

Micromorphological Studies on *Tephrosia Purpurea* (L.) Pers. and *Tephrosia Villosa* (L.) Pers.

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Submitted: 25-11-2023

Accepted: 05-12-2023

ABSTRACT

Genus *Tephrosia* Pers. with c. 400 species is the largest genus in the tribe Millettieae (Fabaceae, Papilionoideae) (Mabberley, 2008) and one of the largest legume genera (Geesink 1984; Schrire 2005). It has a pantropical distribution, occurring mainly in seasonally dry tropical forests, savannas and campos rupestres (open rocky field) vegetation (Schrire, 2005). The species of *Tephrosia* are widely distributed in tropical, subtropical and arid regions of the world (Willis, 1973; Al-Zahrani, 2007). In this paper micromorphology of two species of *Tephrosia* viz. *T. purpurea* and *T. villosa* is studied. The details of trichomes and stomata are studied by using Labomed Lx-400 microscope. Micromorphological variations exhibited are important for the delimitation of the species.

Keywords: Micromorphology, *Tephrosia*, trichome, stomata.

I. INTRODUCTION

Genus *Tephrosia* Pers. with c. 400 species is the largest genus in the tribe Millettieae (Fabaceae, Papilionoideae) (Mabberley, 2008) and one of the largest legume genera (Geesink 1984; Schrire 2005). It has a pantropical distribution, occurring mainly in seasonally dry tropical forests, savannas and campos rupestres (open rocky field) vegetation (Schrire, 2005). The species of *Tephrosia* are widely distributed in tropical, subtropical and arid regions of the world (Willis, 1973; Al-Zahrani, 2007). The genus *Tephrosia* was first described by Persoon (1807), after that the most significant contributions to the taxonomy of *Tephrosia* have been made by De Candolle (1825), Bentham (1862), Baker (1876), Hassler (1919), Wood (1949) and Brummitt (1980).

Baker (1876) has divided genus into three subgenera as *Macronyx* characterized by annuals and simple leaves, second subgenus *Brissonia* characterized by shrubs with leaves imparipinnate, calyx teeth short, deltoid and third subgenus *Reineria* characterized by perennial herbs or shrubs, leaves simple or imparipinnate, calyx teeth

narrow, cuspidate as long as tube. The most recent infrageneric classification was proposed by Brummitt (1980), which based on the work of Wood (1949), Forbes (1948), Cronquist (1954) and Gillet (1958) divided the genus into two subgenera, *Tephrosia* subg. *Tephrosia* Pers. and *Tephrosia* subg. *Barbistyla* Brummitt. The diagnostic characters that supported this subgeneric classification were the presence or absence of indumentum along the style, and the presence or absence of trichomes at the base of the stigma.

Morphologically *Tephrosia* plants are prostrate or erect herbs or in the form of soft or woody shrubs (Hacker, 1990). Stipules caducous. Leaves imparipinnate; stipels absent; leaflet blades opposite, abaxially often sericeous, secondary veins to c. 30 on each side of midvein and closely parallel, margin entire. Pseudoracemes terminal or axillary, sometimes opposite a leaf; bracts usually caducous. Bracteoles absent. Corolla white, cream-colored, or mauve, occasionally orange or red; standard reflexed, suborbicular, outside villous or sericeous. Stamens monadelphous; vexillary stamen somewhat distinct from other 9. Ovary sessile, with trichomes, with numerous ovules. Legume flat, occasionally inflated, dehiscent, apex often beaked; valves twisted. Seeds 5-16 per legume, oblong, ellipsoid, or occasionally globose; radicle folded (Bosman & Haas, 1983).

This paper deals with micromorphological investigation of two species of genus *Tephrosia* Pers. viz. *Tephrosia purpurea* (L.) Pers. and *Tephrosia villosa* (L.) Pers. Details of epidermal structure like trichomes and stomata are studied. In addition maceration of the stems of the two species is studied.

II. MATERIALS AND METHODS

Based on Literature and consulted herbarium information, critical field observations on each taxon collected have been made in the field itself. The species collected have been followed by standard procedures for herbarium and deposited in the Herbarium of the Botany Department,

Vaidyanath College, Parli-Vaijnath. The plants have been photographed in their natural habitats with Sony Cybershot and Nikon cameras. The plant material viz. stem, leaves flowers and fruits have been preserved in the laboratory in 70 % alcohol or FAA for further detailed laboratory studies. The materials of the stem were also studied by maceration techniques. The pieces of stem were boiled in Jeffery’s fluid (Chromic acid 10% and Nitric acid 10% in 1:1 proportion) the macerated cells were studied in detail (Johanson, 1940; Choudhary et al. 1992 and Khandelwal, 2006). The dimensions of the cells were measured with help of Pixel-pro software connected to Labomed Lx-400 Microscope.

MICROMORPHOLOGY OF LEAVES

i) Trichomes

Trichomes are outgrowths of epidermal cells (Roy, 2006). To study the trichomes following procedure have been followed:

1. The trichomes were scraped from leaf surfaces with the help of blade.
2. Trichomes were stained in Saffranin and mounted in glycerin on a slide.
3. The slides were observed under microscope and noted down the type of trichome.
4. The dimensions were taken with the help pixel-pro software connected to Labomed Lx-400 microscope
5. The photographs were taken with the help of

digital camera connected to Labomed Lx-400 microscope.

- 6) The results were recorded for ten fields and the average and range of dimensions of trichomes were recorded.

ii) Stomata

Stomata are microscopic pores on the epidermal surface of aerial parts of higher plants formed by a pairs of specialized epidermal cell termed guard cells, which control opening and closing of the pore by changing their turgidity and thus regulates the gaseous exchange between plants and environment (Roy, 2006).

To study the stomata following procedure was followed:

1. The epidermis was peeled out by means of forceps, kept on slide and mounted in glycerin water.
2. Adjusted the digital camera attached to Labomed Lx-400 microscope and the photographs were taken.
3. Slide with epidermal peel observed under the microscope.
4. The dimensions of the stomata were taken with the help of pixel pro software connected to Labomed Lx-400microscope.
5. The results were recorded for ten fields and calculated the average and range of dimensions of stomata.

Table-I: GPS Locations of Plants

Species	Name of	Latitude	Longitude	Altitude	Field Numbers	Locations
Tephrosia purpurea (L.) Pers.		N 18°84'53"	E 076°51'98"	1512 ft	1017	Parli-Vaijnath to Ambajogai Road, Dist Beed (MS).
Tephrosia villosa (L.) Pers.		N 18°96'64"	E 076°74'81"	1279 ft	1087	Parli-Vaijnath (MS).

Maceration of Stem

1. Tephrosia purpurea (L.) Pers.

Parenchymatous cells are of two types:

- Parenchyma with simple pits: Cells rectangular, squarish or rhomboid, cells thin walled, cell wall continuous, pits few circular or oval, distributed along cell wall at one side, with or without deposition of starch grains, 20.00 – 35.00 × 6.00 – 11.00 µm.
- Parenchyma with rectangular Crystals: cells squarish, or rhomboid, thick walled, Crystals

rectangular, 25.00 – 40.00 × 12.00 – 21.00 µm.

Fibres are of two types:

- Simple fibres, short, slender, thick walled, pointed sharp and tapering at both ends, outline entire, their range is 340.0 – 410.0 × 6.00 – 9.00 µm.
- Simple fibres longer, broader lumen, thick walled, tapering and sharply pointed at both the ends, outline entire, measured range 430.0 – 510.0 × 10.00 – 14.00 µm.

Tracheids are of two types:

- Tracheids simple, elongate, with broader lumen, thick walled, shorter than fibres, blunt at both ends, pits not seen, their range is 210.0 – 270.0 × 12.00 – 21.00 µm.
- Tracheids with elongated crystals: Cells simple, shorter, thick walled, blunt at both the ends, lumen broader, elongated crystals observed, pits not seen, 130.0 – 210.0 × 15.00 – 25.00 µm.
- Vessel elements not seen.

2. Tephrosia villosa (L.) Pers.

Parenchyma of three types:

- Parenchyma with few pits: Cells squarish, rectangular or rhomboid, arranged in rows, pits few, bordered, circular or oval, distributed along cell wall at one side, with or without impregnated with starch grains, 40.00 – 52.00 × 16.00 – 23.00 µm.
- Parenchyma with few pits: Cells rectangular or squarish, cells thick walled, pits few circular or oval, distributed overall the cell, 17.00 – 24.00 × 7.00 – 14.00 µm.

- Parenchyma with rectangular Crystals: cells squarish, or rhomboid, thick walled, Crystals rectangular, 24.00 – 43.00 × 11.00 – 25.00 µm.

Fibres are of two types:

- Simple fibres, short, slender, thick walled, pointed sharp and tapering at both ends, outline entire, measured range 230.0 – 290.0 × 5.00 – 7.00 µm.
- Fibres simple, slender, tapering and sharp ended lumen narrow outline entire, 270.0 – 460.0 × 9.00 – 15.00 µm.
- Tracheids shorter than fibres, slender, blunt at both the ends, with few simple pits 215.0 – 340.0 × 10.00 – 27.00 µm.

Vessel elements are of two types:

- Vessel elements broader, end walls oblique with simple perforations, lateral walls with simple pits, pits alternate, beak short, present on both the ends, 380.0 – 590.0 × 23.00 – 46.00 µm.
- **Pitted Vessel:** Elements broader, thick walled, with oblique perforation plate, plate shifted to lateral side, simple perforation, pits simple, alternate, arranged in rows, with short beak at one end 360.0 – 470.0 × 33.00 – 55.00 µm.

Table-II: Dimensions of Macerated cells.

Type of Macerated Cells	Dimensions observed (µm)	
	T. purpurea	T. villosa
Parenchyma – Type I	20.00 – 35.00 × 6.00 – 11.00	40.00 – 52.00 × 16.00 – 23.00
Parenchyma – Type II	25.00 – 40.00 × 12.00 – 21.00	17.00 – 24.00 × 7.00 – 14.00
Parenchyma – Type III	NA	24.00 – 43.00 × 11.00 – 25.00
Parenchyma – Type IV	NA	16.00 – 32.00 × 10.00 – 17.00
Tracheids	210.0 – 270.0 × 12.00 – 21.00	215.0 – 340.0 × 10.00 – 27.00
Fibres – Type I	340.0 – 410.0 × 6.00 – 9.00	230.0 – 290.0 × 5.00 – 7.00
Fibres – Type II	430.0 – 510.0 × 10.00 – 14.00	270.0 – 460.0 × 9.00 – 15.00
Vessels – Type I	NA	80.0 – 590.0 × 23.00 – 46.00
Vessels – Type II	NA	360.0 – 470.0 × 33.00 – 55.00

MICROMORPHOLOGY OF LEAVES

Taxonomic significance of micromorphic characters in vascular plants is now widely recognized (De Bary, 1884; Solereder, 1908 & Metcalfe & Chalk, 1950). Micromorphology of leaves include study of epidermal tissue system.

1. Tephrosia purpurea (L.) Pers

Leaf showed presence three types of trichomes viz. simple, unicellular, trichomes with bulbous base and pointed end, their average length is 731.66 µm and range 250 – 1200 µm glandular

trichomes which measured 50 – 68 µm long on both surfaces but more common on lower epidermis. Some of the glandular trichomes sessile with quadricellular head measured 21 – 48 µm in length.

Stomata anisocytic (Cruciferous), amphistomatic, 19.16 × 13.33 µm in average and range 17.50 – 20.00 × 12.50 – 15.00 µm.

Upper epidermal cells much larger (average 23.240 × 16.255 µm and range 13.92 – 33.03 × 13.36 – 18.53 µm.) than lower epidermal cells (the average

cell size $13.452 \times 9.182 \mu\text{m}$ and range $10.23 - 16.90 \times 8.14 - 10.64 \mu\text{m}$).

2. Tephrosia villosa (L.) Pers

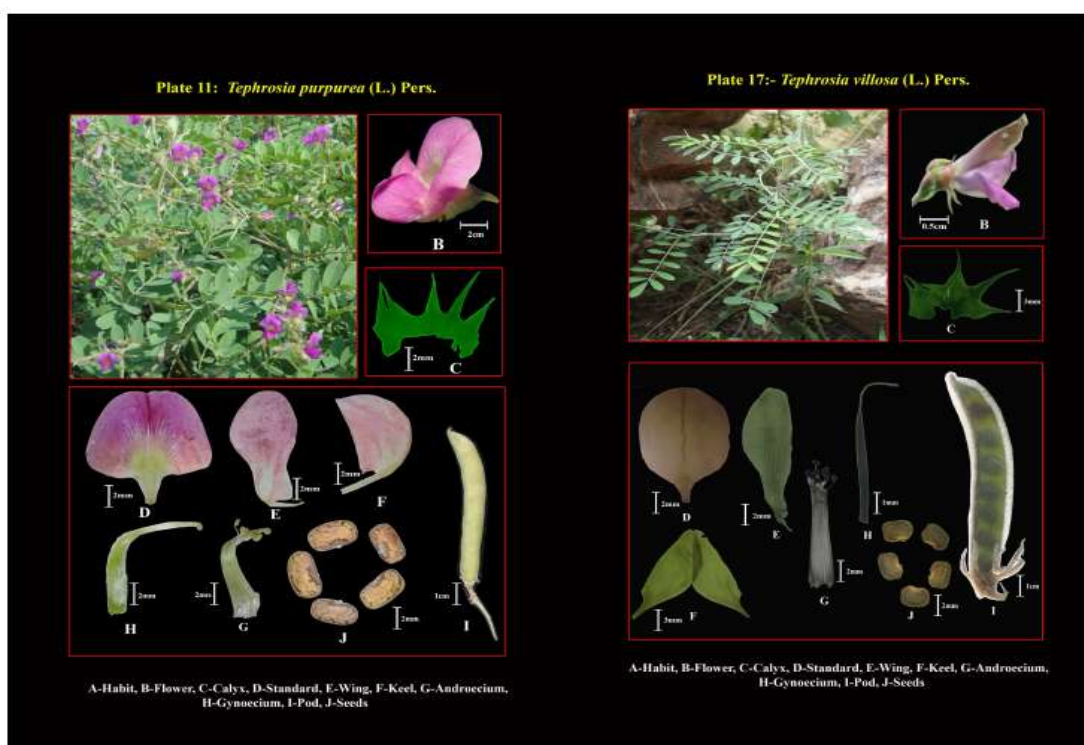
Leaf showed presence of simple, unicellular, trichomes with thick walls, their average length is $644.16 \mu\text{m}$ and range $110 - 1140 \mu\text{m}$, present on both the surfaces, but however, they are more common on lower surface.

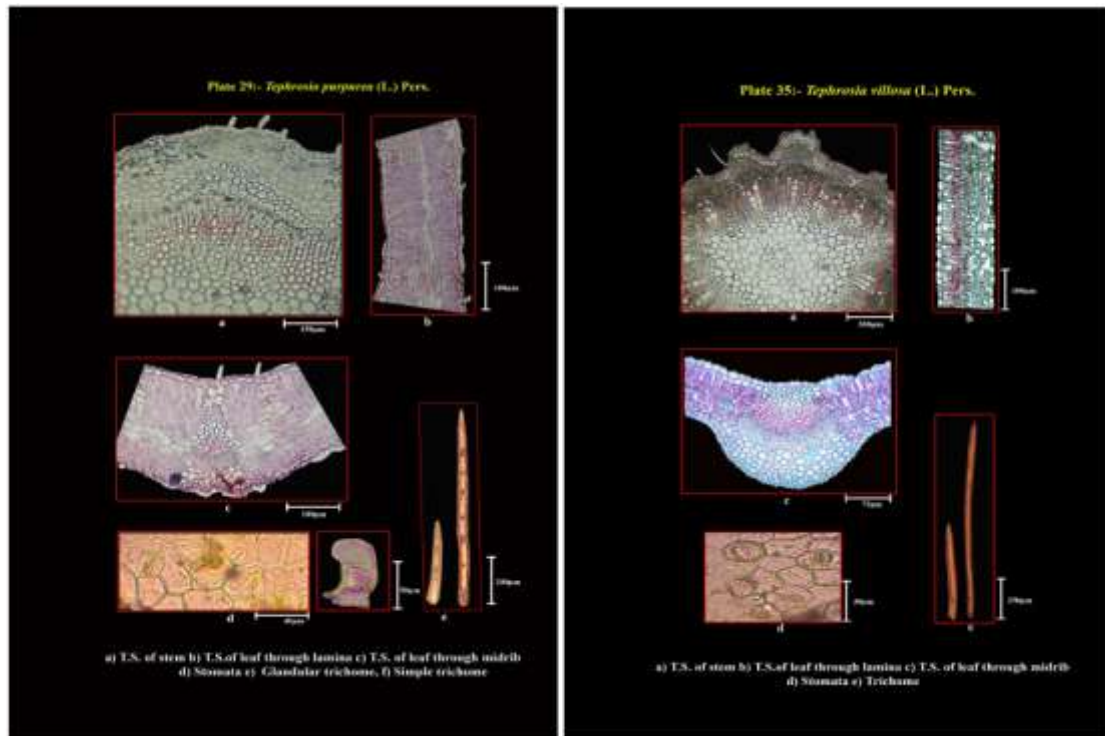
Stomata anisocytic (Cruciferous), hypostomatic, $19.10 \times 12.84 \mu\text{m}$ in average and range $17.50 - 21.23 \times 10.20 - 15.20 \mu\text{m}$.

Upper epidermal cells much larger (average $26.510 \times 20.234 \mu\text{m}$ and range $19.610 - 39.123 \times 16.08 - 27.06 \mu\text{m}$.) than lower epidermal cells (the average cell size $7.844 \times 4.731 \mu\text{m}$ and range $5.34 - 11.05 \times 3.60 - 6.51 \mu\text{m}$).

Type of Epidermal Structures	Observations in	
	T. purpurea	T. villosa
Length of simple Trichomes (μm)	250 – 1200	110 – 1140
Glandular Trichomes	50 – 68	NA
Stomata Type	Anisocytic (Cruciferous)	Anisocytic (Cruciferous)
Stomatal Presence	Amphistomatic	Hypostomatic
Stomatal Dimensions	$17.50 - 20.00 \times 12.50 - 15.00$	$17.50 - 21.23 \times 10.20 - 15.20$

Note: Dimensions were calculated by taking 10 readings for each parameter (μm). NA indicates absence of trichomes as leaves are glabrous.





MACERATED CELLS OF TEPHIROSIA PURPUREA

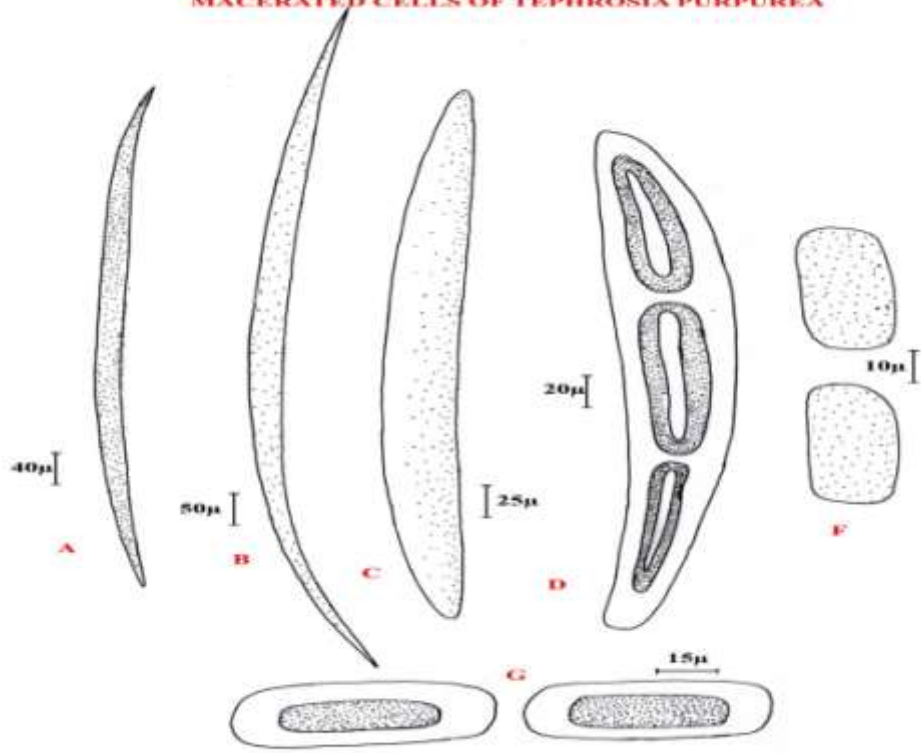


Fig. 11- A & B- fibers, C & D- Tracheids, E, G- Parenchyma cells

MACERATED CELLS OF TEPHROSIA VILLOSA

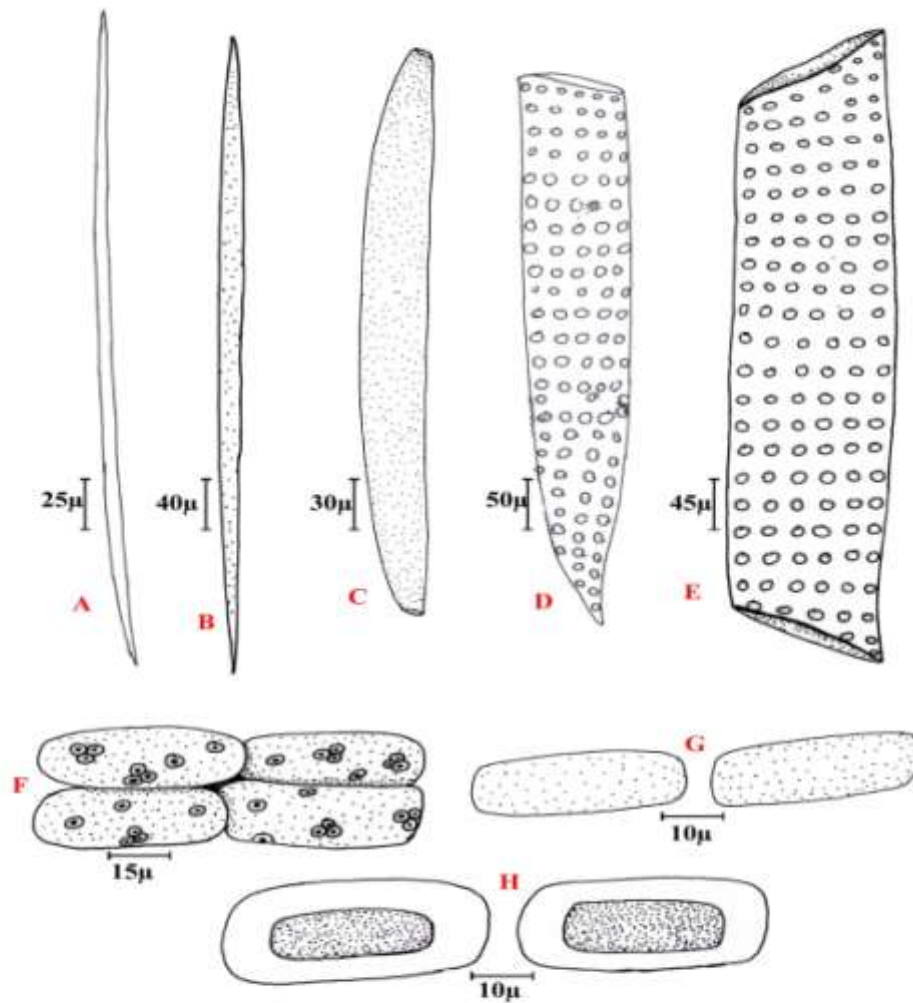


Fig. 17- A&B-Fibers, C- Tracheid, D&E- Vessels, F, G & H- Parenchyma cells

III. DISCUSSION

1. **Maceration:** Two types of parenchyma is seen in *T. purpurea* viz. parenchyma with simple pits and parenchyma with rectangular crystals whereas in *T. villosa*, three types of parenchyma is seen. Fibres are of two types in *T. purpurea* viz. short, slender (ranged 340.0 – 410.0 × 6.00 – 09.00 µm.) and longer with

broader lumen (ranged 430.0 – 510.0 × 10.00 – 14.00 µm.); whilst *T. villosa* showed simple fibres, short, slender, thick walled (ranged 230.0 – 290.0 × 5.00 – 7.00 µm.) and simple fibres with slender, tapering (270.0 – 460.0 × 9.00 – 15.00 µm). In *T. purpurea*, tracheids shorter than fibres, slender, blunt at both the ends, with few simple pits 210.0 – 270.0 ×

12.00 – 21.00 μm . While in *T. villosa*, tracheids simple, slender, blunt at both the ends, with simple pits 215.0 – 340.0 \times 10.00 – 27.00 μm . In *T. purpurea* vessel elements not seen whereas in *T. villosa*, vessels were broader, end walls oblique range 380.0 – 590.0 \times 23.00 – 46.00 μm .

2. **Micromorphology of Leaf:** Two types of trichomes were seen in *T. purpurea*, simple trichomes with a length range of 250 – 1200 μm . and glandular trichomes with 50 – 68 μm . *T. villosa* showed only simple type of trichomes measured 110 – 1140 μm . Simple trichomes in *T. villosa* were much shorter than those in *T. purpurea*. Anisocytic (Cruciferous), amphistomatic stomata were seen in *T. purpurea* measured 17.50 – 20.00 \times 12.50 – 15.00 μm . Whereas anisocytic (Cruciferous), hypostomatic stomata revealed by *T. villosa*. Ranged 17.50 – 21.23 \times 10.20 – 15.20 μm .

IV. CONCLUSION

The qualitative and quantitative characters studied in this investigation are diagnostic to each species. These observations may find helpful for the taxonomic identification and delimitation of these taxa.

ACKNOWLEDGEMENT

Authors are thankful to Dr. R. K. Ippar, Principal, Vaidyanath College, Parli-Vaijnath, Professor and Head, Department of Botany, Dr. B. A. M. University, Aurangabad for providing necessary facilities during this study. Thanks are due to Dr. Ram Gore for providing illustrations.

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