study of leaf morphologies and anatomy of leaf, stem, and root. However, in the present study anatomy of leaf, stem and flower (Gynoecium) were taken to observe the features but it showed that there are no noteworthy differences between them.

Conclusion

The present investigation gives a clear idea about distinguishes the three traits i.e., white, pink and red-eyed flower-bearing traits of *V. rosea* based on morphology, palynology, pollen grain germination and study of anatomical features of leaf, stem and variations in parts of the flower. The results revealed that significant difference was recorded in stomatal frequency, stomatal index, size of the petals, pollen viability and pollen germination. Majority of the *Vinca* sp. used in the laboratory not distinguished properly. The present investigation give a clear idea about the distinguish the *Vinca* sp. at laboratory level.

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Conflict of interest

The authors declare that there is no conflict of interest.

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