CONTRIBUTIONS TO OUR KNOWLEDGE OF INDIAN FRESHWATER PLANTS

PART 2. ON SOME ASPECTS OF THE HABIT, STRUCTURE, LIFE-HISTORY AND AUTECOLOGY OF *LIMNANTHEMUM CRISTATUM* GRISEB. AND *LIMNANTHEMUM INDICUM* THW.*

by EVA MITRA, *Central Inland Fisheries Research Station, Calcutta*

(Communicated by B. S. Bhimachar, F.N.I.)

(*Received July 13; after revision August 25; approved for reading on October 7, 1955*)

INTRODUCTION

Limnanthemum cristatum Griseb. and *L. indicum* Thw. (family Gentianaceae) are widely distributed throughout India, but very little is known regarding their general morphology and autecology, apart from brief general descriptions in various systematic works (cf. Roxburgh, 1824; Bentham, 1869; Hooker, 1890; Prain, 1903; Biswas and Calder, 1937). Srinivasan (1942) has given a detailed account of the internal morphology of the flower, while Mukherjee (1951) has dealt with its cytology. In the present paper detailed descriptions of the two species, their habit, their external morphology, reproduction as also internal morphology and some of the important physico-chemical conditions of *L. cristatum* are described.

HABIT

Gentianaceae, a large family of herbaceous plants, is interesting owing to the range of its geographical distribution, which is from the extra-tropical regions to the hottest part of the tropics, and its habitat. The plants of this family are mainly terrestrial but according to Arber (1920) such marsh plant as *Menyanthes* might form a transition to the typically aquatic genus *Limnanthemum*.

According to Roxburgh (1824), Hooker (1834), Bentham (1869), Prain (1903) and Rendle (1938) species of *Limnanthemum* (*L. cristatum*, *L. indicum*, *L. peltatum*) are all free floating aquatics. Hooker (1890) states that some species like *L. nymphaeoides*, *L. cristatum*, *L. indicum* root at the nodes; *L. aurantiacum*, *L. forbesianum* do not root at the nodes and *L. parvifolium* has stems apparently rooted on mud. According to Biswas and Calder (1937) *L. cristatum* is a rooted aquatic, *L. indicum* has long, floating stems, rooting at the nodes and *L. parvifolium* is apparently rooted on mud at the base. In the course of the present study the author observed that both *L. cristatum* and *L. indicum* are predominantly rooted aquatics, the roots being present on the main tuber as well as at the nodes (Figs. 1 and 2). Only for a short period in their life-history they live as floating aquatics. In these species, each runner that comes out from the main underground rhizome, bears at its apex a fresh rhizome. These floating rhizomes develop roots by which they later attach themselves to the soil, become underground, and give rise to fresh runners. Some-

* Published with the kind permission of the Chief Research Officer, Central Inland Fisheries Research Station, Calcutta.

times, before the floating rhizomes could attach themselves to the soil, they get detached from the main body of the plant probably by wind action or some mechanical disturbance, float up and behave as floating aquatics. This process is repeated by successive generations of rhizomes and very often a number of such free floating plants could be observed in ponds.

To verify whether the plants when detached can live free floating for a considerable length of time, observations were carried out on healthy growing *L. cristatum* in a pond having a depth of 6 to 8 ft. at the centre. Some runners with fully developed young plants at their ends were severed from the mother plants and allowed to float freely within an artificial enclosure made in the deeper part of the pond so that they did not get the opportunity of coming into contact with the bottom mud. A number of young plants were also left in the pond * outside the enclosure as control. From the 5th to 6th day the leaves and roots of plants in the enclosure started decaying. From the 15th to 17th day they started sinking and between 18th and 20th day all of them sank to the bottom. These observations were repeated with confirmatory results. The young plants which were left in the tank as control, without being enclosed, reached the shallower regions and rooted themselves. The experiment was repeated with a few plants of *L. indicum* brought from Kaushalya Ganga, Puri and identical results were obtained. The plant is usually found as rooted aquatics in ponds with the depth of water varying from 3 to 8 ft., the length of the runners often ranging from 2½ to 7 ft. depending on the depth of the tank.

It may be concluded from the above observations that *L. cristatum* and *L. indicum* are both rooted aquatics and that they float only for a limited period of their life-histories. But, if by any chance, a free floating plant is unable to anchor itself to the soil within a limited time, it perishes.

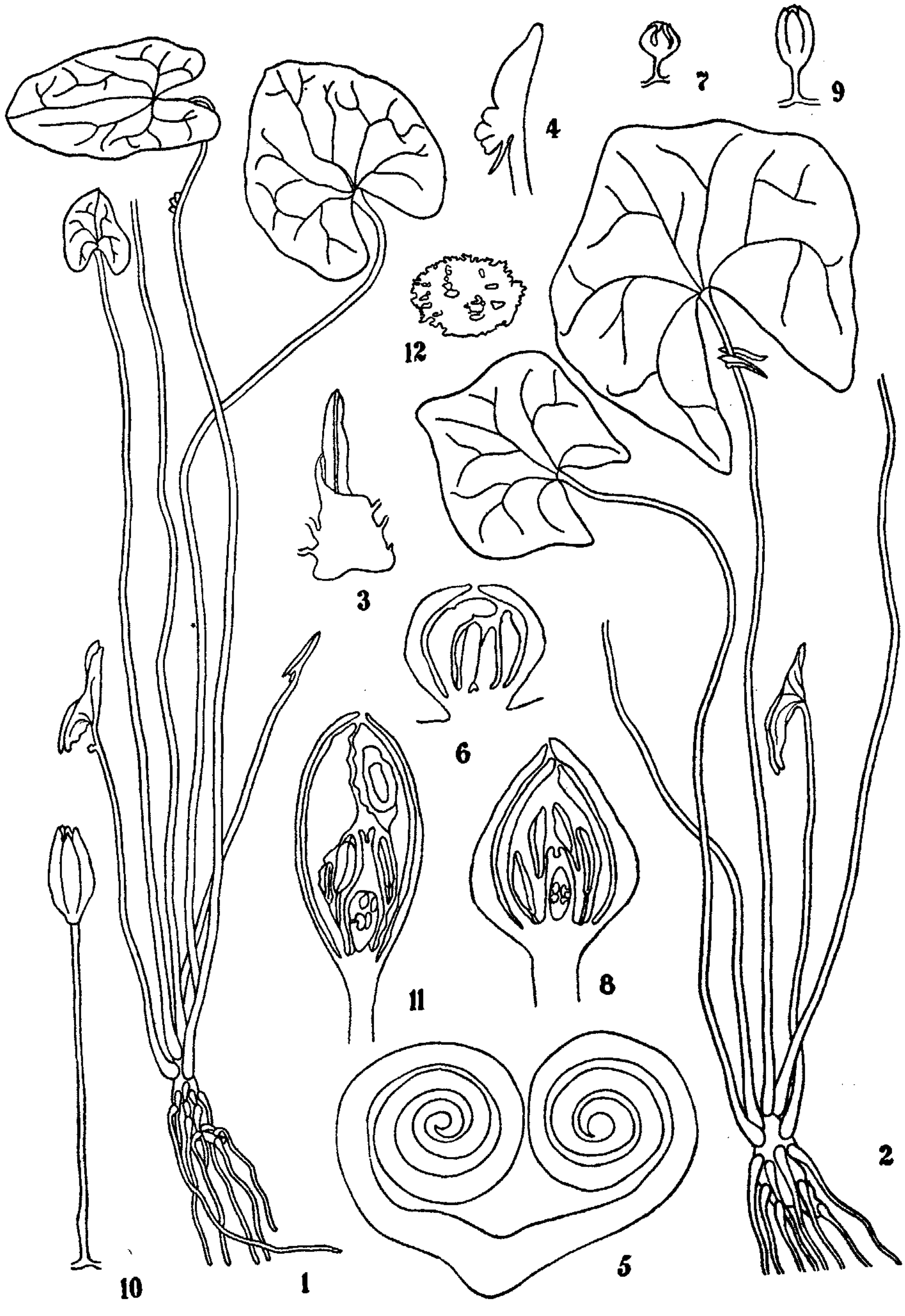
External morphology

The underground tuber has a thick, hard, protective bark-like covering which is absent when the tuber is exposed. The roots develop from the base of the tuber within the soil. The runners come out from that portion of the tuber which is jutting out of the soil. These runners, floating while attached to the rooted tuber on one side, are sometimes very long and develop foliage leaves at their tips. The inflorescence develops on the runner, a little below the foliage leaf giving it the appearance of a lateral outgrowth from the leaf, the entire runner appearing as the petiole. The shoot morphology was first described by Goebel (1891–93) and later by Arber (1920) who states 'In plants of *Limnanthemum*, examined at the flowering season, it is found that a long stalk given off from the rhizome appears to bear both a lamina and flowers or, in other words, that the flowers seem to arise laterally from a leaf stalk. In reality this long stalk is, however, the axis of the inflorescence. The petiole has a short, sheathing base, which in youth surrounds the inflorescence. In development, the foliage leaf pushes the growing point to one side and comes to occupy the terminal position. Goebel considers that this peculiar mode of growth confers a definite biological advantage. The breadth of the leaf surface resting on water gives the inflorescence the necessary support, while the elongated inflorescence axis forms a substitute for both the elongated petiole and peduncle.'

The runners developing from the floating tubers are very thin, delicate and light green in colour. The fully formed runners developing from underground tubers are generally short and thick, ranging from 10·7 to 21·4 mm. in length.

Each leaf is entire, orbicular or deeply cordate developing independently on the runners. In *L. cristatum* the leaves are thinner with prominent venation whereas in *L. indicum* they are thicker with indistinct veins. In both the species the

* The experiment was carried out in a nursery pond at Cuttack (Orissa).



FIGS. 1—*Limnanthemum cristatum* Griseb. Plant ½ Nat. size; 2—*Limnanthemum indicum* Thw. Plant ⅓ Nat. size; 3—Growing point of the leaf coming out of the sheathing base (×25); 4—Leaf primordium enclosed within a sheath-like structure (×25); 5—Involute vernation of young foliage leaf (×57); 6—L.S. of early rudiment of flower bud (×57); 7—Flower bud with the pedicel developed (×57); 8—L.S. of flower bud with stamens and carpels (×214); 9—A fully formed bud (×57); 10—A bud with fully formed pedicel (×57); 11—L.S. of a fully formed bud (×214); 12—A seed (×214).

leaf primordium is about 1 mm. long (Fig. 3) and first develops on the rhizomes within a colourless, spathe-like structure with the primordium of the flower buds just at the base of the small folded lamina (Fig. 4). Below the rudiment of the inflorescence the runner elongates and comes out of the spathe. With this the leaf also increases in size and gradually opens out. In the bud the vernation of the lamina is involute (Fig. 5). When the lamina opens out it reveals 5 veins. The length of the lamina of *L. cristatum* varies from 35 to 93 mm. and width from 61 to 135 mm.; whereas in *L. indicum* it varies from 90 to 125 mm. and 150 to 175 mm. respectively.

In both the species the mature roots developing from underground tuber are 130 to 170 mm. in length and 10 to 12 mm. in width. In *L. cristatum* the adventitious roots that are present at the base of the floating rhizome are 3·4 to 14·8 mm. in length and 1·0 to 4·0 mm. in width. None of the roots possess root caps.

Reproduction

Both the species reproduce vegetatively as well as sexually. But vegetative reproduction is more frequent and abundant than sexual reproduction.

Vegetative reproduction continues almost throughout the year but more vigorously from February to the end of June. Development of vegetative buds on the underground, rooted tubers, which form runners and then develop at their ends, floating tubers. These tubers float only as long they are attached to the rooted parent plants by runners, later they break away from the runner by some external agency and float away. Also very commonly vegetative buds are formed on the floating tubers and in the axils of leaves. These buds individually develop into runners and thus help in the ramification of the plants on which they are formed. Each of these runners again bear in turn on the free ends small tubers with leaves and inflorescence. These floating tubers when come in contact with substratum send out roots and each continues to exist as an independent, rooted plant. Thus repeated branching by means of vegetative buds enable a plant to explore the surrounding expanse of water with a close mosaic of leaves on the surface of the water. According to d'Almeida (1928) *Limnanthemum* is a type of water plant which is intermediate in habit between the *Nymphaea* type which is rooted in the mud and the floating type whose roots do not penetrate the soil but hang freely in the water. Sexual reproduction is carried on by seeds.

Development of the Inflorescence and Flowers

As mentioned elsewhere in this paper, in both the species the inflorescence develops on the runner just at the base of the petiole. The inflorescence in both these species is of the racemose type and may be described as a corymb with a very short main axis as shown by d'Almeida (1928). In *L. cristatum* the early rudiment of the flower bud, when about 1 mm. in length, is almost round in shape and without a stalk. Longitudinal sections of this rudiment show only the primordia of sepals, petals and stamens (Fig. 6). It is without a stalk even when 1·5 mm. in length. When 2 mm. in length a pedicel which is about 1 mm. long is also developed (Fig. 7). Longitudinal section of this pedicelled bud shows the fully developed stamens and the carpels which have just started development with rudimentary ovules (Fig. 8). The pedicel elongates further to about 2 mm. It does not elongate further till the bud attains a length of about 3 mm. (Fig. 9). The bud thereafter elongates gradually to about 5 mm., while the pedicel grows rapidly to about 18 to 19 mm. (Fig. 10). In the fully formed bud the characteristic shape of the petals and the stamens with clearly differentiated filaments and anthers are easily seen (Fig. 11). In the flowers the length of the pedicel varies from 16 to 45 mm. No observation is on record as to how many days it takes from the first stage of flowering up to the formation of seed and germination of those seeds. In nature the flowers of aquatic plants after being pollinated shed their petals, then the pedicels bend and go in the deeper layers of water, where the seeds mature and get dispersed. In a pond due to the presence of innumerable plants and also many other factors the study on the formation and germination of seeds is hampered very much. So studies on this aspect were conducted under controlled conditions.

A flower normally takes 33 to 43 days from the initial stage up to seed formation. From the first stage of flower formation to the opening up of the floral parts takes 15 to 18 days. The flowers remain in full bloom for 3 to 4 days, at the end of which, only the petals fall off. After the shedding of the petals the ovaries gradually increase in size and after 5 to 6 days the pedicels bend down carrying the mature ovaries with the persistent sepals in the deeper layers of water. Then after 10 to 15 days the ovaries dehisce and the seeds are dispersed. Each ovary might be having 5 to 6 ovules but generally only one seed is fully formed. Sometimes two seeds are also found in an ovary but that is very seldom. When the petals of flowers are shed, cloth bags are tied round the mature ovaries and the seeds are collected in the cloth bags. After the seeds have collected in the cloth bags they are immediately planted in a small earthenware tub having 1 inch sterilized soil at the bottom and 2 inches of water. Each seed is globular in shape with a minute stalk (Fig. 12). On the body of the seed very small, spinous projections are present. The length of the stalk varies from 0·25 to 0·5 mm. Seeds are 1·5 to 2·0 mm. in diameter.

The flower of *L. cristatum* is pentamerous (Figs. 13 and 14). In *L. indicum* the number of floral parts varies from 5 to 7 (Figs. 15, 16 and 17).

External morphology of fully developed flowers of *L. cristatum* and *L. indicum* is as follows:—

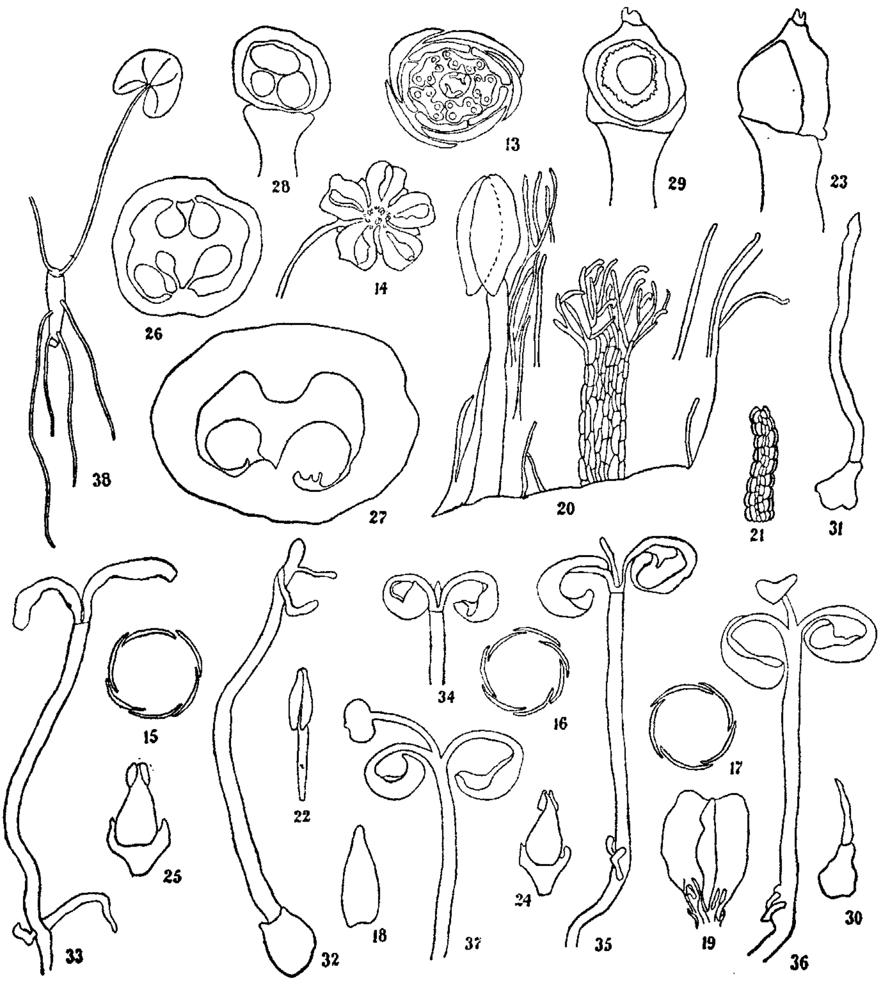
	L. cristatum	*L. indicum*
Calyx:	Sepals 5, united just at the base, free above with rounded apex, green with reddish margins (Fig. 18). Aestivation in bud imbricate.	Sepals 5 to 7, united at the base, green, acute apex, aestivation imbricate (Figs. 16 and 17).
Corolla:	Petals 5 (Fig. 19) joined at the base forming a very short tube, free above, valvate in bud and alternating with the sepals. Colour white except at the base where it is light yellow, each petal 7 to 10 mm. long, 5 to 8 mm. broad. A longitudinal fold present down the middle of each petal. Nectaries present on the corolla in 3 rows (Fig. 20). Outer row consists of white, hairy filaments at the mouth of the tube of the corolla. Middle one is a cluster of light yellow coloured, glandular bodies, which extend from the base up to almost the mouth of the tube and alternating with the filamentous nectaries. The third row is at the base of the tube and consists of small, white filaments. The two layers of cells in the centre of each filament have thickened	Petals 5 to 7, gamopetalous with a short, yellow, tubular portion at the base and 5 to 7 free lobes alternating with the sepals, upper parts of free lobes hairy. Aestivation valvate in bud. Nectaries absent.

	L. cristatum	*L. indicum*
	walls surrounded by a layer of bigger cells, only the outer walls of which are thickened (Fig. 21).	
Androecium:	Stamens 5, alternating with the petals, epipetalous, about 2·0 and 2·5 mm. long (Fig. 22). Anthers bilocular with short, linear filaments.	Stamens 5 to 7, epipetalous, united with the petals just at the base, alternating with the petals, anthers versatile and bilocular.
Gynoecium:	Carpels 2, united, superior, unilocular ovary (Fig. 23). In young flowers the stigma is sessile and directly on the beak of the ovary. Green in colour with its apical portion bifid. In mature flowers the beak of the ovary elongates and curves slightly, bringing the free lobes of the stigma on one side of the ovary (Figs. 24 and 25). Placentation marginal (Fig. 26). The ovules are anatropous (Fig. 27). Ovules 6 to 10 in number, colourless when young, brown when half mature (Fig. 28), black when fully mature as seeds, which are spherical and have very thick testa (Fig. 29).	Carpels 3, united, superior, ridged, unilocular ovary. Stigma sessile, trifid. Placentation parietal. Ovules and seeds similar to those of *L. cristatum*.

Germination of seeds in *L. cristatum*

Twenty-five seeds were planted in a small earthenware tub, 6 inches in diameter, with 1 inch of sterilized soil and 2 inches of tap water. The earthenware tub was kept inside the laboratory, where the sunrays fell on it during the morning hours only, and the remainder of the day it was in the shade. The pond mud was sterilized by drying it stone hard in the strong sun for 2 weeks and then the normal temperature was brought back by keeping it in the laboratory within the small tub for a few days. During this period the mouth of the tub was covered with a cloth tied round it, so that the soil was not infected with any other external element. Later when the seeds were available they were planted in that tub.

Out of 25 only one seed germinated on the 92nd day. When germination started the seed was covered with mud and the hypocotyl was just protruding from the soil (Figs. 30 and 31). On the fifth day of the growth of the hypocotyl the small thin roots developed just below the hypocotyl apex (Fig. 32). Beyond the root the hypocotyl continued to elongate and on the 8th day morning the cotyledonary leaves opened out from the tip of the hypocotyl, hence it showed an epigeal germination. Up to this stage of development the seedling was in contact with the soil. Later the same afternoon the seedling broke off just below the roots from the seed and continued to survive as a floating aquatic (Fig. 33). On the 10th day morning a tip was found to develop in between the two cotyledonary leaves (Fig. 34). On the 12th day the tip had elongated more in size (Fig. 35). On the 15th day the foliage leaf though small in size was fully formed (Fig. 36). From the 17th day the seedling behaved as a rooted aquatic instead of a floating one (Fig. 37). So when the seed germinated the seedling behaved as a rooted aquatic for 7 days,



FIGS. 13—Floral diagram of *L. cristatum* (×214); 14—Flower of *L. cristatum* (×57); 15, 16, 17—Floral diagram of *L. indicum* (×214); 18—Sepal of *L. cristatum* (×57); 19—Petal of *L. cristatum* (×57); 20—Epipetalous stamen and 3 rows of nectaries (×214); 21—Filament of a nectary (×214); 22—A stamen (×57); 23—An ovary with the persistent sepals removed (×214); 24, 25—Mature ovaries (×57); 26—Placentation (×59); 27—Anatropous ovules (×312); 28—L.S. of half mature ovary (×214); 29—L.S. of mature ovary with one seed (×214); 30—Hypocotyl coming out of seed (×12); 31—Hypocotyl elongated (×12); 32—Roots developed a little below the hypocotyl apex (×12); 33—Hypocotyl elongated and the apex unfolding the cotyledons (×12); 34—Foliage developing in between the cotyledons (×12); 35—Foliage leaf more developed (×12); 36—Foliage leaf unfolded (×12); 37—Foliage leaf fully formed (×12); 38—*L. cristatum* grown on sand. Nat. size.

from the 8th day it behaved as a floating aquatic for 9 days and on the 10th day when root, foliage all were present the seedling started to behave again as a rooted aquatic.

Bertha Chandler (1910) worked on the germination of the seeds of *Utricularia emarginata* Benj. and had shown that the seeds flourished best in a shallow pan, having a thin layer of mud at the bottom with enough water to cover the mud. She had also shown that the best method of cultivation was to keep the plant in partial shade in still water.

Out of 25 seeds that were planted in the earthenware tub only one showed germination under similar conditions as shown by Chandler (1910), and which underwent no long dormant period. But the rest of the seeds had not shown germination and might have been in a dormant condition. Observations are being continued on their behaviour. Parija (1934) had shown that the seeds of Water Hyacinth remained dormant for at least one season, that is, November to June, and retained their viability for several years. Stiles (1936) writes that A. Fischer observed that mature seeds of a number of water plants such as *Sagittaria sagittifolia, Alisma plantago-aquatica, Hippuris vulgaris* and various species of *Potamogeton, Scirpus* and *Sparganium* did not germinate in pure water but did so readily in impure water containing bacteria.

Internal morphology of *L. cristatum*

The vegetative parts were fixed in formal acetic alcohol and after passing through the usual xylol-alcohol grades were embedded in paraffin and 10μ thick transverse and longitudinal microtome sections were cut. For the anatomical study the sections were stained in safranin and light green. Camera lucida drawings have been made.

I. Root

1. T.S. of mature floating root—Fig. 39.

Epidermis—One layered, thin walled cells.
Hypodermis—One layered and parenchymatous.
Cortex—Many layered, loosely arranged, rounded cells with intercellular spaces in between.
Aerenchyma—Big air-spaces partitioned by one layered partition walls.
Endodermis—One layered, closely arranged, small cells.
Central Cylinder—Vascular bundles are arranged radially. There are 7 groups of vessels and each group having 3 to 6 vessels and few phloem cells arranged in between the xylem groups. Small parenchymatous cells present in between the xylem and phloem cells.
Pith—Composed of parenchymatous cells.

2. L.S. of floating root tip—Fig. 40.

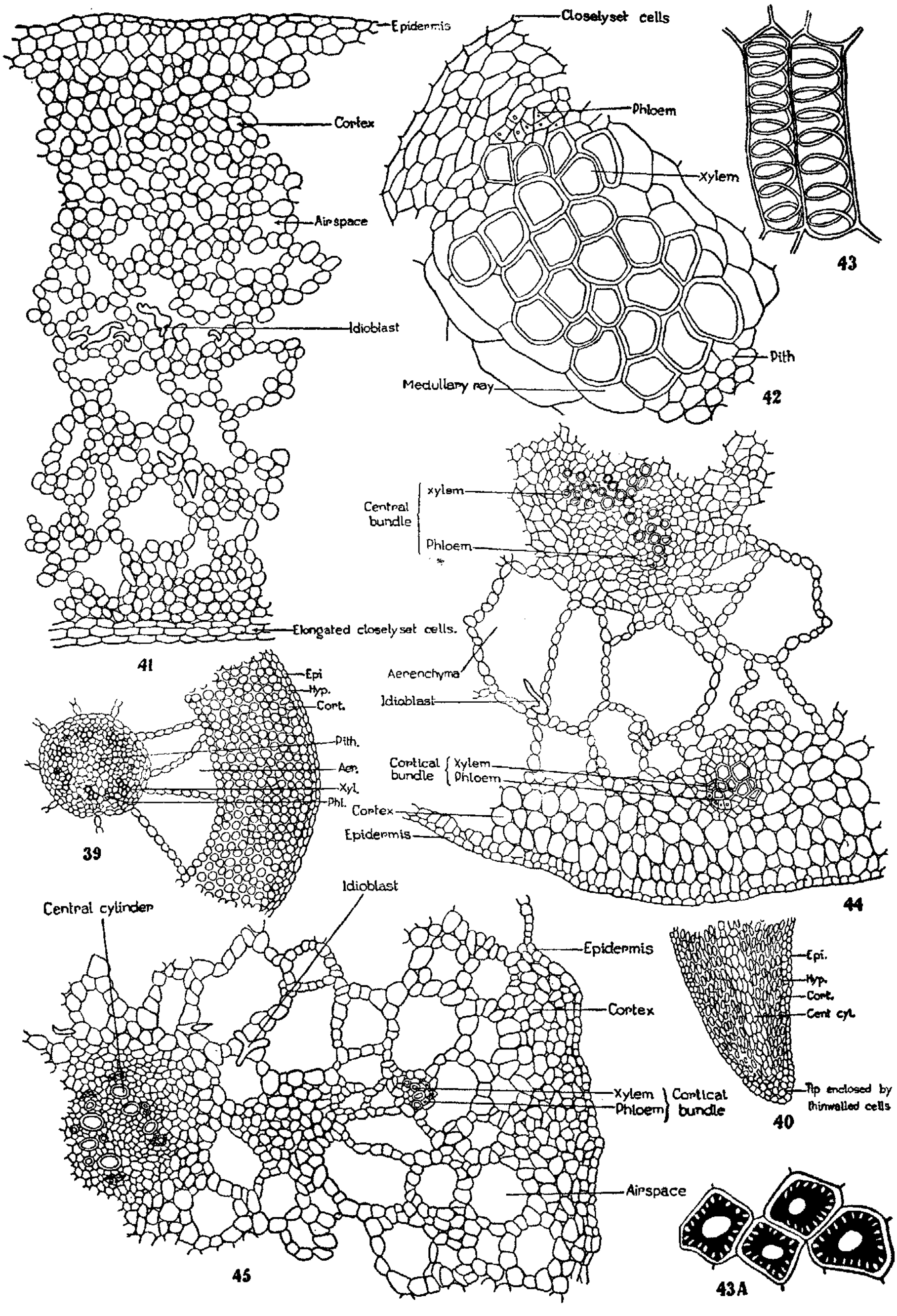
Epidermis—One layered, thin walled cells.
Hypodermis—One layered, parenchymatous cells.
Ground tissue—Many layered, loosely arranged cells with intercellular spaces.
Central Cylinder—Few layered, closely set, slightly elongated cells present. In this meristematic region the vessels have not yet formed.

The aerenchymatous tissues have also not yet developed and instead only the small intercellular spaces are present. The tip is enclosed by thin walled cells which in the absence of root cap protects the meristematic root tip against external injury.

II. Tuber

3. T.S. of underground tuber.

Cortical region—Fig. 41.
Epidermis—One layered, thin walled cells.
Hypodermis—A few layered, closely set, parenchymatous cells.



FIGS. 39—T.S. of floating root (×214); 40—L.S. of root tip (×214); 41—T.S. of cortical region of underground tuber (×214); 42—Central vascular cylinder of underground tuber (×960); 43, 43A—Vessels of underground tuber (×960); 43—Spiral thickenings; 43A—Pitted vessels; 44—T.S. of floating tuber (×214); 45—T.S. of runner (×214).

Cortex—Many layered, loosely arranged, rounded cells with scattered air-spaces which are larger toward the central region. Branched, thick walled idioblasts projecting in the intercellular spaces are present in a scattered manner.

After the cortex 3 to 4 layers of elongated, closely set cells are present.

Central Cylinder—Fig. 42.

The xylem vessels are arranged in groups just below the closely set cells. Few phloem tissues are present above each group of vessels which are either pitted or thickened spirally (Figs. 43 and 43A). The groups of vessels are separated from each other by medullary ray cells.

Pith—Composed of thin walled, rounded cells with minute intercellular spaces.

4. T.S. of floating tuber—Fig. 44.

Epidermis—Single layered, thin walled cells.

Cortex—Thin walled, irregular shaped cells with minute intercellular spaces. Small vascular bundles are scattered in the cortical region. Each bundle is composed of a few vessels with phloem towards the epidermal region and surrounded by small, parenchymatous cells.

Aerenchyma—A layer of aerenchymatous tissue is present. Idioblasts are present in this layer.

Central Cylinder—Vessels are arranged in a scattered manner with phloem towards the epidermal region and small, parenchymatous cells in between and also surrounding them.

In comparison to the underground tuber the floating tuber has more of air-spaces in the form of aerenchyma. The compact arrangement of the cells of the underground tuber gives more of mechanical support that is needed. In the underground tuber the xylem vessels are in groups in the central region only, and each group of vessels has a group of phloem tissue. But in the floating tuber the xylem vessels are scattered in the central region with one group of phloem tissue only and additional bundles are present in the cortical region.

III. RUNNER

5. T.S. of runner—Fig. 45.

Epidermis—One layered, thin walled.

Cortex—Composed of loosely arranged, rounded cells with small air-spaces near the epidermal region and larger ones toward the central cylinder. Small vascular bundles are scattered in the cortical region. Idioblasts are present.

Central Cylinder—(Fig. 46)—Xylem vessels are arranged in 2 strands. Phloem tissues are present on the outer sides of both the strands. Thick walled, parenchymatous cells are present all round and in between the xylem vessels.

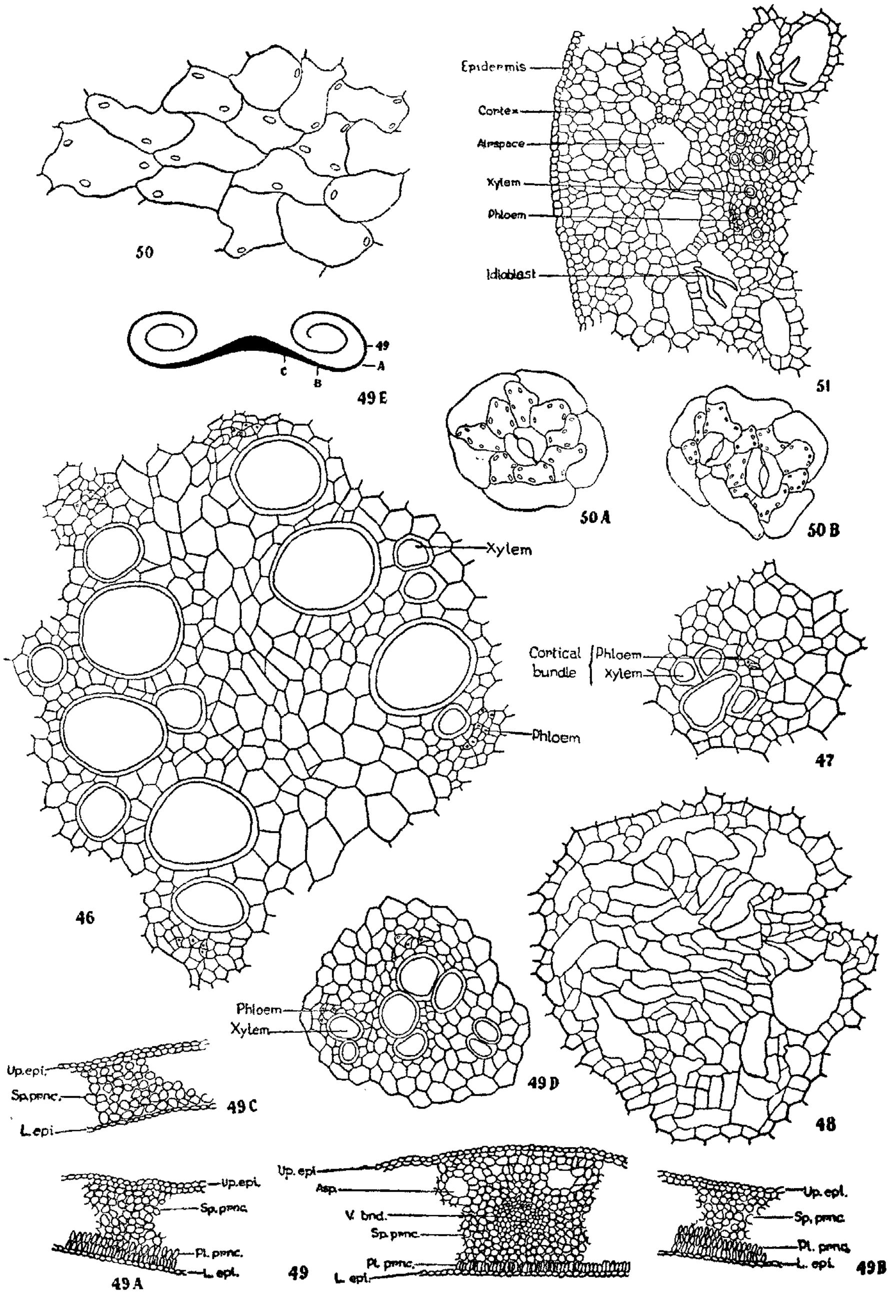
Cortical bundle—(Fig. 47)—Xylem vessels are very few in number and phloem is present only on the outer side of the vessels. The bundle is enclosed by parenchymatous cells.

Diaphragm—(Fig. 48)—The bigger air-spaces are covered with thin walled, cellular trabeculae known as diaphragm.

IV. LEAF

6. T.S. of leaf—Fig. 49.

Epidermis—One layered, thin walled cells on both adaxial and abaxial sides. In aquatic plants the epidermis instead of being protective in



FIGS. 46—Central vascular bundle of runner (×960); 47—Cortical bundle of runner (×960); 48—T.S. of runner showing diaphragm (×214); 49—T.S. of leaf with one layer of palisade parenchyma (×214); 49A—T.S. of leaf with 2 layers of palisade parenchyma (×214); B = T.S. of leaf with 3 layers of palisade parenchyma (×214); C = T.S. of leaf with palisade parenchyma absent (×214); D = T.S. of leaf with the central vascular bundle (×214); E = A diagrammatic sketch of leaf showing the different regions in it with varying numbers of palisade parenchyma layers; 50—Lower epidermis of leaf (×960); 50A, 50B—Upper epidermis of leaf (×960); 51—T.S. of pedicel (×214).

function is modified for the absorption of gases and nutrients directly from water and for those functions the surface of the epidermal cells are increased (Fig. 50) by the irregular forms. Stomata are absent from the lower epidermis (Fig. 50) and also the chloroplasts are few in number in individual cells. Stomata are present on the upper epidermis. Each stoma has two guard cells and subsidiary cells varying in number from 7 to 8 and each of them having chloroplasts in them (Figs. 50A and 50B).

Palisade parenchyma—It is either absent or varies in number of layers in different parts of a leaf just next to the lower epidermis. It is absent at the end of a leaf (Fig. 49C). It is present in one layer (Fig. 49) just after that, the number of layers of palisades increasing toward the central region (Fig. 49E) of the leaf. The presence of three layers (Fig. 49B) is much less frequent than the two layers (Fig. 49A).

Spongy parenchyma—Composed of thin walled, rounded cells with small air-spaces, present in between the upper epidermis and palisade parenchyma.

Central Cylinder—Fig. 49D.

Two groups of vessels present in the central region of the leaf with phloem tissue on the upper side of each group. Parenchymatous cells surround the vascular tissue and also present in between them. Vessels are all annular thickened.

V. PETIOLE

T.S. of petiole is same as that of the T.S. of pedicel.

VI. PEDICEL

7. T.S. of pedicel—Fig. 51.

Epidermis—One layered, thin walled.

Hypodermis—One layered, parenchymatous.

Cortex—Many layered, irregular shaped cells with minute intercellular spaces in between. Some big air-spaces are scattered toward the inner region. Idioblasts are present in the air-spaces.

Central Cylinder—Vessels are scattered in the central region with phloem on one side.

As the different vegetative parts of an aquatic plant are all existing in a similar aquatic environment they do not show much of variation in their anatomical structure.

It is already known (Haberlandt, 1914) that the presence of the firm cellulose walls makes it possible for a plant to preserve a constant shape and attain to outward differentiation. The presence of the aerenchymatous tissues makes the plant flexible against the transverse tensions which result when the structure is subjected to bending stresses and thus protects it from being torn or being stripped off bodily. Special stiffening arrangements are particularly necessary in the case of organs which are provided with wide air-spaces. The richly branched, cellular trabeculae that are present within the air passages of aquatic plants are known as diaphragms. In *L. cristatum* few diaphragms are present and in their absence the thick walled, branched or unbranched idioblasts present in the air passages take their place and perform the mechanical function and also prevent flooding of the air passages.

Schwendener (1874) has demonstrated that the species which are restricted to stagnant or slow flowing water (*Potamogeton crispus*, *P. densus*, *P. pectinatus*) developed no specialized mechanical cells either in the cortex or in the central mestome cylinder, the latter itself being able to cope successfully with the very slight demands that are made upon the tensile strength of the stem. In *L. cristatum* there is complete absence of mechanical tissues and each of the central cylinders is surrounded by closely set, thin walled cells which meet the little mechanical demands made by the vegetative parts.

As shown by Metcalfe and Chalk (1950) the intraxylary phloem is absent and central stele is present with some cortical bundles. The vessels in the underground tuber have pitted and spiral thickenings. The rest of the parts have spiral vessels more commonly.

It is well known that water plants require more ventilating system than terrestrial plants for the need of gaseous exchange. In *L. cristatum* aerenchymatous tissue is found to exist almost throughout the whole body of the plant. These air chambers act as organs for the storage of air and allow ready diffusion of gases within the body of the plant, to facilitate both respiration and photosynthesis. These air-spaces also serve to increase the power of flotation by lowering the specific gravity of the plant body. Reduction in the water conducting strands is very outstanding as the absorption goes on throughout the whole surface of the plant body. As shown by Majumdar (1938) each of the cortical bundles has an endodermis surrounding it without a clearly distinguishable pericycle. He also states that the endodermal cells have well developed casparian strips but the present author has not observed their presence. As shown by Majumdar (1938) the endodermal cells have starch grains in them.

Some physico-chemical conditions of existence

Lundëgardh (1931) writes that the phenomena one observes in nature are always the resultant of the interaction of a number of more or less independent factors and the investigator must consider these all together. All the factors making up the environment have an influence upon the growth of the species present there, but only in proportion to their relative intensity. Many factors exist together and each of them exerts a direct or an indirect influence on the life of all the vegetation present in a pond. In nature it is almost impossible to find out how a single factor is helping in the growth of a plant. So experiments were carried out in earthenware tubs keeping all factors, except one, constant and then the effect of that one varying factor was found out.

L. cristatum, with the different vegetative and reproductive stages, was collected from several ponds in Calcutta (Bengal), Balasore and Kujang (Orissa).* In Calcutta the ponds were all Corporation tanks and had a depth of approximately 15 to 20 ft. The water in these ponds came from the river Ganges through inlet pipes. The ponds in the other places were all used as fishery nursery tanks and were not open to the public. They had accumulated rain water in them with a depth ranging from 3 to 8 ft. The plants occurred in these tanks almost throughout the year. The physico-chemical characters in some of these tanks, recorded in the course of other investigations, show that the temperature of the water varied from 21·2° to 35·2°C. (time of collection between 10-30 and 11-30 a.m.), *p*H from 7·3 to 9·2, dissolved oxygen from 2·6 to 17·7 p.p.m., free carbondioxide from nil to 10·56 p.p.m., carbonate from nil to 36·0 p.p.m., bicarbonate from 82·0 to 234·0 p.p.m., nitrate from 0·036 to 1·92 p.p.m., and phosphate from nil to 2·5 p.p.m.

L. indicum, with the different vegetative and reproductive stages, was collected from fishery tanks at Baripada (Mayurbhanj District), Kaushalya Ganga and Nuapara (Puri District) in Orissa. In these tanks the depth of water varied from 4 ft. 6 in. to 12 ft. In these waters the *p*H varied from 7·3 to 7·8, dissolved oxygen

* Sri S. M. Bannerjee and Sri V. Ramchandran very kindly permitted me to use their unpublished chemical data.

from 6·7 to 8·4 p.p.m., free carbondioxide from trace to 6·0 p.p.m., bicarbonate from 60 to 78 p.p.m., phosphate from 0·12 to 0·21 p.p.m., nitrate from 0·03 to 0·53 p.p.m.

The ponds from which *L. cristatum* and *L. indicum* were collected had the *p*H ranging from 7·3 to 9·8. Acid waters are seldom found in the places of collection referred to above. It was therefore felt desirable to ascertain experimentally whether these plants could thrive under acid conditions also. Certain experiments on this aspect carried out with *L. cristatum* are briefly reported here.

Experiment I.—In an earthenware tub in which *L. cristatum* was growing healthily, the *p*H of the water was lowered by the addition of commercial sulphuric acid and it was maintained between 5·5 and 6·5 by the addition of acid frequently. After 15 days the plants showed rotting of root tips and the death of older leaves. The young leaves were still healthy. After another 15 days the roots started reviving and the plants grew as healthily as they grow in alkaline waters. Plants, kept in the control tub in which the *p*H of the water ranged between 8·7 and 9·0, remained healthy throughout the period of observation.

Experiment II.—In another earthenware tub the *p*H of the water was maintained between 4·0 and 5·5 by adding acid as in the previous case. On the 6th day the older leaves were half decomposed and the root tips were also affected. From the 10th day majority of the plants were dead and even those that were living had only small, unhealthy leaves which had yellowish green colour. Some of the plants continued to survive in this unhealthy state for a period of two and a half months which was the duration of the experiment. The control tub with the *p*H ranging between 8·8 and 9·2 had healthy plants all along.

Experiment III.—In another earthenware tub the *p*H of the water was lowered further with acid and was maintained between 3·0 and 4·0. On the 4th day most of the root tips were decomposed and most of the leaves were half decomposed. On the 9th day the majority of the plants were dead and decomposed and the few rooted plants that survived had unhealthy leaves which were yellowish green in colour and slimy to touch. The control tub which had the *p*H ranging from 8·7 to 9·3 had healthy plants all along the experiment.

TABLE No. 1

Observations on Limnanthemum cristatum *plants growing in aquatic medium of different Hydrogen ion concentration*

*p*H of water	9·0–8·7	6·5–5·5	5·5–4·0	4·0–3·0
After 5 days of the experiment.	Healthy plants.	Healthy plants.	Older leaves half decomposed and root tips also affected.	Most of the root tips and leaves decomposed.
After 15 days.	,,	Rotting of root tips and death of older leaves.	Majority of the plants dead and others living in an unhealthy state.	Majority of the plants dead and decomposed and the surviving plants very unhealthy.
After 30 days.	,,	Roots started reviving and plants healthy.	Surviving plants in an unhealthy state.	Surviving plants very unhealthy.

It appears from the above observations (Table No. 1) that though the plants grow healthily in fairly alkaline waters, that is, *p*H ranging between 7·3 and 9·2, they can tolerate acidic waters up to a *p*H of 5·5. In more acidic waters (*p*H between 5·5 and 3·0) only a few plants survive and that also in a very unhealthy state.

In Calcutta (Bengal) most of the ponds are having *L. cristatum* in abundance. In one place within a distance of about 500 yards two ponds are present, one of them having healthy growth of the plant and the other is showing complete absence of it. The pond in which it is completely absent sustains healthy growth of *Vallisneria spiralis* L. The water analyses of the two ponds are as follows:—

TABLE NO. 2

Water analyses of the ponds with and without L. cristatum

	Water of the pond in which the plant grows abundantly	Water of the pond in which the plant is absent
*p*H of the water	7·6	7·8
Dissolved oxygen ..	14·8 p.p.m.	12·0 p.p.m.
Alkalinity	60 p.p.m.	190 p.p.m.
Phosphate	trace	0·07 p.p.m.
Nitrate	trace	0·32 p.p.m.
Free ammonium	nil	nil
Albuminoid ammonia ..	9·0 p.p.m.	8·7 p.p.m.
Oxygen consuming capacity ..	14·0 p.p.m.	19·0 p.p.m.

There is no marked difference in the waters of the two ponds, only the pond from where *L. cristatum* is absent alkalinity and nitrate are much more in concentration than where it is present. Observations are now being continued to find out why the plant *L. cristatum* thrives in one pond healthily and is absent from the other.

From another pond in which the plant *L. cristatum* is growing healthily, the bottom mud is collected and after mixing sand with it in different proportion an experiment was set up in earthenware tubs to find out how the plants would grow in different types of substrata (vide Table No. 3).

In nature the growth of plants in an environment is the resultant of the interaction of a number of more or less independent factors. Among all the factors soil is the most important one for a rooted plant. As Lundëgardh (1931) states that from the standpoint of ecology the soil might be defined as that part of the earth's crust which bears life. The rooted water plants, though they are absorbing nutrients from the water even then, obtain a part of their inorganic nutrient from the soil. It is known already that the presence of nutrient salts in the soil furthers the growth of the root system. In pure sand there is lack of nutrient salts and so *L. cristatum* grown on sand did not show healthy growth or survival of the root system. While growing on sand, for a month the plants had quite a good and healthy appearance with flowers but they did not show any further vegetative growth, because when the root system fails to grow healthily the other vegetative parts also do not show healthy growth and development.

TABLE No. 3

L. cristatum *grown in earthenware tubs on different types of substrata*

Dates	Earthenware Tub 1 Pure pond mud.	Tub 2 Mud—50% Sand—50%	Tub 3 Mud—25% Sand—75%	Tub 4 Mud—75% Sand—25%	Tub 5 Pure sand.
19-8-1954	Ten healthy plants planted in each tub.				
25-8-1954	Plants healthy in all tubs.				
3-9-1954	Plants healthy in all tubs.				
13-9-1954	Good growth	Better growth	Good growth	Better growth	Not very healthy
15-9-1954	Do.	Do.	Do.	Best growth	Unhealthy.
18-9-1954	Do.	Do.	Do.	Do.	Do.
28-9-1954	Healthy but not as in tubs 2 and 4. A few flower buds still present.	Very healthy. Few of the flowers in bloom and a few more buds present.	Healthy with two flower buds but not as healthy as the plants in tubs 2 and 4.	Very healthy. Few of the flowers in bloom and some buds still developing.	Unhealthy. Only a very few small foliage leaves present in an unhealthy state.
After 40 days.	The growth of the vegetative parts is same as the plants in tub No. 2.	The foliage leaves show normal shape and size and very healthy but only the root system is little less branched than those growing in tub No. 4.	The foliage leaves slightly smaller than the leaves on the plants in tubs 2 and 4 and also the root system less branched.	Healthy root system and the other vegetative parts of the plants also very healthy	Only the main root is present without any branching and that is also very thin and delicate. The foliage leaves also are very small in size.

The plant is found to be growing best in a slightly sandy soil, that is, mud 75% and sand 25%. When the substrata was pond mud 50% and sand 50% the growth of the plant was still good but when the proportion of sand increased more than 50% the growth of plants was not as healthy as up to 50%. While growing on pure sand there is neither vegetative nor sexual reproduction and after existing for a month in a healthy condition by using the reserve material as well as the nutrients available from sand and water the condition of the plants started deteriorating. After 40 days the leaves were quite small and the root system was also very scanty (Fig. 38). The plants grown on pure sand might not be dying but they survived in a very unhealthy state and they neither showed vegetative growth nor production of flower buds. Their survival in that unhealthy state might be due to non-availability of the nutrient elements from the substratum. Troug (1953) has stated that the soil acts as the custodian of nutrient elements and the living phase is helped by these elements very much and if a soil was not frugal with its resource of nutrient elements, then a protective vegetative cover would fail to grow. As nutrient elements are absent from sand, the plants growing on sand will show neither healthy growth nor healthy survival.

Summary

1. Experiments have been conducted to verify the observations that *L. cristatum* and *L. indicum* are both rooted aquatics and that they float only for a limited period of their life histories. If by any chance a free floating plant is unable to anchor itself to the substratum within a limited period, it perishes.

2. By studying the external morphology of both the species it is found that the runners come out from that portion of the underground tuber which is jutting out of the soil. The inflorescence develops on the runner a little below the foliage, giving it the appearance of a lateral outgrowth. Each leaf is entire, orbicular or deeply cordate. Floating tuber similarly bears runner, foliage and inflorescence.

3. Both the species reproduce vegetatively and sexually. Vegetative reproduction is commonly carried on by vegetative buds and sexual reproduction by the formation of flowers which ultimately develop seeds. The flowers are in racemose clusters. The flower of *L. cristatum* is pentamerous and of *L. indicum* 5 to 7 merous.

4. Development of inflorescence and flowers of *L. cristatum* has shown that a flower normally takes 33 to 43 days from initial stage up to seed formation.

5. Germination of seed of *L. cristatum* has been worked out. After its formation a seed generally takes 92 days to germinate in tap water.

6. Internal morphology of *L. cristatum* has been studied and it is observed that the vegetative parts do not show much of variation in structure as they are all existing in a similar aquatic environment.

7. Some physico-chemical conditions of existence of *L. cristatum* and *L. indicum* have been studied under natural conditions in detail. It is observed that the tanks in which *L. cristatum* flourished healthily *p*H varied from 7·3 to 9·2, dissolved oxygen from 2·6 to 17·7 p.p.m., free carbondioxide from nil to 10·56 p.p.m., carbonate from nil to 36·0 p.p.m., bicarbonate from 82·0 to 234·0 p.p.m., nitrate from 0·036 to 1·92 p.p.m., and phosphate from nil to 2·5 p.p.m. The tanks in which *L. indicum* grew healthily *p*H varied from 7·3 to 7·8, dissolved oxygen from 6·7 to 8·4 p.p.m., free carbondioxide from trace to 6·0 p.p.m., bicarbonate from 60 to 78 p.p.m., phosphate from 0·12 to 0·21 p.p.m., and nitrate from 0·03 to 0·53 p.p.m.

Under experimental conditions varying one factor only, such as the *p*H of the water and the substratum separately, the conditions of existence of *L. cristatum* have been studied. It appears from the observations that though the plants grew healthily in fairly alkaline waters, that is, *p*H ranging between 7·3 to 9·2, *L. cristatum* can tolerate acidic waters up to a *p*H of 5·5. In higher acidic waters (*p*H between 5·5 and 3·0) only a few plants survive and that also in a very unhealthy state. By varying the composition of the substratum it has been found that *L. cristatum* grow best in a slightly sandy soil, that is, mud 75% and sand 25%.

Acknowledgements

The authoress is grateful to Dr. I. Banerji, F.N.I., Head of the Department of Botany, Calcutta University, for his valuable suggestions while correcting the manuscript. She is also grateful to Dr. B. S. Bhimachar, F.N.I., Chief Research Officer, Central Inland Fisheries Research Station, Calcutta, for his encouragement and for communicating this paper for publication.

References

Arber, Agnes (1920). Water Plants. Camb. Univ. Press, London.

Bentham, George (1869). Flora Australiensia, 4, London.

Biswas, K., and Calder, C. C. (1937). Handbook of common water and marsh plants of India and Burma, Delhi.

Chandler, Bertha (1910). *Utricularia emarginata* Benj. *Annals of Bot.*, **24**, 95.

d'Almeida, J. F. R. (1928). On the short morphology of *Limnanthemum*. *Journ. Ind. Bot. Soc.*, **7**, 1.

Goebel, K. (1891–93). Morphologische and biologische studien. 6. *Limnanthemum*.

Haberlandt, G. (1914). Physiological Plant Anatomy, London.

Hooker, W. J. (1834). Hydrochariteae. *Jour. of Bot.*, **1**, London.

Hooker, W. D. (1890). Flora of British India, 4, London.

Lundĕgardh, Henrik (1931). Environment and plant development, London.

Majumdar, G. P. (1938). A preliminary note on Polystely in *Limnanthemum cristatum* and *Ottelia alismoides*. *Curr. Sc.*, **8**. Feb.

Metcalfe, C. R., and Chalk, L. (1950). Anatomy of the Dicotyledons, 2, London.

Mukherjee, A. (1951). Cytology of *L. cristatum* Griseb. *Curr. Sci.*, **20** (12).

Parija, P. (1934). Physiological investigations on water hyacinth (*Eichhornia crassipes*) in Orissa with notes on some other aquatic weeds. *Ind. Jour. Agri. Sc.*, **4**, **3**.
Prain, D. (1903). Bengal Plants, **2**, Calcutta.
Rendle, A. B. (1938). The classification of flowering plants, **2**. C.U. Press, London.
Roxburgh, W. (1824). Flora Indica, Care's edition, **2**.
Schwendener, K. (1874). Das mechanische, Prinzip.
Srinivasan, A. R. (1942). Cytomorphological features of *L. cristatum* Griseb and *Enicostema littorale* Blume. *Proc. Ind. Acad. Sci.*, **14**, Sec. B.
Stiles, W. (1936). An introduction to the principles of plant physiology. Methuen & Co., Ltd., London.
Troug, Emil (1953). Mineral nutrition of plants. Univ. Wincon. Press.

Issued March 10, 1956.