Tapering of aerial stem in *Equisetum*

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Abstract

Known to have descended from widespread, arboreal extinct sphenopsids, the fern genus *Equisetum* is poorly represented in the modern flora by just 15 species of herbaceous habit but these have retained the characteristic ancestral features like articulated and canalized, distally attenuated aerial stem arising from underground rhizomes which are also well-differentiated into nodes and internodes. The present study reports the tapering of aerial stem towards apex in *E. diffusum* and *E. ramosissimum* from the eastern part of west Himalaya. Morphometric observations reveal that the internodal length and diameter of each internode together contribute to tapering in these taxa. Number of leaves on nodes varies considerably in both species but this variation is strikingly different in these two species. Random change in the number of leaves on adjoining nodes is not observed. Basal tapering of stem in *E. ramosissimum* is compared with some extinct members of the group and concluded that this feature is an example of perfect coordination between apical segmentation and activity of the intercalary meristem during stem growth. Tapering in the underground rhizomatous stem does not occur. Additional studies are required to understand the random changes in the number of leaves that occur in adjoining nodes in underground stem by investigating apical segmentation in runners of this genus.

Keywords: Aerial stem in *Equisetum*, Apical activity, Internodal diameter, Internodal length, Taper angle

Introduction

Although many morpho-anatomical aspects of the aerial stem of the sphenopsid fern *Equisetum* were studied in detail in the first half of last century (Campbell, 1928; Eames, 1936; Golub & Wetmore, 1948), but only after Bierhorst's (1959) incisive studies on the constitution of aerial stem of *Equisetum*, did it become

clear that the aerial stem in this sphenopsid is conical in shape and not cylindrical. Bierhorst (1959, 1971) also suggested that the diameter in the distal internodes of some species of this genus gradually decreases, and in the process the number of scale leaves at each node plays significant role in shaping the axis like a cone. Unlike all other lower vascular plants, the aerial stem in the species of *Equisetum* is distinctively differentiated into nodes and internodes. The present study discusses the role of the articulated nature of sporophyte in achieving the conical shape of aerial stem in two *Equisetum* species.

Gradual reduction in diameter of the aerial axis (stem) towards apex is of universal occurrence in terrestrial plants and to explain this phenomenon in trees four theories/ hypotheses have been put forward: (i) Uniform stress hypothesis, (ii) Nutritional theory (eco-physiological factors), (iii) Water conducting theory and (iv) Hormonal theory. The main and widely accepted theory i.e., uniform stress hypothesis of Metzger (1893) proposed that 'the stem was a beam of uniform resistance to bending, anchored at the base and functioning as a lever arm'. Most of the experiments carried out to test this proposal were on forest trees and it is of general acceptance that the tree shape in relation to its strength requirements and support function is satisfactorily interpreted by this theory.

Except tree ferns, most of the lower vascular plants (ferns and lycophytes) are of herbaceous habit where the main stem is an underground rhizome but in *Equisetum* the prominent aerial stems arise from the underground rhizomatous stem which in some species attain considerable height (*E. gianteum* L*., E.*

myriochaetum Schltdl. & Cham.). Either in tree ferns or in giant horsetails, studies on reduction in diameter of stem towards apex remained overlooked though one can expect that this feature must have evolved concomitantly with adaptation to terrestrial habit in first land vascular plants including extinct sphenopsids (to which lineage the extant *Equisetum* belongs).

Fifteen extant species assigned to *Equisetum* are grouped into two subgenera, each comprising seven species: i. sub-genus *Equisetum* and ii. sub-genus *Hippochaete* (Hauke, 1963, 1978; PPG1, 2016), with *E. bogotense* is now considered as of independent evolutionary lineage (PPG1, 2016; Elgorriaga et al., 2018). Differentiation of sporophytic plant body into underground rhizomatous stem and erect aerial axes, jointed stem and markedly canalized internodes especially in above ground shoot are among the features shared by both the sub-genera and *E. bogotense*. Slender, relatively long, unbranched aerial axis in sub-genus *Hippochaete* externally differentiate it from sub-genus *Equisetum*, the aerial axis in the members of which is short and profusely branched. Position and structure of stomata also support this sub-division. In this study we considered morphological distinction between two sub-genera as an opportunity to investigate stem taper in *E. diffusum* belonging to subg. *Equisetum* and *E. ramosissimum* belonging to subg. *Hippochaete* which are reported to have independent lines of origin (Elgorriaga et al*.,* 2018). As no reports on this aspect of growth in *Equisetum* species is available, we have attempted to know how this feature is achieved in two species of *Equisetum*.

Material and methods

This study is a part of detail phenological studies on two species of *Equisetum* (*E. diffusum* D. Don and *E. ramosissimum* Desf.) growing abundantly in the eastern part of Kumaon (Western Himalaya, India). We selected 45 mature plants of each species for this study from three localities: (i) from SSJ University Campus, Pithoragarh, India, (ii) from near Kujoli village near SSJ University Campus, Pithoragarh and (iii) from near Naini Saini airport, Pithoragarh (West Himalaya). Whenever required, observations were also made on plants which were still in the stage of growth. Number of nodes on each full grown plant were counted as well as the number of leaf teeth on leaf sheath at each node. The length of internodes (between two nodes), and of complete plant was measured with the help of metric scale. Every internode grows independently by the activity of an intercalary meristem present within the 'leaf sheath cup' or "node" between it and the adjoining lower internode. Every internode terminates in a node, thus it is the part of that particular internode but does not participate in changes in diameter. Diameter of the apical end of internode (just below the node) may be larger or smaller. Diameter of internode of an aerial stem was measured with the help of vernier calliper at its base just above the intercalary meristem (just above the leaf sheath cup of the lower internode) and just below the swollen node.

It was necessary to count the number of nodes on aerial stem and to measure the length of individual internode mainly because, in the process of attenuation of the aerial stem is involved each individual internode constituting the axis. Averages of 45 plants/measurements for each species are presented here. It is often difficult to find the aerial stems of same height and with same number of nodes; therefore, averages were taken from 45 plants of different height and with different number of nodes (Table 1). To count leaf number, leaf sheath from every node of all the 45 stems of *E. diffusum* and *E. ramosissimum* were removed mounted
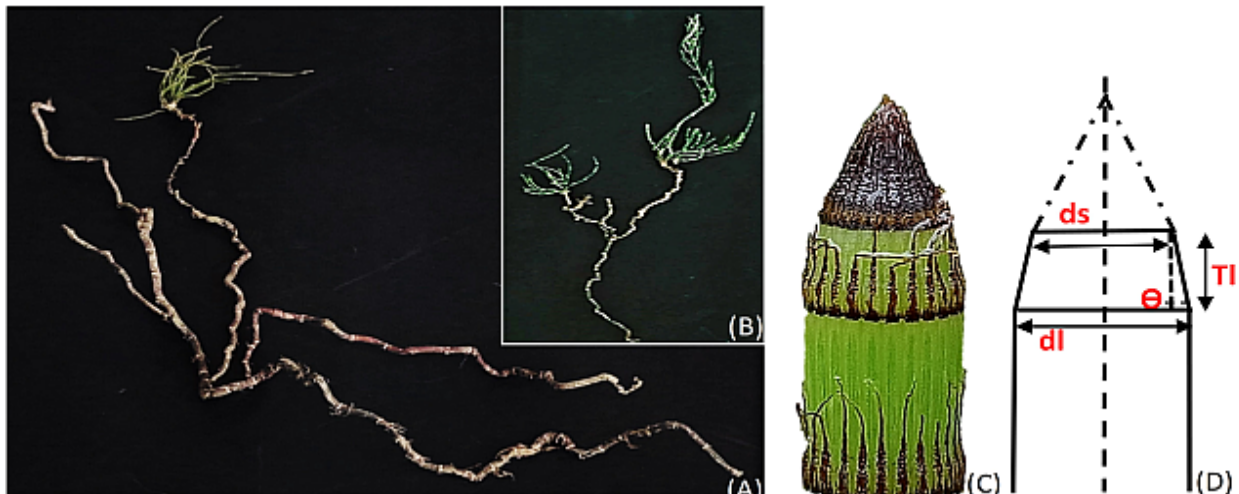

Fig. 1. Underground rhizomatous stem of *E. ramosissimum* (A), and *E. diffusum* (B); apex of aerial stem of *E. ramosissimum* (C), 2-D approximation of apex of aerial stem of *Equisetum* to measure taper angle (D)

temporarily on a slide, were examined under stereo zoom microscope. As the number of leaves varies from node to node in one stem and also in different stems, averages from different nodes of different stems were taken (from base to apex).

Aerial stems in these species arise from the nodes of underground rhizomatous stem, which is also differentiated into nodes and internodes and grows just below the soil surface (subterranean) or exhibits plagiotropic growth habit (Fig. 1). To measure the length of internodes and diameter of internodes and to count number of nodes on rhizomatous underground stems, the later were dug out cautiously to collect the maximum possible rhizomatous part. Length of internodes was measured in 10-internode long pieces of ten rhizomatous stems from different plants using metric scale and the diameter at both ends of internodes was measured with the help of vernier calliper. Leaf sheaths from 10 nodes of 10 pieces from 45 underground rhizomatous stems were taken to count the leaf number on every node.

A large number of equations, formulae and models have been developed to measure the stem taper particularly in trees (Mc Tague & Weiskittal, 2021), we preferred the common taper formula to measure the tapering of aerial axis in two species of *Equisetum*.

$$TPcm = (dl - ds)/ Tl$$

where TPcm is the reduction in the cross sectional diameter per cm of length for an internode having taper length of Tl. Dl = diameter of the internode base and Ds = diameter of the internode apex (Fig. 1C, D).

Taper angle was measured by applying equation:

$$\Theta = \tan^{-1} (TPcm/2)$$

Cumulative taper angle for the aerial axis is the sum of successive additions of taper angles of each internode.

Results and discussion

The underground rhizomatous stem in both the taxa investigated in this study (*E. diffusum* D. Don and *E. ramosissimum* Desf.) grows indefinitely and is well differentiated into nodes and internodes (Figs. 1A, B). Branching in underground stem is very irregular and rare. For example, if a lateral branch arises from node 'X', the other may arise from the next one ahead or may arise after a gap of 5-10 nodes or even after a longer space. These lateral branches act like the main underground stem in both the species (Fig. 1). The aerial stems arise from the nodes of underground stem irregularly i.e., if an aerial stem arises from node 'X', the other may arise either from the next node ahead or after a gap of 5-6 nodes or even after a longer space or the terminal internode of the underground stem may transform into the aerial axis. The length of internodes (between two nodes) is highly variable in investigated parts varies from 0.8 to 4.0 cm (average 2.27 cm) in *E. diffusum* (Table 2) and from 0.5 to 4.6 cm (average 2.88 cm) in *E. ramosissimum* (Table 3). Diameter of internodes at their two ends (posterior end and anterior end) also varies: In 10–internode long part of underground stem of *E. diffusum* it varies irregularly from 0.14 to 0.32 cm at the posterior end and from 0.19 to 0.30 cm at the anterior end (Table 2) whereas in *E. ramosissimum* the diameter at the posterior end varies from 0.40 to 0.50 cm and at anterior end from 0.52 to 0.60 cm (Table 3). Number of leaves present on nodes is highly variable and irregular e.g., 6 to 8 leaves are present on ten nodes in underground stem of *E. diffusum* but not

Table 1. Average number of nodes and average height in selected aerial stems of two species of *Equisetum*

E. diffusum			*E. ramosissimum*			
Number of aerial stems	Number of nodes per stem	Height of aerial stem (cm)	Number of aerial stems	Number of nodes per stem	Number of aerial stem	Height of aerial stems (cm)
06	26	57.4	21	29	02	188.0
06	23	56.3	15	26	19	182.8
18	22	50.4	09	24	01	176.6
15	20	50.9			02	175.2
					07	170.4
					08	162.6
					03	145.69
					03	138.7

Average number of nodes on 45 aerial stems = 22

Average height of 45 aerial stems = 51.6 cm

Average number of nodes on 45 aerial stems = 27

Average height of 45 aerial stems = 172.83 cm

Table 2. Length of internodes, number of leaves on nodes and diameter of different internodes (at the anterior and posterior ends) in the underground rhizomatous stem of *E. diffusum*

Internode number (from posterior end to anterior end)	Length of 10 internodes in underground stem (cm)	Average no. of leaves present on the node on respective internode	Diameter of internode at posterior end (cm)	Diameter of internode at anterior end (cm)
1	1.0	8	0.2	0.2
2	2.0	7	0.2	0.2
3	1.0	8	0.14	0.19
4	0.8	8	0.3	0.25
5	4.0	7	0.32	0.3
6	3.0	6	0.23	0.23
7	2.2	6	0.22	0.2
8	2.2	7	0.2	0.22
9	3.0	7	0.2	0.23
10	3.5	7	0.2	0.2

in any regular arrangement (Table 2). Similarly, 8 to 12 leaves present on 10 nodes in the underground stem of *E. ramosissimum* are also arranged irregularly on different nodes (Table 3).

The articulated aerial stem in two species differs markedly in general morphology. The average height of aerial axis in *E. diffusum* is 51.57 cm and bears an average number of 22 nodes. At each node are present 4-8 lateral branches in a whorl. On the other hand, the slender-unbranched aerial axis in *E. ramosissimum* attains an average height

Table 3. Length of internodes, number of leaves on nodes and diameter of different internodes (at the anterior and posterior ends) in the underground rhizomatous stem of *E. ramosissimum*

Internode number (from posterior end to anterior end)	Length of 10 internodes in underground stem (cm)	Average no. of leaves present on the node on respective internode	Diameter of internode at posterior end (cm)	Diameter of internode at anterior end (cm)
1	3.6	11	0.45	0.52
2	4.5	12	0.42	0.55
3	4.4	11	0.45	0.53
4	2.9	10	0.43	0.54
5	3.7	11	0.44	0.53
6	3.0	8	0.50	0.54
7	3.0	8	0.50	0.54
8	2.0	9	0.50	0.60
9	1.1	10	0.44	0.60
10	0.5	9	0.40	0.50

of 172.83 cm in Himalayan climate and bears an average of 26 nodes (Fig. 2D). The aboveground axis of *E. diffusum* is made up of 24 internodes which are jointed with one another at 22 nodes. The lower two internodes are smaller than internode no. 3 (3.96 cm) but the upper ones subsequently become smaller (Table 5), the terminal being only 0.76 cm long. Length of internodes from base to apex is highly variable in *E. ramosissimum* (Fig. 2D; Table 6), which increases from the lowest (2.05 cm) to internode number 10 (9.24 cm) beyond which the length decreases gradually in upper internodes. However, the decrease in length is not in any regular proportion (Table 6). Terminal being only 2.15 cm long. Internodes in both the species are very weakly attached to one another, can be detached by applying little force or sometimes are dislodged by speedy winds.

The average number of leaves at the lowest node (here marked as 1) in *E. diffusum* is 7 (Figs. 2C, 5A; Table 4) and in *E. ramosissimum* is 10 (Figs. 2E, 5B; Table 4). In *E. diffusum*, the leaf number

remains constant on basal 10 nodes (7) beyond which the number of leaves decreases gradually (Fig. 2C; Table 4). Only 3 leaves are present on distal three nodes (Fig. 2C). In *E. ramosissimum*, the number of leaves on upper nodes (2nd upward) increases to 23 up to node number 13 to 16 beyond which the leaf number decreases gradually which on ultimate node (no. 26) is 17.

Diameter of aerial stem from base to apex is highly variable in two species: For example, in *E. diffusum* the basal internode is 0.26 cm thick at its base as well as at its apex but the next two (numbers 2 and 3) are 0.26 cm thick at the base and 0.25 cm at its apex. Thickness of internode no. 4 is 0.27 cm at base and 0.26 cm at its apex. Thickness of internodes decreases gradually in upper internodes, terminal being only 0.061 cm thick at the base and 0.01 cm thick at the tip (Fig. 4A; Table 5). Diameter of internodes also varies from base to apex in *E. ramosissimum*, the diameter of basal internode is 0.44 cm both at the apex and at its base but in the next above internode,
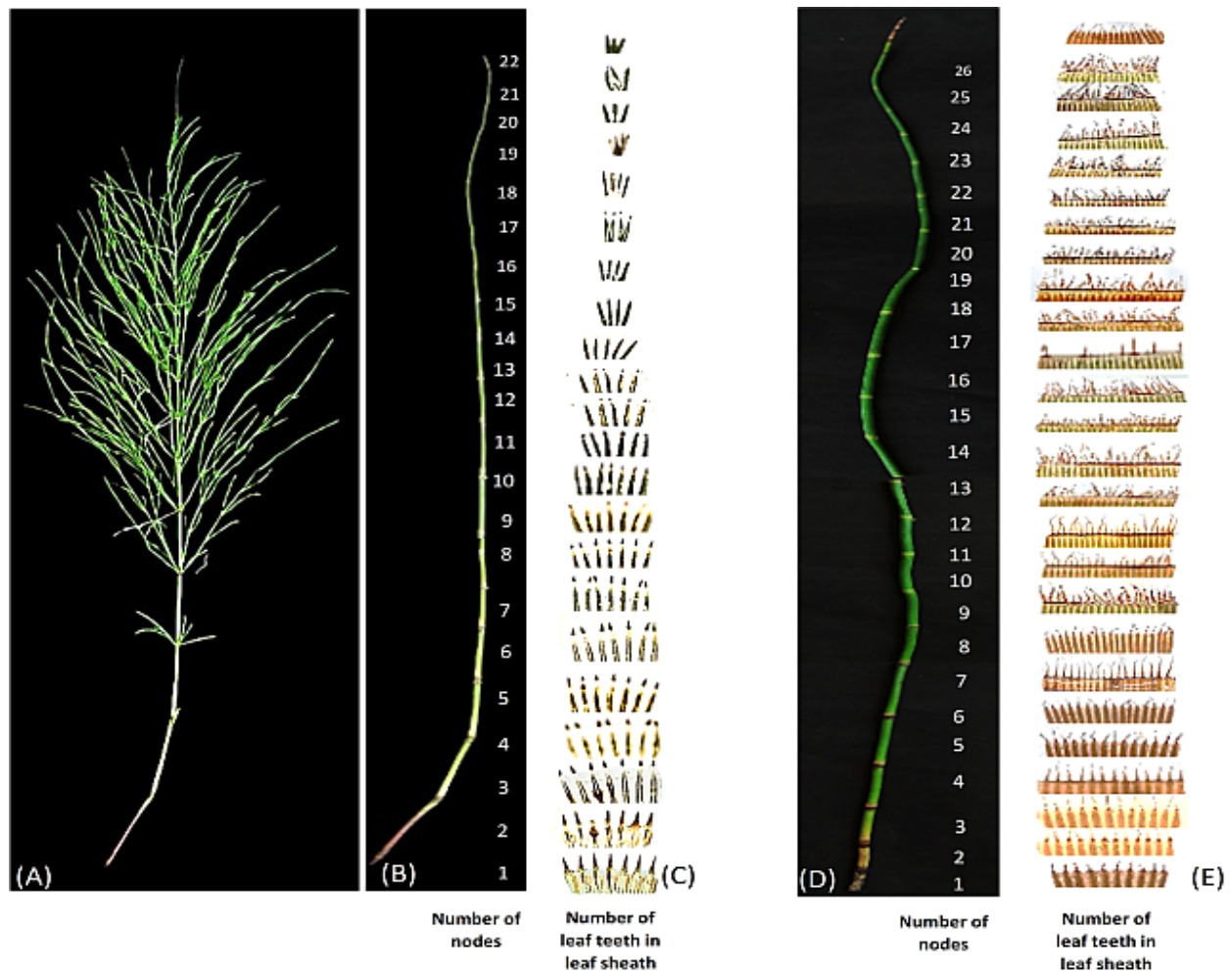
Fig. 2. Branched aerial stem of *E. diffusum* (A), number of nodes on aerial stem (B), number of leaf teeth in each leaf sheath on different nodes (C), unbranched aerial stem of *E. ramosissimum* with 26 nodes (D), number of leaf teeth in each leaf sheath on different nodes (E)
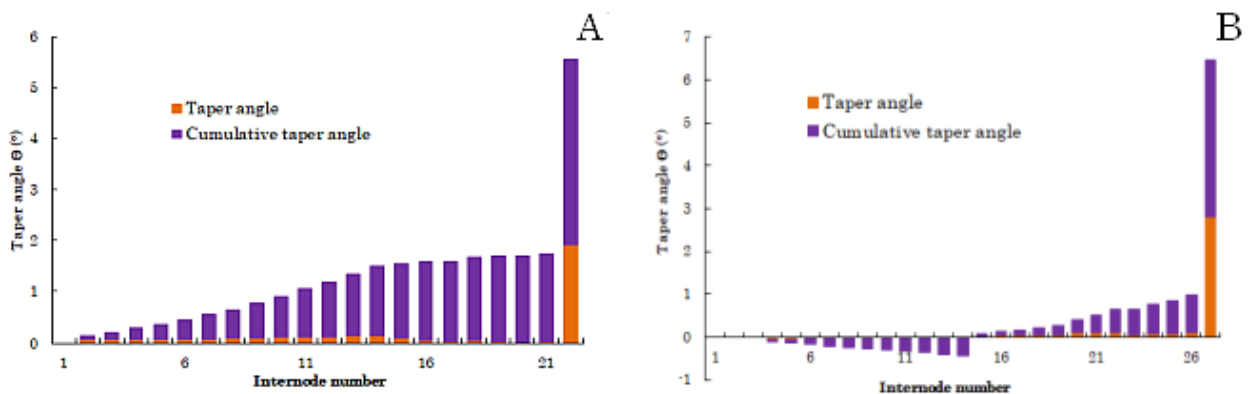
the basal diameter is 0.46 cm and at its apex it is 0.45 cm (Fig. 4B; Table 6). Increase in diameter continues up to internode number 11 which measures 0.51 cm at the base and 0.50 cm at its apex. Same thickness is observed in next two upper internodes (numbers 12 and 13). Decline in diameter starts from internode number 14, the basal diameter of which is 0.50 cm and at the distal end it is 0.49 cm. The terminal internode is 0.22 cm thick at its base and 0.01 cm at the apex (which is covered by whorls of scaly leaves), some adjacent intermediate internodes, though may be of equal diameter.

Length of internodes, which determines the taper angle in the aerial stem, varies significantly from base to apex. The basal internode in *E. diffusum* is 2.36 cm long but the longest internode (no. 3) is 3.96 cm long (Table 5). Length of internodes in this species decreases upward, being only 0.76 cm in the terminal internode (no. 22). On the other hand the basal internode in

Table 4. Average number of leaves on different nodes in the aerial stem of *E. diffusum* and *E. ramosissimum*

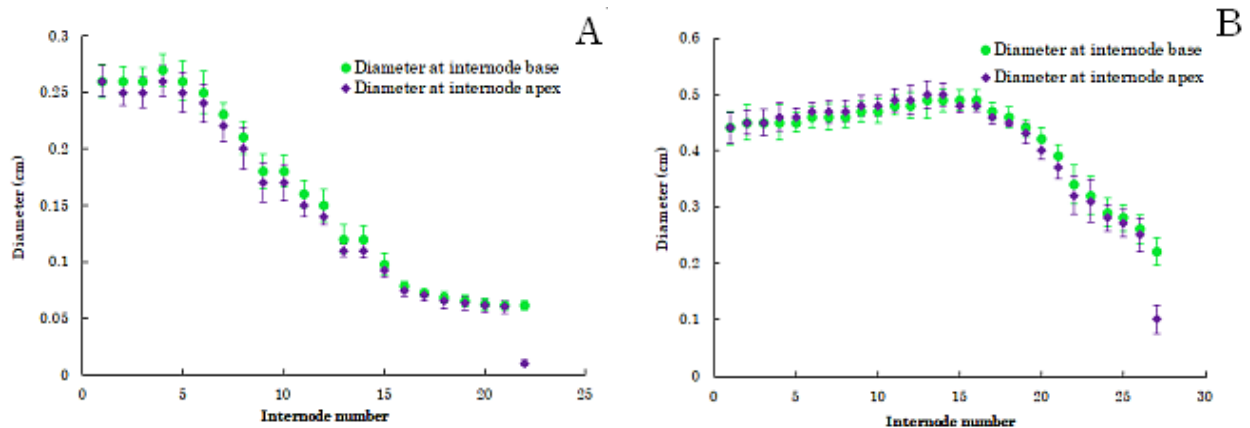| *E. diffusum*		*E. ramosissimum*	
Node number (from base to apex)	Number of leaves	Node number (from base to apex)	Number of leaves
1 - 10	7	1	10
11, 12	6	2, 3	12
13, 14	5	4	13
15 - 19	4	5	14
20 - 22	3	6	16
		7	17
		8	19
		9	20
		10 - 12	22
		13 - 16	23
		17 - 20	22
		21	21
		22, 23	20
		24	19
		25	18
		26	17

Fig. 3. Relationship between taper angle and cumulative taper angle in: *E. diffusum* (A), *E. ramosissimum* (B)

E. ramosissimum is 2.05 cm long whereas the next above is 3.48 cm. Internode number 10 is longest measuring 9.24 cm where after, the length in upper internodes gradually decreases; the terminal one being only 2.15 cm long (Table 6).

Taper angle value for lowest internode (no.1) is zero in *E. diffusum*. However, the values vary for subsequently upper internodes from 0.0762° (on internode no.2) to 0.0311° (on internode no 21). Largest taper angle formed on the

Table 5. Length of internodes, diameter, taper angle, and cumulative angle in the aerial stem of *E. diffusum*

Internode number (from base to apex)	Length of internode (cm)	Diameter of internode (cm)		Taper angle Θ (°)	Cumulative taper angle (°)
		at base	at apex		
1	2.36 ± 0.3772	0.26 ± 0.0146	0.26 ± 0.0134	0	0
2	2.36 ± 0.1166	0.26 ± 0.0128	0.25 ± 0.0115	0.0762	0.0762
3	3.74 ± 0.2204	0.26 ± 0.0116	0.25 ± 0.0131	0.0721	0.1483
4	3.96 ± 0.2158	0.27 ± 0.0139	0.26 ± 0.0136	0.0739	0.2222
5	3.86 ± 0.2891	0.26 ± 0.0176	0.25 ± 0.0176	0.0773	0.2995
6	3.70 ± 0.2767	0.25 ± 0.0190	0.24 ± 0.0167	0.0830	0.3825
7	3.44 ± 0.1208	0.23 ± 0.0102	0.22 ± 0.0139	0.0876	0.4701
8	3.26 ± 0.1435	0.21 ± 0.0143	0.20 ± 0.0181	0.0953	0.5654
9	3.00 ± 0.1449	0.18 ± 0.0152	0.17 ± 0.0168	0.1060	0.6714
10	2.70 ± 0.2121	0.18 ± 0.0143	0.17 ± 0.0156	0.1164	0.7878
11	2.46 ± 0.1691	0.16 ± 0.0120	0.15 ± 0.0101	0.1267	0.9145
12	2.26 ± 0.1288	0.15 ± 0.0137	0.14 ± 0.0067	0.1323	1.0468
13	2.16 ± 0.0400	0.12 ± 0.0124	0.11 ± 0.0058	0.1491	1.1959
14	1.92 ± 0.0374	0.12 ± 0.0116	0.11 ± 0.0067	0.1461	1.342
15	1.96 ± 0.1326	0.098 ± 0.0096	0.092 ± 0.0051	0.0987	1.4407
16	1.74 ± 0.1630	0.078 ± 0.0048	0.074 ± 0.0050	0.0662	1.5069
17	0.73 ±0.1700	0.072 ± 0.0037	0.070 ± 0.0050	0.0332	1.5401
18	1.72 ± 0.1529	0.068 ± 0.0048	0.065 ± 0.0064	0.0596	1.5997
19	1.44 ± 0.2204	0.065 ± 0.0049	0.063 ± 0.0058	0.0447	1.6444
20	1.28 ± 0.2332	0.062 ± 0.0048	0.061 ± 0.0057	0.0238	1.6682
21	1.20 ± 0.2408	0.061 ± 0.0039	0.06 ± 0.0054	0.0311	1.6993
22	0.76 ± 0.1720	0.061 ± 0.0039	0.01 ± 0.0024	1.9217	3.621

Fig. 4. Diameter of internodes in aerial stem in: *E. diffusum* (A), *E. ramosissimum* (B)

terminal internode measures 1.9217°. The cumulative taper angle in the species measured is 3.621° (Fig. 3A, 5A; Table 5).

On the other hand, the taper angle value for basal three internodes is zero in *E. ramosissimum* but on internodes between

Table 6. Length of internodes, diameter, taper angle, and cumulative angle in the aerial stem of *E. ramosissimum*

Internode number (from base to apex)	Length of internode (cm)	Diameter of internode (cm)		Taper angle Θ (°)	Cumulative taper angle (°)
		at base	at apex		
1	2.05 ± 0.4609	0.44 ± 0.0284	0.44 ± 0.0271	0	0
2	3.48 ± 0.4509	0.45 ± 0.0320	0.45 ± 0.0213	0	0
3	4.92 ± 0.4684	0.45 ± 0.0231	0.45 ± 0.0237	0	0
4	5.82 ± 0.4115	0.45 ± 0.0320	0.46 ± 0.0248	-0.0492	-0.0492
5	6.68 ± 0.3196	0.45 ± 0.0165	0.46 ± 0.0162	-0.0429	-0.0921
6	7.24 ± 0.3709	0.46 ± 0.0188	0.47 ± 0.0160	-0.0396	-0.1317
7	7.66 ± 0.2600	0.46 ± 0.0233	0.47 ± 0.0201	-0.0374	-0.1691
8	8.32 ± 0.3992	0.46 ± 0.0196	0.47 ± 0.0191	-0.0344	-0.2035
9	8.92 ± 0.3337	0.47 ± 0.0182	0.48 ± 0.0177	-0.0321	-0.2356
10	9.24 ± 0.3535	0.47 ± 0.0214	0.48 ± 0.0182	-0.031	-0.2666
11	9.00 ± 0.3224	0.48 ± 0.0169	0.49 ± 0.0200	-0.0318	-0.2984
12	8.94 ± 0.4883	0.48 ± 0.0231	0.49 ± 0.0252	-0.032	-0.3304
13	8.60 ± 0.4501	0.49 ± 0.0332	0.50 ± 0.0243	-0.0333	-0.3637
14	8.28 ± 0.4684	0.49 ± 0.0207	0.50 ± 0.0193	-0.0346	-0.3983
15	7.76 ± 0.6569	0.49 ± 0.0182	0.48 ± 0.0135	0.0369	0.0369
16	5.63 ± 0.3075	0.49 ± 0.0170	0.48 ± 0.0104	0.0509	0.0878
17	7.00 ± 0.2181	0.47 ± 0.0151	0.46 ± 0.0124	0.0409	0.1287
18	6.54 ± 0.1720	0.46 ± 0.0182	0.45 ± 0.0063	0.0438	0.1725
19	5.92 ± 0.1881	0.44 ± 0.0159	0.43 ± 0.0181	0.0484	0.2209
20	5.72 ± 0.2989	0.42 ± 0.0200	0.40 ± 0.0156	0.1002	0.3211
21	5.52 ± 0.3891	0.39 ± 0.0214	0.37 ± 0.0193	0.1038	0.4249
22	5.06 ± 0.4781	0.34 ± 0.0347	0.32 ± 0.0332	0.1132	0.5381
23	4.42 ± 0.3781	0.32 ± 0.0340	0.31 ± 0.0368	0.0648	0.6029
24	3.62 ± 0.3652	0.29 ± 0.0248	0.28 ± 0.0228	0.0791	0.682
25	3.34 ± 0.1913	0.28 ± 0.0230	0.27 ± 0.0243	0.0858	0.7678
26	2.70 ± 0.1720	0.26 ± 0.0248	0.25 ± 0.0295	0.1061	0.8739
27	2.15 ± 0.2459	0.22 ± 0.0242	0.1 ± 0.0244	2.7959	3.6698

4 and 14 it varies from -0.0492° to -0.0346°. Positive values of taper angle are obtained for internodes beyond internode no.15 which vary from 0.0369° to 0.1061° (internode no.26). A relatively large taper angle (2.7959°) is formed on terminal internode (Figs. 3B, 5B; Table 6). The cumulative taper angle for the complete length of aerial axis in this species is 3.6698° (Fig.3B).

Any significant difference in the diameter of internodes at their two ends (anterior or posterior) in the underground stem of two species is not observed, also the internodes of anterior or posterior parts of underground stem are of nearly equal diameter suggesting that thinning towards anterior end does not occur. Further, the leaf number neither decreases regularly on successive anterior nodes nor does in the posterior nodes.

These morphometric studies on the constitution of aerial axes in two species of *Equisetum* of the present study agree
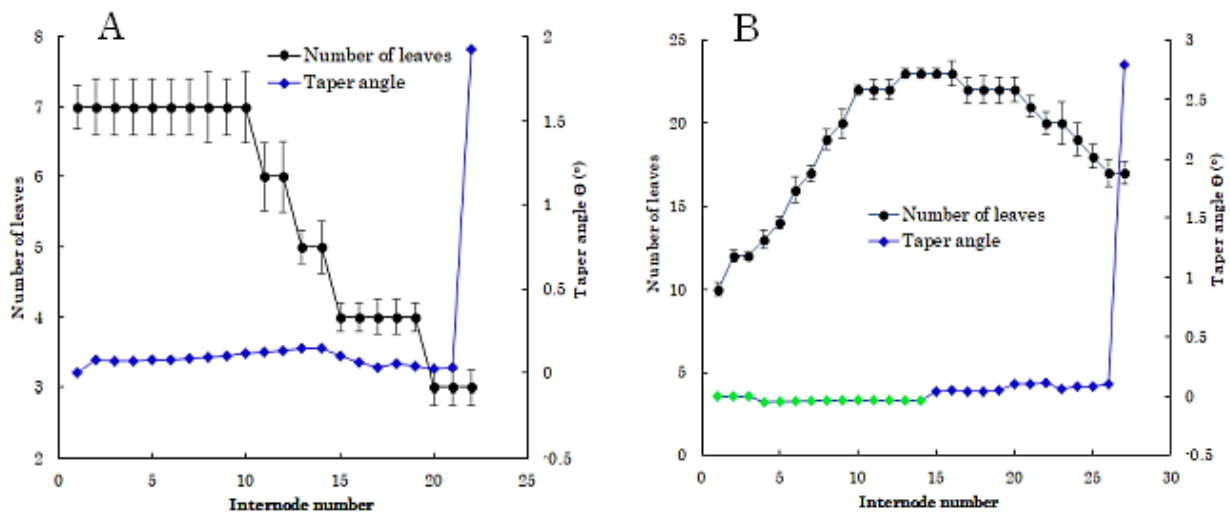
Fig. 5. Corelation between number of leaves on nodes of those internodes on which taper angles are formed: *E. diffusum* (A), *E. ramosissimum* (B)

partly with the morpho-anatomical studies of Bierhorst (1958) on nine species of this genus. Apart from the number of leaves present on every node, Bierhorst also emphasized on the thickness of internodes in shaping the aerial stem like a cone. Our observations reveal that the length of each internode also contributes independently in determining the shape of aerial stem in this fern. Leaves in *Equisetum* are the product of apical cell (Golub & Wetmore, 1948; Bierhorst, 1971; Gifford & Foster, 1989) and, their number for any particular node is decided much earlier in the growth of aerial axis (i.e., at the time of formation of a particular node), whereas length and thickness of internodes depend on the activity of intercalary meristem (present at the base of adjacent upper internode). Average number of leaves on first 10 nodes (from base) is 7 in *E. diffusum,* this number subsequently decreases on upper nodes, being only 3 on the last node (number 22). Except for the basal internode (number 1), thickness of which is equal at both the ends, the internodal thickness at two ends in subsequent upper

internodes (2nd upward) is different i.e., the distal (upper) end of every internode is always thinner than its basal end. Further, thickness of distal (upper) internodes gradually decreases though, occasionally, the thickness of adjoining two internodes may be equal. This leads distal attenuation of the axis in *E. diffusum*. The other factor which apparently associated with the phenomenon is the variable length of internodes.

The results for *E. ramosissimum* are altogether different from *E. diffusum* both in the number of leaves on different nodes and in internodal diameter at both the ends of each internode. In *E. ramosissimum* the number of leaves is higher on middle nodes and the diameter of middle internodes is not only larger but the diameter at upper end (distal end) of any particular internode is also larger than its lower end (basal end). These two attributes are encountered up to node number 14 causing attenuation of axis towards base. Number of leaves decline from node 17 upward being lowest (17) on terminal node (number 26). This is

accompanied by the reduction in the internodal diameter at both the ends of every internode as well as of subsequent upper internodes which results into distal attenuation of the aerial stem. Thus, less than upper half of the aerial axis is tapered towards apex and more than lower half towards the base.

Niklas (1989) compared basal tapering of unbranched *E. hyemale* shoot with the tapered base of extinct *Archaeocalamites* which is often treated as a common ancestor for Calamitaceae and Equisetaceae (Boureau, 1964; Bierhorst, 1971; Gifford & Foster, 1989). Any precise information on basal tapering of calamitacean axis is not available, instead secondary growth in thickness as known in the calamitacean trunk is considered an obvious biomechanical support system evolved to strengthen the stout, tall and profusely branched axes (Eggert, 1962). Features like arboreal habit and secondary growth in the trunk are though lost in the long evolutionary history of equisetalean lineage (Bateman, 1991; Stewart & Rothwell, 1993), distal attenuation of axis in *Equisetum* is retained. Any special tissue for biomechanical support is not present in the aerial stems of herbaceous extant taxa of this genus but observations on *E. hyemale* indicate that flexural rigidity of the shoot is significantly influenced by nodal septa (Niklas, 1989). Interestingly, of the two taxa, tapering towards stem base occurs only in *E. ramosissimum* and not in *E. diffusum*. Tapering towards apex in *Calamites* aerial shoot is figured by some authors (Meyen, 1987; Gifford & Foster, 1989) but any detail account on tapering of aerial shoot towards apex in *Calamites* and *Archaeocalamites* is not available. However, this feature is clearly known in first land plants like *Rhynia gwynne-vaughanii* Kidston & Lang and *Aglaophyton major* (Kidston & Lang) Edwards (Kidston & Lang, 1917, 1920; Edwards, 1986) suggesting that attenuation of aerial axis in land flora appeared much earlier than the origin of sphenopsids and evolved concomitantly with shifting of plants towards land surface. Whether this feature was evolved to overcome the environmental stresses like wind exposure or to facilitate mineral and nutrient transport, is not known clearly but it was apparently relayed through different plant groups (including extant equiseta) with the expansion in plant body plans.

Most literature pertaining to research on stem taper is restricted to trees especially forest trees (Morgan & Cannell, 1994; Mencuccini et al., 1997; Gomat et al., 2001; McTague & Weiskittel et al., 2021) but the shoot axis in any of the investigated tree is not as distinctly articulated as in *Equisetum*. Therefore, observations on *Equisetum* appear to be of interest because the optimal results on thinning are obtained through naturally segmented axis. Length and differential internodal diameter (difference in the diameter of an internode at its two ends) determine the taper angle on that particular internode: in the event when the differential diameter of an internode is zero i.e., the internode is cylindrical, any possibility of attenuation can not be expected, no matter how long the internode is. This is observed in basal three internodes of *E. ramosissimum*. The lowest internode in this taxon is thin as compared to the next two above. This is the beginning of tapering towards base in this particular species. However, in upper internodes (internode numbers 4 to 14), the upper ends are thicker than their lower ends resulting into taper angle

values in negative. Thus the taper angle formed on internode number 4 (-0.049°) is larger than that on internode number 14 (-0.034°). Variation in internodal length between internodes 4 to 14 determine the magnitude of taper angle formed on these internodes. In the event when the differential internodal diameter of any two internodes on the aerial stem is same but the length of these two internodes is different, the taper angle on short internode will be large i.e., the taper angle is inversely proportional to the length of that particular internode. On the other hand, lower ends of each internode beyond internode number 14 are thick compared to their upper ends resulting into upward tapering of the aerial stem which is in contrast to tapering towards base in internode nos. 4 to 14. Variable lengths of internodes between 15 and 26 also affect the values of taper angles for different internodes, for example, the taper angle formed on 7.76 cm long internode number 15 is 0.0369° but on 2.7 cm long penultimate internode, it is relatively large (0.1061°). An abrupt attenuation of ultimate internode (number 27) results into a large taper angle (2.79°) which adds largely to the cumulative taper angle (3.66°) of a 65.38 cm long upper half of the aerial axis in *E. ramosissimum.*

Except on lowest internode, which is cylindrical in shape, a taper angle is formed on every internode in *E. diffusum* also, the magnitude of which is determined by the length of every internode and its differential internodal diameter. Therefore, the value of taper angle differs for every internode. An average cumulative taper angle up to penultimate internode in *E. diffusum* is 1.6993° but a sudden increase in the cumulative taper angle (3.621°) is on account of an abrupt attenuation towards apex in the ultimate

internode (number 22). Tapering of aerial stem in *E. ramosissimum* is strikingly different from *E. diffusum*: in the former, only 40.23% of distal part of 172.83 cm long aerial stem is tapered upward whereas lower 59.76% part tapers downward (basal tapering) but in *E. diffusum* complete aerial stem (51.6 cm) tapers upward. Any major difference in the cumulative taper angle is not observed in the two species which are morphologically very distinct.

Among the features of interest in the aerial and underground stem of *Equisetum* is variation in leaf numbers on different nodes even sometimes on adjoining nodes but only few morphologists paid attention on this change. In small parts of aerial stem of *E. kansanum* (= *E. laevigatum* ?), Browne (1939) described changes in the number of leaves in some nodes but Golub & Wetmore (1948) found frequent changes in leaf numbers in *E. arvense*. Based on anatomical studies on aerial stem of nine species of this genus, Bierhorst (1959) stressed on the importance of change in leaf number on nodes in the constitution of aerial stem in *Equisetum* including tapering of aerial stem. We found that the change in leaf number on different nodes does occur but in an orderly manner and, that the number of leaves on nodes is not a determining factor of tapering of aerial axis. For example, an average of 7 leaves are present on lower 10 nodes in *E. diffusum* but upward thinning of every internode resulted into a cumulative taper angle of 0.7878° on internode number 10, more precisely, even if there is no change in leaf number, there is always a reduction in diameter of upper internodes. Number of leaves on upper internodes may be reduced from node to node or may remain unchanged on upper few nodes and then

reduce again on remaining upper nodes but this is not in accordance with gradual tapering of respective internodes which is a fact in the aerial stem of *E. diffusum* (Fig. 5A).

The relationship between leaf number on nodes and tapering of internodes bearing these nodes in the aerial stem of *E. ramosissimum* is very complex. In contrast to *E. diffusum*, the number of leaves is lowest on basal node (no. 1) and highest on middle nodes (nos. 13-16) but the diameter of internodes bearing these nodes increases subsequently from lower internodes to internode no. 14 in *E. ramosissimum*. Although the average diameter of internode nos. 15 and 16 is equal at their two ends and the number of leaves on nodes of these two internodes is also same (23), taper angles of different magnitudes are formed due to the difference in the length of these two internodes (15 and 16). From here starts upward tapering of aerial stem in this species. Higher up in the axis, four subsequent nodes (17 to 20) bear same number of leaves (22) but the taper angles formed on the internodes bearing these nodes vary from $0.0409°$ to $0.1002°$. Due to reduction of one leaf each from every node beyond node number 21, only 17 leaves are present on ultimate node no. 26 but the change in taper angle on respective internodes is not proportionate to this change in leaf number. For example, a taper angle of $0.1038°$ is formed on 5.52 cm long internode no. 21 but a relatively large taper angle of the magnitude of $0.1132°$ is formed on 5.06 cm long internode number 22 and again, a relatively small taper angle ($0.0648°$) is formed on 4.42 cm long internode no. 23. It is thus apparent that there is no relation between number of leaves present on any particular node and magnitude of tapering of that particular internode which bears that node.

Tapering towards both ends (base and apex) from nearly middle of the aerial axis is an example of high level of coordination between activities of the apex and intercalary meristems of the middle internodes during the growth of aerial axis in *E. ramosissimum*. Equal number of leaves (23) are cut off by the apex early in the growth of the axis. On four nodes (13 to 26 in present case) but the diameters of two ends of these internodes bearing these nodes are decided by the cambium of each internode (present at the base of each internode) much later in the growth of the aerial axis.

Internode nos. 13 to 16, present approximately in the middle of the aerial stem, act as a transition zone as far as tapering of the aerial stem in this particular species is concerned. Each of the four nodes present on these internodes bears 23 leaves. Interestingly, the lower two internodes (13 and 14) taper towards base and the upper two (15 and 16) taper upward (Fig. 5B). Values of taper angles formed on internode nos. 13 and 14 are - $0.0333°$ and $−0.0346°$, respectively, and on internode nos. 15 and 16 are $0.0369°$ and $0.0509°$, respectively (Fig. 5B). Number of leaves on different nodes of lower half of the aerial stem is not related with tapering of internodes bearing those nodes though the change in leaf number is frequent. We were unable to detect any one leaf larger than others of the whorl as reported by Bierhorst (1959) in nine species of the genus. Though the two forms (aerial and underground) of the same plant are similar in general morphology (articulated stem differentiated into nodes and internodes, ridged internodes and presence of scale leaves on nodes), the growth habits in two forms are entirely

different. Detailed studies are required particularly to investigate behaviour of apical cell in cutting off of leaf initials in the underground stem of this genus. Another aspect on which further studies are required is to explore reasons characterise the thickening of the stem just below the apical region in this plant of archaic lineage.

Acknowledgement

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