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TRAPA NATANS: NATURE AND PHYTOCHEMISTRY OF THE "SUBMERGED LEAVES".

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ABSTRACT

As the morphology of submerged leaves is variously interpreted, they are subjected to a detailed morphological, anatomical and phytochemical studies. The position of origin at the leaf base, pinnate tristichous nature of leaflets and the limited life span prove the submerged leaves to be leafy in nature. The absence of root cap and exarch protoxylem which are the typical characters of roots goes against the root-concept. The morphological similarities of young submerged floating leaves to the other submerged leaves, ultrastructural studies like cuticular combs and pore like structures as in floating leaves, phytochemical observations and molecular biological data indicate the submerged structures to be true leaves. The role of environmental factors in shaping the characters is suggested.

Introduction

Trapa natans Roxb., a free floating aquatic plant, is a native to Europe, Asia and Africa. It is commonly known as Water Chestnut or Shinghara (in india) due to its edible nuts. In addition, the vegetative parts are used in medicine for a number of diseases. The plant possesses an underwater rhizome and two types of leaves: submerged and floating. The floating leaves are rhomboid, fan shaped with toothed edges and are crowded at the tips of branches. Submerged leaves arise in pairs from the leaf base and consist of a rachis on which needle like leaflets are arranged spirally in a tristichous manner. The morphology of the submerged leaves are interpreted differently by various authors. Roxburgh (1819), Clarke (1879) , Bercu (2004), Arima, Tanaka & Kubota (1989), Neduka (2012), Cronquist(1981) and Willis(1973) considered them as submerged leaves coming out in place of stipules from both the sides of leaf base. Due to the tetrarch/ pentarch condition of the xylem in the central vascular cylinder in rachis, Barneoud (1848), Reut & Plachno (2022) and Seago, Eyres & Volny (2016) considered them as aquatic roots. Cooke (1967) and Timonin (1984) named them as green root-like spreading "pectinate organs" (floating roots). As these differing views create confusion in the mind of a biologist, the submerged leaves of *T. natans*, were subjected to a detailed anatomical and phytochemical studies to assess their true morphological nature.

Materials and methods.

The plants were collected from ponds of Kasor, near Anand, Gujarat. The plants were compared with the Herbarium of Maharaja Sayajirao University (BARO). Hand sections of leaves were taken, stained with Safranin and mounted in Glycerine. The sections were then observed under microscope and photographs were taken with CMOS 5.2megapixel Microscope camera with basic software. The size (dimensions) of various cells and crystals were measured using stage and ocular micrometers. The quantitative data are based on the average of 20 readings.

Results

a. Anatomy

The submerged leaves arise in pairs, one each on either side of the leaf scar of the floating leaves. They are 5-6cm long, 2-3mm thick and consists of a central rachis bearing three rows of leaflets in a tristichous arrangement(Fig 1A &B). The rachis, in T.S., is cylindrical with a large vascular bundle in the center and two smaller bundles on either side (Fig.1C). At distal regions there occurred only the central vascular bundle (Fig.1D).

The vascular tissues in the central bundle contain 4-5 tracheids or groups of 2-3 tracheids arranged in different radii (Fig .1E). Most of the tracheids are of the same size, though some tracheids are slightly smaller in size seen either on the inner side of larger tracheids. If the smaller tracheids are considered as protoxylem, in most of the sections, they are seen inner to the larger tracheids (metaxylem). Therefore it is difficult to consider the xylem as exarch or endarch and it is better to consider them mesarch. The central region of the vascular bundle is occupied by a large tracheid , which can be named metaxylem(similar to that of a dicot root). Phloem tissues appeared as small patches of cells between the radial rows of xylem. The smaller vascular bundles on both the sides can be considered as leaf traces. Xylem in these vascular bundles is represented by one large metaxylem and a few protoxylem elements seen towards the periphery. Each one of the three bundles is encircled by a parenchymatous endodermis, surrounded by smaller set of closely arranged parenchyma. Epidermis of the rachis is not very distinct consisting of small barrel shaped cells. Below this layer is 3-4 layers of lamellar collenchyma. Between the collenchyma and central vascular bundle are network of air chambers each separated by a single layer of parenchyma rich in starch grains. On these walls, towards the chamber, are seen large number of sphaeraphides. Towards the distal side of rachis air chambers are minimal.

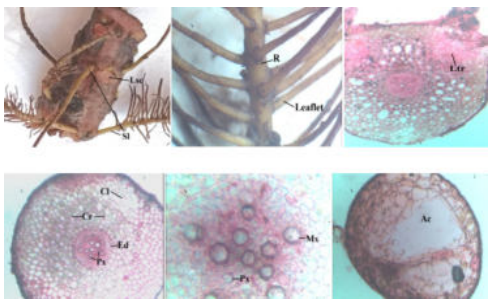

Fig 1 A-F. Anatomy of the submerged leaves, A. Part of main stem showing submerged leaves emerging from two sides of leaf base, Lsc= Leaf scar, Sl= submerged leaves; B. Close-up of submerged leaf, R=rachis, C. T.S through lower portions of rachis showing 2 leaf traces, D. Distal portion of rachis, Cl=Chlorenchyma, Cr= Cortex, Ed=Endodermis, Px= Protoxylem, E. Vascular cylinder of rachis, Mx= Metaxylem, Px= Protoxylem, F. T.S of leaflet, Ac= Air chamber.

Leaflets are thread like, cylindrical and reach a length of 4cm and a diameter of 0.5-1mm , arranged spirally around rachis in 3 distinct rows (fig.1B). Each leaflet is elongated and tubular enclosing two rows of unequal air cavities, one large and the

other smaller(Fig.1F). In TS, the leaflet is circular in outline containing ground tissue encircled by a not too distinct epidermis. The air chambers are sunken in this ground tissue and are separated by a parenchymatous partition mostly two cells in thickness. An incipient vascular bundle composed of one or two small tracheids are seen on one side at the end of the septum separating the two air spaces. The outer cells representing the cortex are slightly smaller and are rich in chloroplasts. The inner layers of ground tissue are larger, almost 30-40m in diameter and contain large number of starch grains and fewer chloroplasts. Epidermis is represented by almost rectangular cells.

b. Phytochemistry

The submerged leaves of Trapa are chemically similar to the floating leaves (Pooja, unpublished) in having similar flavonoids, phenolic acid and mucilage but it does not contain anthocyannins and tannins. The flavonoids identified are quercetin and kaempferol. The phenolic acids located are vanillic acid, trans-ferulic acid, trans-p-coumaric, cis-p-coumaric and cis-ferulic acid. Mucilage amounted to 5.4%.

Discussion

The study provides ample evidences in favour of considering the submerged leaves as true leaves. The exarch nature of the protoxylem, quoted by Bercu (2004) and Reut & P□achno (2022) as evidence of root like nature of these structures, is not seen in the present study. Though the tracheids are arranged in a radial manner the protoxylem remained inside making it a mesarch condition. Therefore these leaf like organ cannot be considered as a true root.

In addition there are many other evidences which favour the leafy nature of these organs. They are the following :

1. Unlike roots which increase in length and girth with age , the pectinate organs are uniform in nature and size. They have a definite life and fall off like other leaves.

2. The pinnate nature of the pectinate organ in having a rachis and spirally arranged leaf like organs are not seen in any root and thus these organ should be basically leafy in nature.

3. If they were roots, they should come out from any part of the rhizome but here they come out from specific places from either side of the leaf base , exactly from where the stipules come out, and not from anywhere else in the stem

4. The roots are never caducous or deciduous, but permanently fixed into the plant but here the leafy structures fall off after sometime

5. Collenchyma below the epidermis of rachis is indicative of the leafy nature and not of roots.
6. Root cap, typical of any root, is absent here.

7. Arima (1989) reported that floating leaves, which were submerged were narrow or belt shaped or some had needle shape. The lamina emerges when the apical portion get floating and full lamina emerged only when the leaves are fully exposed to sunlight. This indicates the submerged leaves retained the initial shape of floating leaves (needle shape) because they are under water.

8. Bitonti and co-workers (1966) compared the shoot meristems of floating and submerged buds that produce the different leaves using the random amplified polymorphic DNA (RAPD) approach and found that there is DNA polymorphism in the two types of leaves. The light microscope study of chromatin organization revealed an articulate structure of the nuclei, with prominent chromocentres in both floating and submerged buds. However, the meristematic nuclei of floating buds, as compared to those of submerged buds, displayed peculiar features which seem to be related to a higher proportion of A-T-rich sequences and a higher level of DNA methylation of the nuclear genome. These results show that organs of an individual plant living in different environmental conditions undergo changes in genome organization which may predispose those organs to function more effectively in different environments, a clear case of epigenetics.

9. Based on the studies on the ultrastructure of epidermal surface in floating and submerged Leaves, Nedhuka (2012) found out that the submerged leaves have cuticular combs and pore like structures (in place of stomata) on the adaxial sides similar to the floating leaves which have stomata on upper epidermis and high cuticular combs along perimeter at each cell on lower epidermis.

10. From the germination study of seed, Seago (2016) observed that from epicotyl a stolon with reduced leaves grows to the water surface. Only when the stolon reaches the surface does a typical rosette rhombic leaves result and thus both submerged and floating leaves are homologous in nature.

The nature of the morphological, anatomical, molecular biological and phytochemical features of submerged leaves of Trapa natans proves overwhelmingly that they are leaves adapted for the submerged nature. The argument that the root vascular cylinder in rachis is exarch in nature is based on improper observations. The fact that the plant survives before and after the fertile season mainly due to these submerged leaves is a fact to be observed in support of these structures. The morphological differences between the floating and submerged leaves are due to epigenetic factors such as light intensity, salt concentration etc around them.

Conclusions

As the morphology of submerged leaves is variously interpreted, they are subjected to a detailed morphological, anatomical and phytochemical studies. The position of origin at the leaf base, pinnate tristichous nature of leaflets and the limited life span prove the submerged leaves to be leafy in nature. The absence of root cap and exarch protoxylem which are the typical characters of roots goes against the root-concept. The morphological similarities of young submerged floating leaves to the other submerged leaves, ultrastructural studies like cuticular combs and pore like structures as in floating leaves, phytochemical observations and molecular biological data indicate the submerged structures to be true leaves. The role of environmental factors in shaping the characters is suggested.

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