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Terminology of venation pattern in *Viola tricolor* Leaf architectures

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Abstract

A study of terminology in *Viola tricolor* species is presented. For this purpose, has been studied the topology of venation pattern. Vein systems have been classified with "types" for each order. In the present study the venation is pinnate and first order veins, type include brochidodromous, second order veins, type include cascade and third order veins, type has been seen both lattice and percurrent. Ultimate of veins branching has been seen areoles structures. The areoles are variable in size. They are triangular, tetragonal or polygonal in outline and with simple, linear and uni-veinlets with dichotomously branched vein endings. There is no relationship between the size of areoles and the number of vein endings. In present cases, where areoles are devoid of vein-ending, a loop like structure is seen which is formed either due to the union veins. This is a common feature in *Viola tricolor*. In general, studied of terminology is necessary for leaf architecture, which incorporates lamina development and gives greater emphasis to the patterns formed by groups of venation element.

Keywords: *Viola tricolor,* topology, terminology, Vein systems.

Introduction

Leaves are highly polymorphic organs and provide sets of diverse features. The vein's and vein lets which form the vasculature, called the "venation" is an important feature of mature leaves. Levin (1929) & Strain (1933) discussed the taxonomic significance of vein-islet areas and vein-endings. Hickey (1973), Hickey& Wolfe (1975) and Melville (1976) classified the architecture of dicotyledons and angiospermous leaves respectively.

Leaf architecture was represented the placement and form of those element constituting the out ward expression of leaf architecture, including venation pattern, marginal configuration and leaf shape.

Leaf architectureis described using a hierarchyof venation "elements", which are grouped into "patterns", which are located in "zones"and "segments" of the leaf lamina.Inleaf architecture, "venation pattern" refers to the interrelationships between veins or between veins and the margin. The amount of lamina development between veins, or the thickness of the veins themselves, is a separate phenomenon and should not be incorporated in a classification of venation pattern. The terminology of Hickey (1973, 1979) and Dilcher (1974) has as its cornerstone the recognition of vein orders. the recognition of vein orders is essential in describing leaf architecture and The fundamental rule for the determination of the order of a vein is its relative size at its point of origin"(Hickey, 1979:32).

Within and across species, leaves are enormously diverse in venation architecture. Ever since a seminal review introduced a new integrative science of leaf venation (Roth-Nebelsick et al., 2001), there has been increasing recognition across plant biology and ecology of the importance of leaf venation. Leaf venation systems vary strongly across major plant lineages, with many early groups having dichotomously branching, open systems, but reticulation evolved frequently. Angiosperms have greatest diversity in vein structure but share key architectural elements, that is, a hierarchy of vein orders forming a reticulate mesh (Hickey, 1973; Ellis et al., 2009; McKown et al., 2010). Typically there are three orders of lower-order veins, known as 'major veins', often ribbed with sclerenchyma (Esau, 1977). One or more first-order veins run from the petiole to the leaf apex, with second-order veins branching at intervals, and third-order veins branching between.

Ramified structure is a very important feature in leaves classification and determines the type and function of plant resistance against environmental elements. Access to this information by examining terminology in the field of leaf architecture is possible. A simple set of working assumptions may be stated to distinguish different order of venation pattern in a leaf, and to incorporate these distinctions in a terminology:

1. Progressive growth is indicated by decreasing thickness along a vein and/or by free ends.
2. Simultaneous development is indicated by veins of equal width along their length which terminated against a lower order vein at each end.
3. Thicker veins are ontogenetically older than thinner ones in the immediate neighborhoods.

Materials and Methods

The seeds of viola tricolor were brought from the genetic resources center, Iran and grown in the university botanical garden(day temperature maximum 28± 2ºC, night temperature minimum 16 ±2ºC, relative humidity 60 ± 5 %, 52 μmol $m^{-2}s^{-1}$ Photosynthetic Photon Flox Density, photoperiod 8 to 16h). The leaves were collected at vegetative stage then their architectural features were analyzed. In this study was presented proper protocol to transparent of leaves. According to this protocol, leaf samples were placed in 5% HCL for 6 hours. However, in order of development this time becomes longer. Approximately, one other way to view leaf surface is boiling in KOH 10% for 4-5 minutes. Then, slides prepared from leaves and examined by the microscope. A terminology as defined by HICKBY (1973) and HICKEY & WOLFE (1975) are followed for the description of leaf architecture.

Results

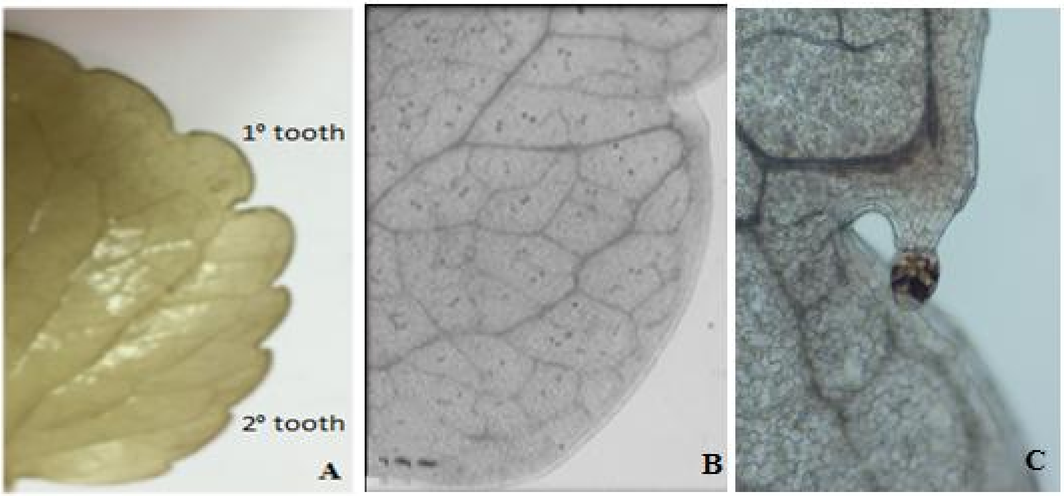
Morphological description:

Leaves are simple and alternate in all cases. Lamina is more or less symmetrical. The apex is acuminate or rounded, the base is cordrate and the margin is crenate type, where it is clearly lobed. The venation is pinnate where a single primary vein serves as the origin for the higher order venation. The first, second and third degree veins are major and the higher order veins, the minor venation pattern.

Fig. 1 Morphology of Leaf

Leaf margin

The teeth can be separated in to different size groups and called compound. The number of teeth is 3-4/cm in the middle 50% of the leaf (Fig 2; A). Tooth spacing refers to the interval between corresponding points on the teeth or crenation is irregular types. Tooth shape in apical and the basal is convex (Fig 2; B). The shape of the sinus of the tooth is angular. Tooth apex is papillate type that having clear, nipple-shaped, glandular apical termination (Fig 2; C).



Basal and apical angle of leaf

Base angle is the angle from the vertex to the points where a line vertical to the midvein at 0.25*lm* from the base intersects the margin and the Apex angleis the angle from the apical termination of the midvein to the pair of points where a line vertical to the midvein and 0.75*lm* from the base intersects the margin. The base and apical angle were detected obtuse (angle 90 - 180°) type.

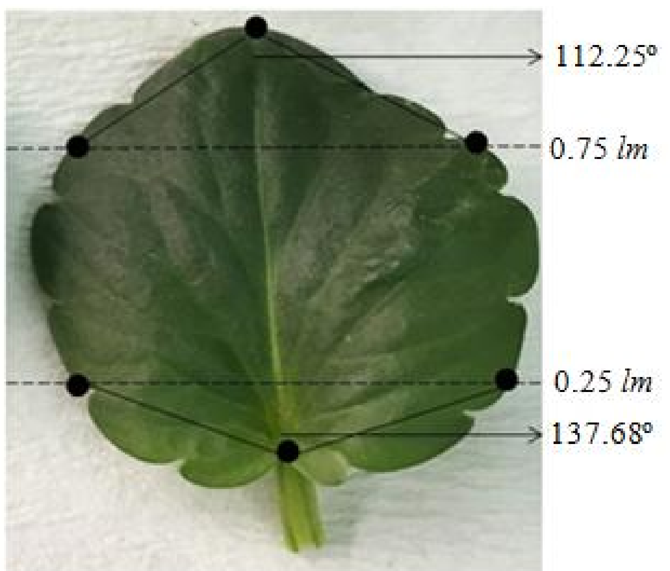


Fig.3

Hierarchy in leaf architecture

A. Elements

The veins themselves are termed "elements" and, depending on their behavior and size, can be divided into ramifying or non-ramifying; first, second, third order elements; in present sample has been seen ramifying structure; first, second, third order elements have been reported.

B. Patterns

The way in which venation elements interact with each other provides "patterns". Depending on the order of the elements concerned, and their location, these patterns can be divided into first, second, third order patterns. These pattern has been studied and were detected.

C. Zones and Segments

These both refer to areas of the lamina which are defined by the course of elements and filled in with patterns. "Zone" is the most general category, and it may be divided into "segments".

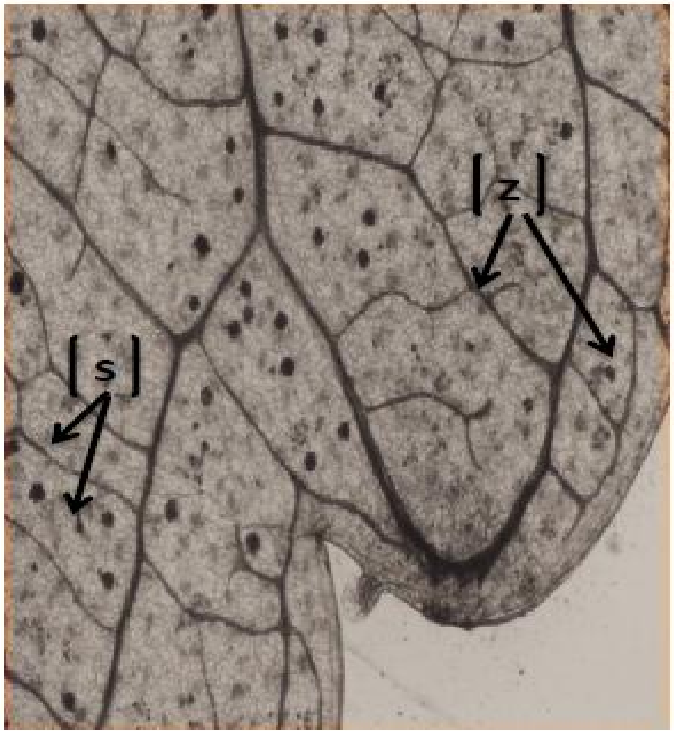


Fig. 4: S; segment. Z; zoon.

Basic venation elements

Midrib

Primary vein exists per leaf, which is called"midrib". The midrib is the central vein of a leaf, acting more or less as an axis of symmetry, and it is strongest vein in the leaf and the main point of reference for the following vein terminology. The number of midrib is important to determine vascular system types include palmate or pinnate. In the present study the venation is pinnate.

Laterals

Any ramifying veins developed along the midrib, and when applicable, the largest pair at the midrib base, are referred to as "laterals". The largest of these are here termed "first order laterals". Higher order laterals, *i.e.* second or third order laterals (here termed "extra-laterals"). Higher order laterals can usually be recognized by their behavior as well as by their relative thickness. The order of lateral vein was visible in the *Viola tricolor* leaf.

External Veins

All ramifying veins developing from the basiscopic side of lateral veins are termed "first order external veins". External veins normally develop along the distal parts of the laterals. Three order of external vein has been reported in these samples**.**

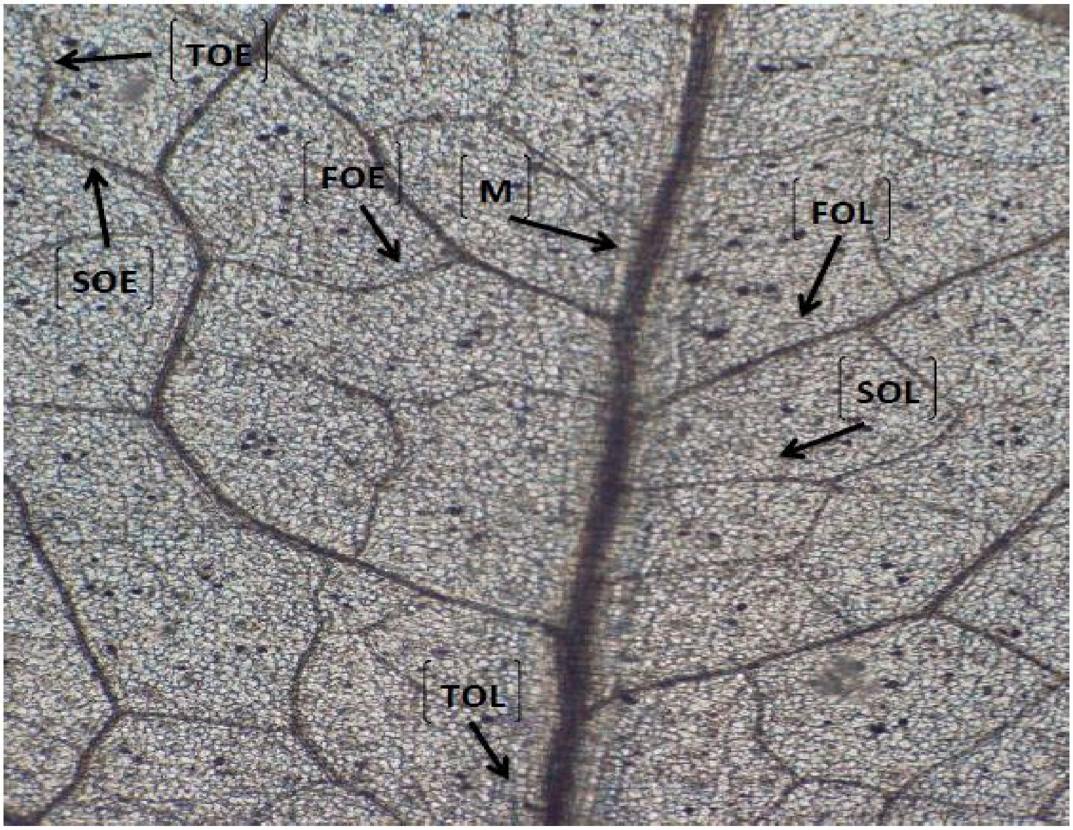


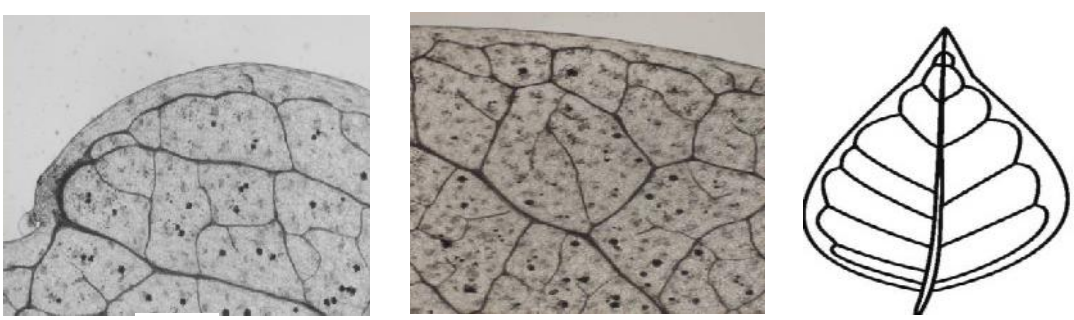
Fig. 5: M (midrib), FOL (first order lateral), SOL (second order lateral), TOL (third order lateral), FOE (first order external), SOE (second order external), TOE (third order external).

First Order Venation Pattern

Designation of first order vein is important characteristic to determine a total structure of venation pattern. In order to Identification the type of first order vein, a few matters are considered that includes:

1. The relationship between the branches of veins in the leaf margin.
2. The relationship between the terminal parts of veins with leaf margin.
3. Veins arrangements that mean they are placed parallel to each other or have a ramifying structure.

These data are important for identifying leaf architecture characteristics and also are used for taxonomic significant. These "basic" venation patterns, formed by the midrib. In the present study the venation is pinnate where a single primary vein serves as the origin for the higher order venation. First order venation is brochidodromous type. This term is a kind of camptodromous venation reserved for that condition where adjacent first order laterals directly join one another and not terminating at the margin.



A B C

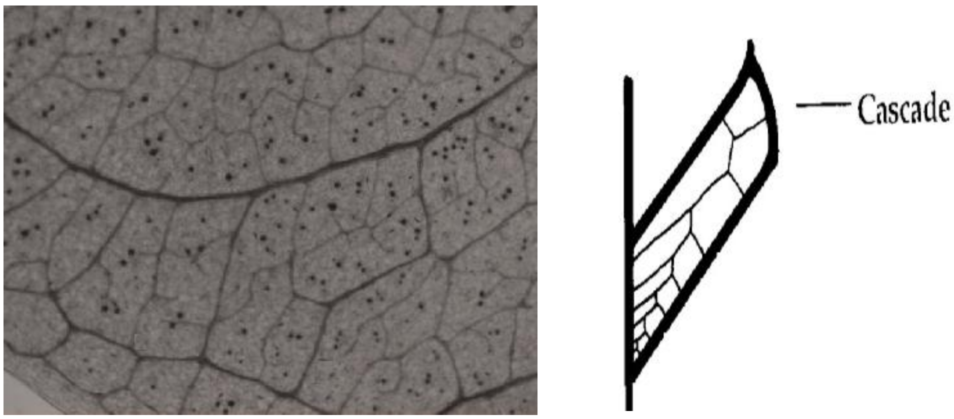
Fig. 6: A, B, Brochidodromous leaf venation: second order veins are joined,. C, schematic picture of brochidodromous.

Second Order Venation Pattern

Second order venation pattern refers to the most basic pattern formed in the region between first order lateral veins, externals, or loops. The word "pattern" as used here refers to a group of veins showing some coherence in their order.To identify the Vein patterns in this order should be considered two parameters:

1. veins placement to each other between two first lateral; Present study indicates that the veins are arranged in ramifying form
2. Presence or absence of the angle between branching veins; in this studied sample was seen ramifying structure with producing angle.

Based on the points mentioned above Second order venation in the present sample has been reported cascade venation. This form is a special case where interangular veins are not directly percurrent, but are staggered so that the end of a vein originating at the midrib terminates along a vein originating on a lateral, and vice-versa. Growth producing this pattern is initiated in the angle between midrib and laterals.



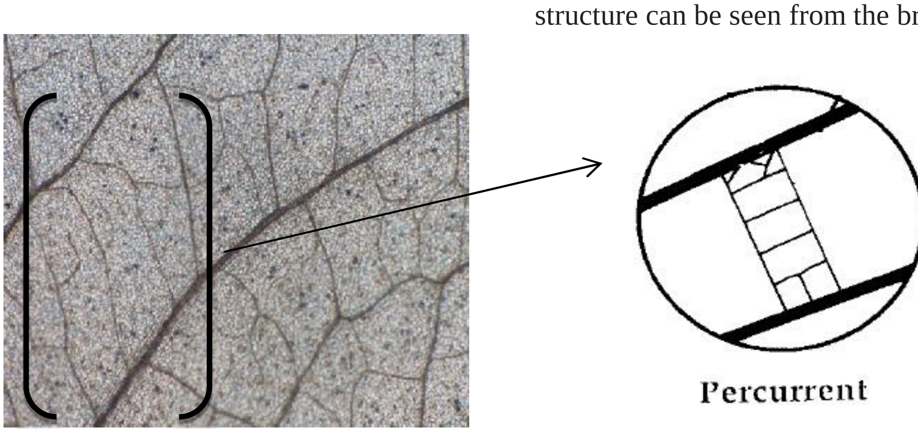
A B

Fig. 7: A, cascade venation types of second order venation pattern. B, schematic picture.

Third Order Venation Pattern

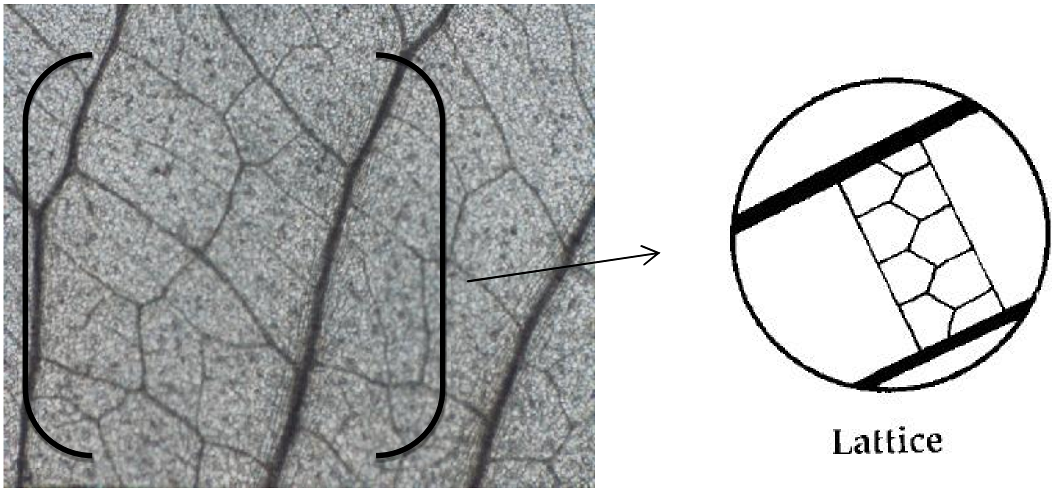
Third order refers to repeating patterns of venation which fill the segments defined by veins of the second order pattern. In the present study third order venation pattern has been seen both percurrent (Plate 6) and lattice(Plate 7) venation. percurrent venation is non-ramifying veins which join two adjacent lateral veins and veins in this form are parallel to each other.

Lattice venation Found as segment "fill", between percurrent veins, but also found between first order laterals. It is formed by a zigzag vein connecting two rows of offset veins projecting from the two veins defining the segment. In this sense it is similar to the construction of a cascade vein, but there is no marked trend in the size of its constituent veins in any particular direction, and it is not oriented towards the axil between two major veins. In general, a network structure can be seen from the branching veins.



A B

Fig. 8; A, percurrent type of the third order venation pattern. B, schematic picture



A B

Fig. 9; A, lattice type of the third order venation pattern. B, schematic picture.

Loops

Loops refer to closed circuits of ramifying venation. They are often composite structures formed by the linkage of several elements. The thickest vein in the loop is the "base" from which the "origins", the next thickest veins, depart. The opposite end of the loop from the base is the "arch". Loop structure has been seen in looping zoon, this is refers to the area of lamina between the lateral part of venation pattern and the leaf margin.

a) Lateral loops

These are loops formed by first order lateral veins growing to join an adjacent first order lateral or an external branch of it. The midrib forms the base, and the arch include first order external and second order external.

b) External loops

1. First-order external loops. These loops have first order externals as their origins and are based on first order laterals. The arch is a second order external.

2. Second-order external loops. These have second order externals as their origins and are based on a first order external. The arch is a third order external.

3. Third-order external loops. These have third order externals as their origins and are based on a second order external.All three cases have been seen in present study.

c) 1nternalloops

These loops are found within the interlateral segments defined by the lateral loops

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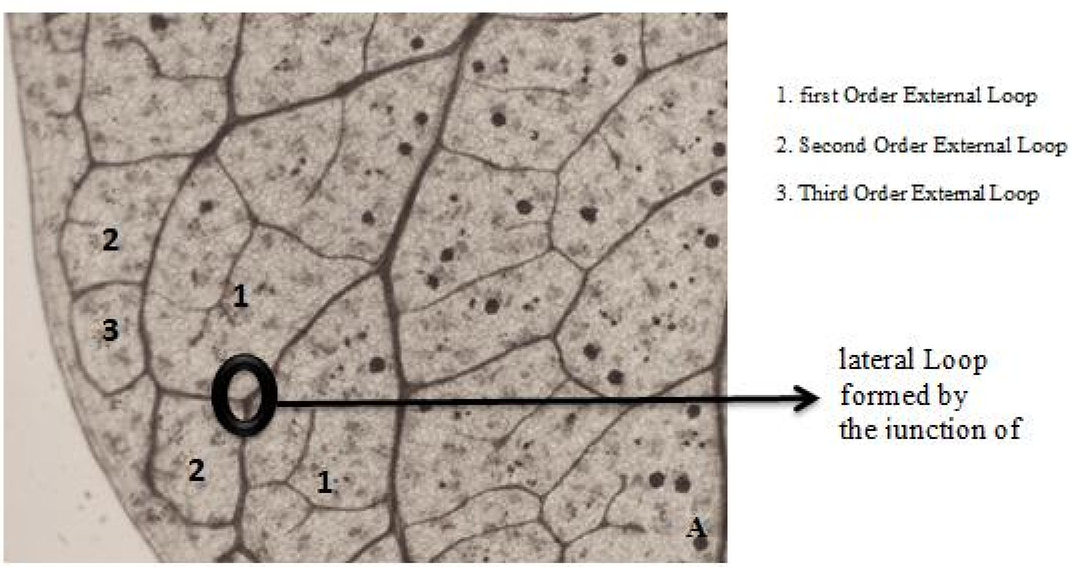


Fig 10

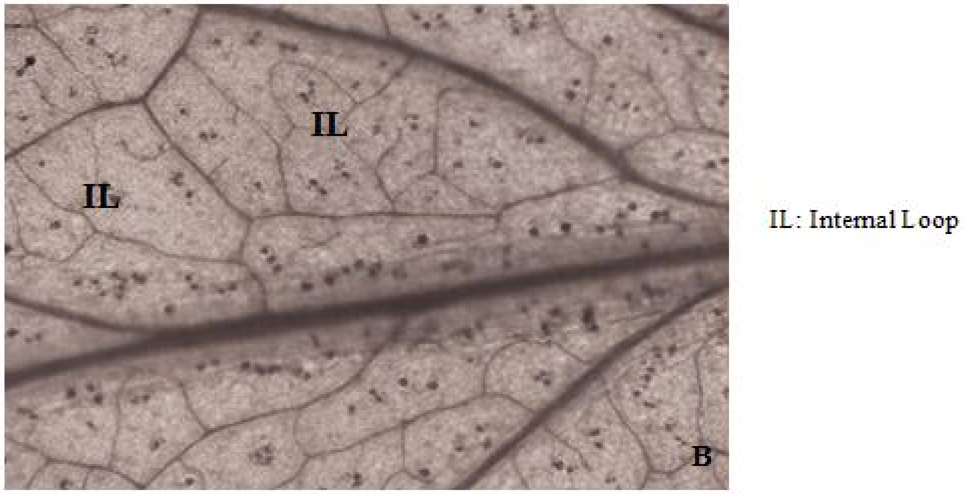


Fig 11

Areolation

An index of loopiness is areolation, the smallest areas of the leaf tissue bounded by veins which form a contiguous field over most of the area of the leaf are called areoles and the appearance and characteristics of the areoles are termed areolation. Areoles vary in their shape and size within given leaves. In the present study the areoles are perfect and shape may be polygonal, tetragonal or triangonal.

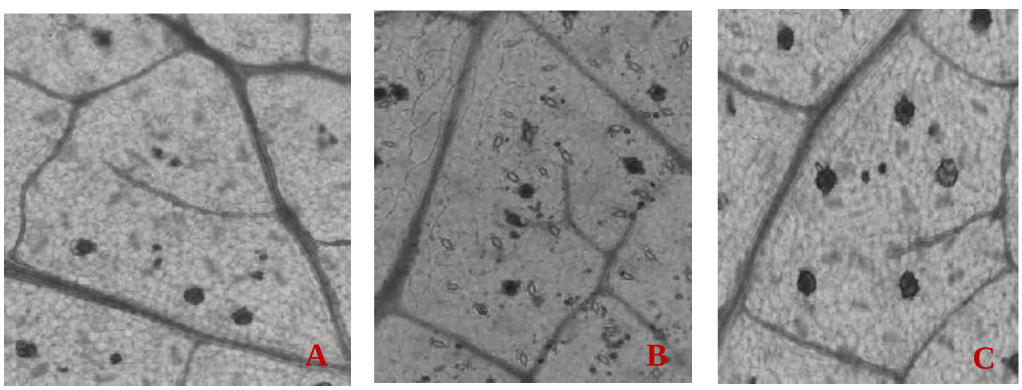


Fig 12; A, triangonal shape of areole.B, tetragonal shape of areole.C, polygonal shape of areole.

Veinlets

The ultimate veins of the leaf which occasionally cross the areoles to become connected distally are either simple or branched. Simple vein- endings may be linear (Fig. 13,B, C) or curved (Fig. 13, D). The branched ones may divide dichotomously once or twice and branches may be symmetrical (Fig. 13, F) or asymmetrical (Fig. 13,E).the veinlet's are mostly uniseriate. They may be long and thin (Fig. 13, B) or thick and short (Fig. 13, C). In most of the cases where areoles are devoid of vein endings, a loop like structure is seen which is form due to union of veins or tracheids. Loop formation thus decreases the distance between the vein and help in transporting system.

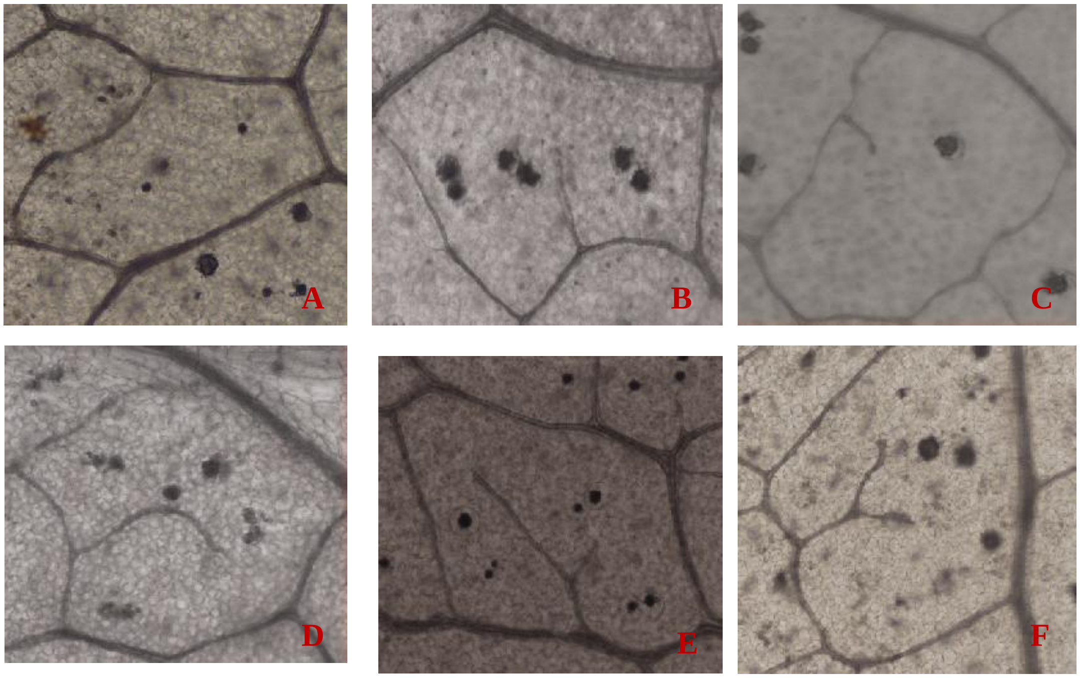


Fig. 13; A, loop like areoles. B, C, linear vienlets. D, curve veinlets. E,F, uniseriate type of brunched veinlets.

Tracheids

The veinlets are associated with terminal tracheids which increase in cell diameter and are extra-ordinarily variable in size, shape and nature. The tracheids are either uniseriate (solitary) or biseriate.Uniseriate (solitary) tracheids are dilatedand elongated. Biseriatetracheids are elongated.

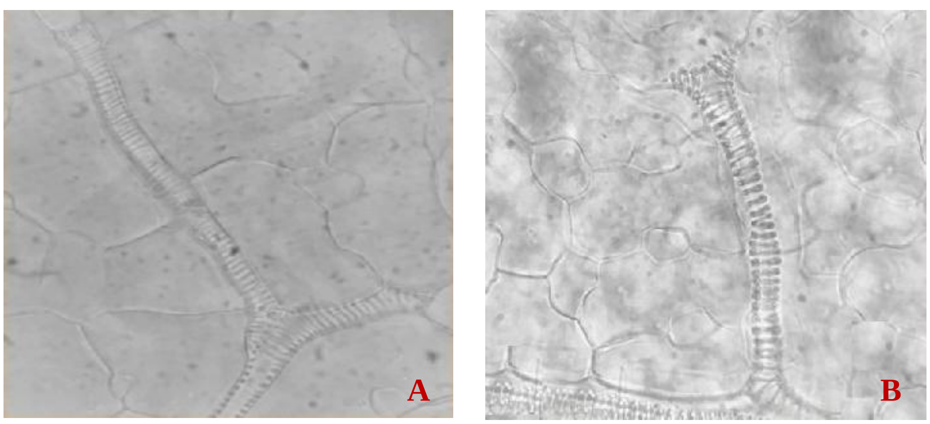


Fig. 14;A; biseriatetracheids. B;uniseriate (solitary) tracheids.

Stomata

The stomata in *Viola tricolor* are anisocytic type that named unequal celled type. In this stomatal arrangement there are at least three accessory cells that one of them are small and two other cells are larger. Stomata density at adaxial surface is less than abaxial surface. The size of stomata in abaxial surface is smaller than the adaxial surface.

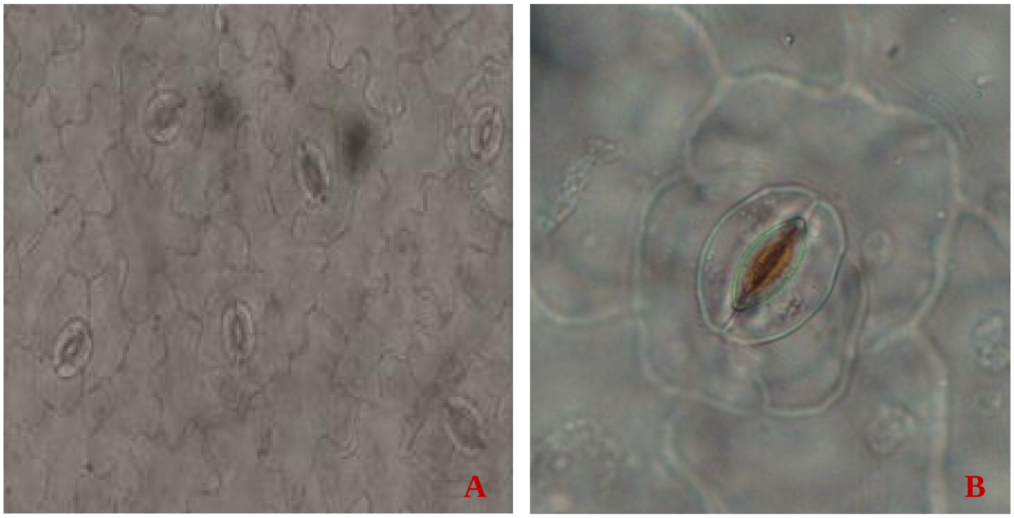


Fig. 15; A, B, anisocytic types of stomata in adaxial surface.

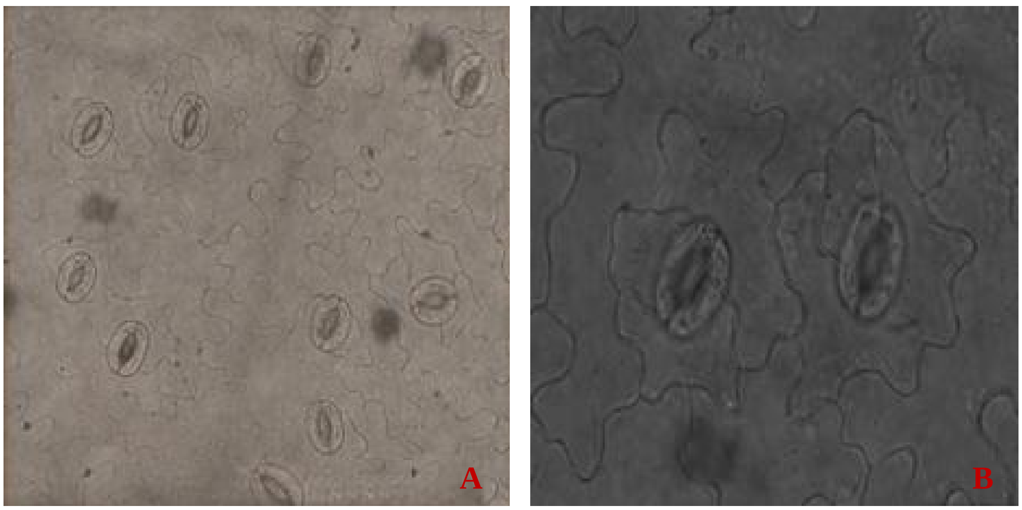


Fig. 16; A, B, anisocytic types of stomata in abaxial surface.

Table 1. Epidermal characteristics of leaves of *Viola tricolor*.

Parameters		*Viola tricolor* adaxial	abaxial
Stomatal frequency (mm-2)		15.33±0.75	25.33±0.53
Epidermal cell frequency (mm-2)		31.33±0.54	47±0.56
Stomatal index		33.08±0.37	34.72±0.29
S/E ratio		0.49	0.53
Stomatal size	Length (µm)	43.68±0.38	34.75±0.44
	Breadth (µm)	35.25±0.67	24.28±0.32
Epidermal cell size	Length (µm)	91.13±0.09	92.5±0.35
	Breadth (µm)	37.14±0/35	40.43±0.57

Discussion

Leaf venation in angiosperm varies both in pattern (Hickey 1973) and regularity (Hiokey & Doyle 1972). According to Peay (1954), the veins of first, second and third order form major venation pattern and those of subsequent orders constitute minor venation patterns. Vein systems have been classified with 'types' for each order. In cultivar of *viola tricolor*, the major venation pattern is pinnate type, first order veins, type include brochidodromous, second order veins, type include cascade and third order veins, type has been seen both lattice and percurrent. The classification proceeds with progressively higher order veins until the areolation which terminates the vein system and ultimately veins are branched into the areola.Hickey (1973) classified the vein endings into simple and branched. Branched ones divide once, twice dichotomously. In the present study has been reported that areoles are variable in size and they are triangular, tetragonal or polygonal in outline and with simple, linear and uni-veinlets with dichotomously branched vein endings. Most of the areole has been seen without any veinlets and considered a loop like structure. So, could be reported Loop formation is a common feature where there are few vein endings or none in *Viola tricolor*. The recognition of vein orders is essential in describing leaf architecture but some structure, such as zoon and segment play an important role in studying and determining of leaf venation system that have been identified in this study.Mostly tracheids at the vein endings increase the cell diameter and are extraordinarily variable in size, shape and orientation. In the same areole both uniseriate and biseriatetracheids are observed at the vein-endings. So, after the three lowest vein orders have been demarcated, Could be seenthe higher orders of venation present in the leaf (Fig. 17) and have been reported some numerical data of venation pattern in the present study (Table 2).

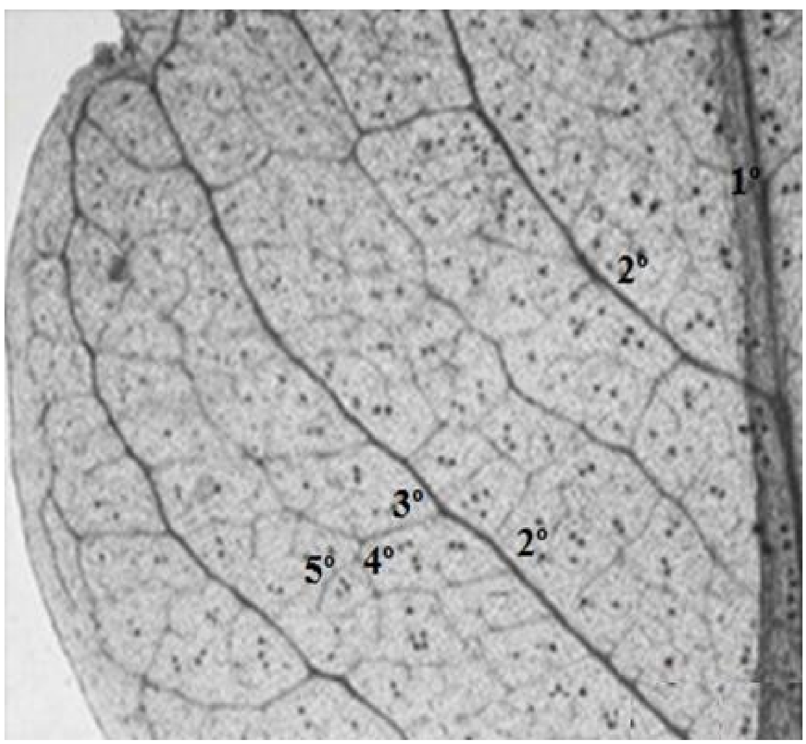


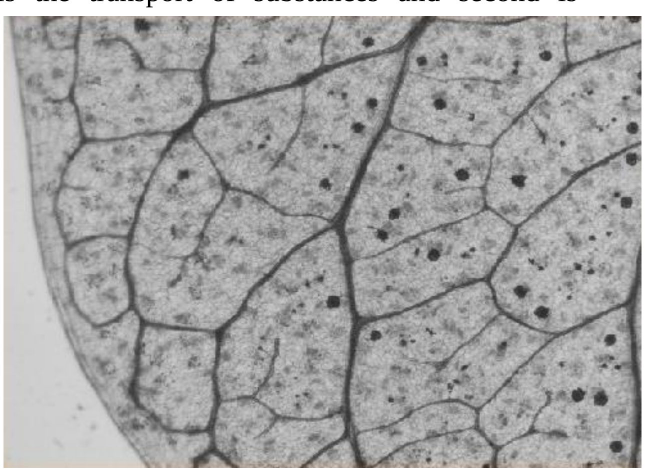
Fig. 17, five order venation pattern in *Viola tricolor*.

Table 2: Numerical Data on the Venation Patterns of the different *viola tricolor* leaves.

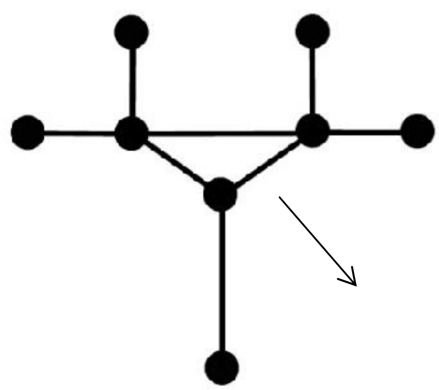
Name of samples	*Viola tricolor*
Leaf area (mm^2)	6
Leaf length (mm)	35
Leaf width (mm)	25
Number of 2^o a large one side of midrib	4
Angle between 1° & 2° vein	50º- 60º
Angle between 2° & 3° vein	80º- 85º
Angle between 3° & 4° vein	95º- 100º
Vein islets (areoles)/ mm2	6.06
Veinls entering areole / mm2	4.16
Veinlets entering areole per mm2	2
Vein lets termination number/ mm2	3.72
Highest vein order	5
Average size of areoles(mm^2)	0.7

In general, the present study refers to leaf architecture terminology of Hickey (1973, 1979) and Dilcher (1974) that need to consider several factors of vein pattern in different order. This factors including: (1) ranking of veins orders that including first order venation is brochidodromous type and Second order venation is cascade type and third order venation has been seen both lattice and percurrent venation ; (2) study of vein relationships with leaf margin development; (3) Studying and understanding the position of important elements of architecture such as "loops"; (4) studying of low order veins structure such as "areole" and type of vein endings because low level importance of patterns.

There are two main functions of leaf venation. The first is the transport of substances and second is mechanical stabilization. It is to be expected that the architectural structure of leaf venation influences these main tasks and other functional properties, so large and mechanically stiff midribs and appropriate vein arrangement were favorable, because the greatest mechanical stress occurs along the longitudinal axis of a leaf. Based on the analysis that performed in the present study, a ramified structure with cycle will be termed "network" or "closed". Because present structure has redundancy more than one. Redundancy is a parameter used to describe the path architecture in a network by quantifying the presence of alternative pathways. In this sample, due to presence mesh, two possible routes lead from one point in the network to another.



A



B

Fig. 18, A, Network system of venation pattern in *Viola tricolor*. B, Graph represents a network by insertion of a cycle. , To illustrateroutes. ▬ ●

According to the image above, gets several benefits may be reported of redundant closed system:

(1) For a redundant closed system, however, several routes exist and the water can take the shortest path to a certain mesophyll location.
(2) Another interesting aspect is represented by the pressure values at the marginal regions of the model. A marginal vein guarantees a sufficient water supply to the leaf margin, which is prone to high water stress. The fluid pressure distribution of model as stated above in fact show moderate fluid pressure values at the marginal region.
(3) Another important aspect of redundancy in a transport network is the improvement of safety. In the case of network, elimination of one or more vein path due to injury is compensated for because fluid can reach the region beyond the damage site by numerous by passes.

Network with a high of redundancy should be corresponding resistant to vein damage. Also, a closed network would be able to provide for a homogeneous pressure distribution by rerouting the water flow to sites with higher rates of water loss.

Recognizes three distinct orders of venation patterns in an attempt to produce a terminology which expresses more closely the ontogenetic development of the leaf.

Conclusion

A total of *Viola tricolor* species were collected and preserved, observed, measured and characterized based on leaf architecture terminology. The leaf attachment, venation order, structures distinction such as zoon, segment, loop, areoles and they types are diagnostic characteristics in the study.

The principal characteristics of the leaf venation pattern of a species are, in general, genetically fixed; this provides the basis for using the leaf venation as a taxonomic tool in plant identification and classification and important anatomical features to assess the shape and resistance of leaf. Many geometric properties of leaf venation are coupled to functional traits.

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