EXPERIMENTAL AND ANALYTICAL STUDIES OF THE OPHIOGLOSSALES

II. Analytical Study of some Abnormalities in *Helminthostachys zeylanica* (L.) Hook.

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Several abnormalities in *Helminthostachys zeylanica* (L.) Hook. have been described, which included bifurcation of the leaf stipe, pinnation of the lamina lobes, over-all reduction and condensation of the lamina, bifurcation of the fertile spikes, reduction and condensation of the same and sterilization and lamination of the spikes. It is proposed that these teratologies, perhaps, resulted as a consequence of nutritional disturbances and interference in the genetic constitution of the plants. It is stressed that such abnormalities should not be necessarily considered from phylogenetic or similar points of view.

Introduction

Helminthostachys zeylanica (L.) Hook., an unusual monotypic eusporangiate fern, has been reported to occur most frequently in the forests of Gorakhpur Division (Roy and Kumar 1959; Dixit and Tripathi 1966). During our regular and intensive surveys of the forests of Ramgarh (Kusmi), Tilkonia (situated 7 miles east of Gorakhpur City) and Lakshmipur (65 km north-west of Gorakhpur), the authors have met with certain very interesting and peculiar abnormalities in the vegetative and reproductive organization of *Helminthostachys*. These departures from the normal and characteristic growth pattern of the fern are so conspicuous and distinct that they compel attention. These anomalies include, among many things, the bifurcation and lateral branching of the spikes; formation of compressed, small and globular spikes; considerable reduction of the frond size and sterilization of the spike into phylloid structures of various shapes and sizes; deep lobes and serrations in the leaf blade and branching of the petiole, etc. Bower (1926) has illucidated various abnormal modifications of the leaf and accessory branchings of the fertile spike in *Ophioglossum* and *Botrychium*. Unusual branchings and abnormal orientations of the spikes of *Ophioglossum vulgatum* have been occasionally reported (Bower 1896; Mahabale and Deshpande 1934; Vashist 1929). Bower (1926) has also pointed out certain (few) accessory branchings of the spike in *Helminthostachys*. Goebel (1918) has described some

variations in the arrangement of the sporangia on the spike. However, in *Helminthostachys zeylanica* such and other teratological phenomena in the morphology of the plant have not received due attention. In a previous contribution Sharma *et al.* (1967) have already stressed the need of a critical and detailed study of such aberrations from causal or morphogenetical standpoint because it may sometimes yield valuable information. The present treatment is devoted to a detailed investigation of the morphology of the abnormal features encountered in *Helminthostachys zeylanica* from the causal and developmental angles.

Material and Methods

These abnormal plants of *Helminthostachys* were collected from the forests of Ramgarh, Tilkonia and Lakshmipur. Some of the anomalous plants were dug out from the forest soil with their creeping rhizomes intact and were transplanted in the Botanical Garden of the University. Important and curious structures were photographed on site. Material for anatomical study was fixed in FAA, sections were cut at 10–12 μ and stained in safranin and fast green.

Observations

Morphology of the Normal Plant:

The adult plant usually possesses an unbranched creeping rhizome which bears leaves alternately in two rows on its upper surface. Thick, fleshy roots spring from the flanks and the under surface of the rhizome. The petiole of the ternate leaf arises from the upper surface of the rhizome in relation to a flap-like basal stipule. The leaf blade is usually ternate, each of its divisions being again divided: the venation is open. The margins of laminar lobes are, as far as known, always entire. The fertile spike rises from the adaxial face of the leaf (more or less at the juncture of the petiole and the leaf blade). It is more complex in this genus than in other genera of the Ophioglossaceae. The sporangia are borne on outgrowths of the nature of sporangiophores, which are disposed in serried ranks right and left along lines closely corresponding to the lines of sporangia in *Ophioglossum* and *Botrychium*. Each sporangiophore has its own vascular supply and bears a varying but small number of irregularly disposed sporangia, while its apex ends in irregular vegetative lobes. The sporangia are quite large and open by longitudinal slits. As the sporangia approach maturity the upper part of each sporangiophore may further grow out into an irregular rosette of laciniae of vegetative tissue. The large eusporangiate sporangia are nearly globular, short stalked or nearly sessile structures borne in irregular groups. The sporangium wall is massive enclosing a thick layer of tapetum which disorganizes at maturity.

Abnormalities in the Vegetative Structures:

(i) *Branching of the petiole.*—The petiole, as described earlier, arises singly from the rhizome through the stipular sheath. Curiously enough the authors noticed one plant of *Helminthostachys* in the Tilkonia forest growing at the base of a tree having three sets of leaf blades originating from a single petiole (Fig. 1). The petiole continued to be unbranched up to nearly three inches high and then it forked into two branches, one strong and the other thin and long. A little higher the stronger shank again furcated into two additional branches of similar form. All the three petiolar branches, thus developed as a result of two 'pseudo-dichotomies', terminated into three sets of ternate leaf blades. These leaf blades were all sterile and showed no trace of any fertile component. The leaf blades were, however, of the normal healthy type. In the authors' view the petiolar branching of this type is being reported for the first time in this fern.

(ii) *Unusual orientation of the leaf stipes on the rhizome.*—The rachis of the young leaf in *Helminthostachys zeylanica*, and for that matter in all the members of the Ophioglossaceae, is upright from the start and does not exhibit circinate vernation (cf. the true fern). But in Lakshmipur forest the junior authors, while collecting rhizomes of this plant for detailed morphological study, noticed unusual growth pattern of the rhizome and the petiolar bases on it. The rhizomes were placed almost obliquely in the soil and the leaf petioles (stipes) had to travel considerably parallel to the axis of the rhizome before they could emerge upright above the ground (Fig. 2). This was usually accomplished by a curvature of about 90° in the petiole-base which, as a result, could give the leaves their normal erect stance. Furthermore, it has been observed in such cases that the ternate leaf blade is oriented in a fan-shaped fashion, instead of being spread in all directions.

(iii) *Over-all condensation of the frond.*—It would be hard to identify an individual plant as shown in Figs. 3A and 10 with *Helminthostachys zeylanica*. The authors, after careful examination of this plant in the Kusmi forest, brought it over to the Botanical Garden for further investigation in order to

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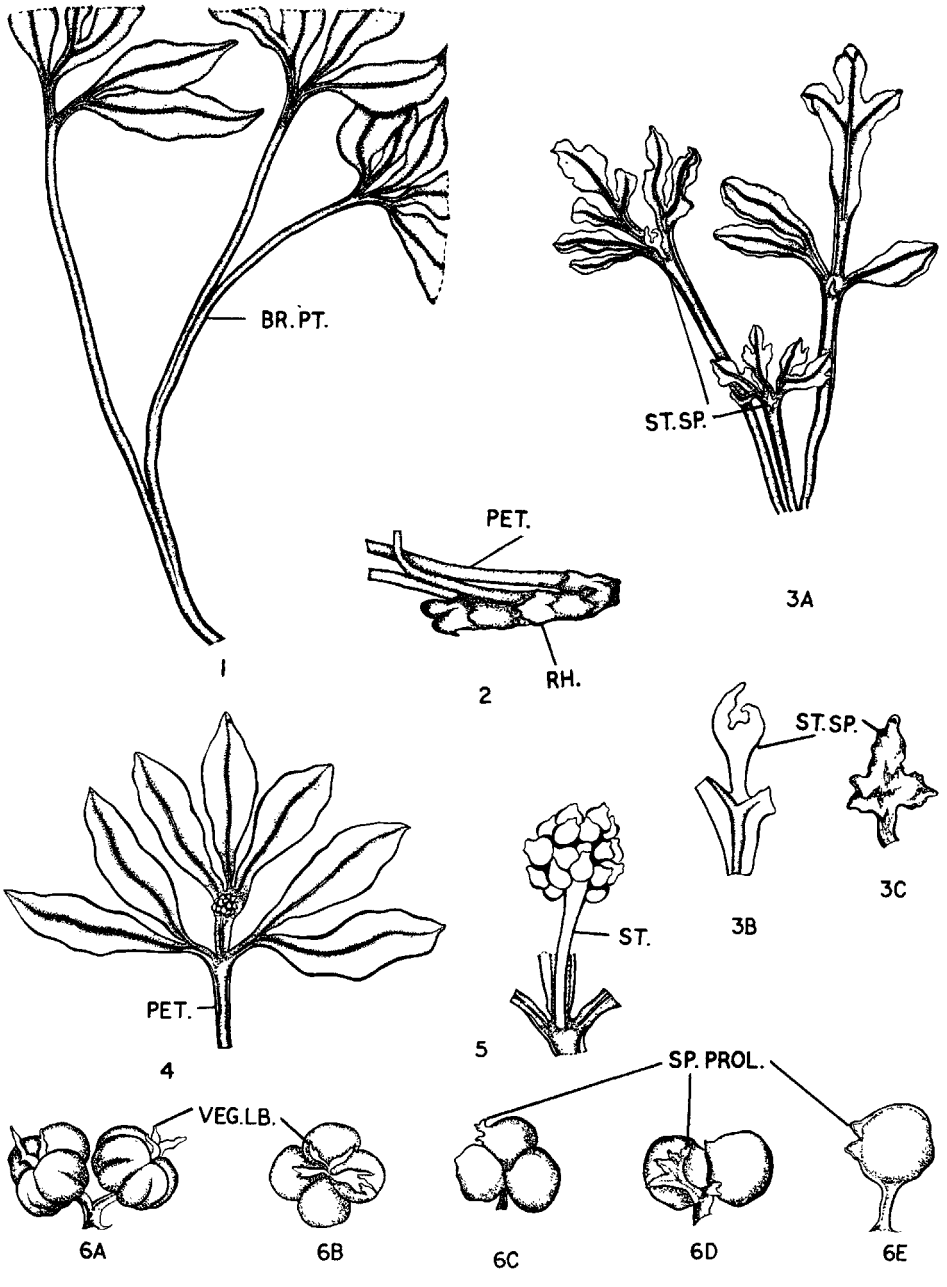
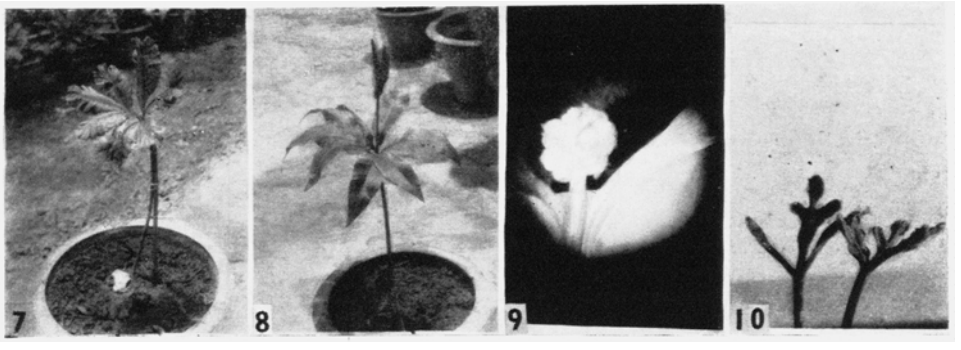
FIGS. 1–6E. *Helminthostachys zeylanica* (L.) Hook., showing different types of anomalies. (*BR.PT.*, branched petiole; *PET.*, petiole; *RH.*, rhizome; *SP. PROL.*, sporangial prolifications; *ST.*, stipe; *ST.SP.*, sterile spike; *VEG.LB.*, vegetative lobe). 1, petiolar ramification. $\times \frac{1}{3}$. 2, a rhizome with abnormally oriented leaf stipes on it. $\times \frac{2}{3}$. 3A, three fleshy leaves, each showing an over-all suppression of their vegetative and reproductive components. $\times \frac{5}{6}$. 3B, 3C, sterilized and laminate spikes from the leaves in Fig. 3A, the former with a semilunar and forked appearance and the latter looking arrow-shaped. 3B, $\times 3\frac{1}{3}$; 3C, $\times 6\frac{2}{3}$. 4, a frond with a globular short stalked fertile spike. $\times \frac{2}{3}$. 5, the short stalked globular spike of Fig. 4 enlarged. $\times 5$. 6A–6E, different sporangial structures. 6A, 6B, normal 'sporangiophores', each with four sporangia and sterile outgrowths. $\times 6\frac{2}{3}$. 6C, 6D, sporangiophores with groupings of three and two sporangia with irregular outgrowths arising from the sporangial walls. 6C, $\times 10$; 6D, $\times 8\frac{1}{3}$. 6E, a sporangiophore bearing solitary spornagium with two thalloid outgrowths emanating from its walls. $\times 5$.


BR. PT.
1
ST. SP.
3A
PET.
RH.
2
ST. SP.
3B
3C
ST.
5
PET.
4
SP. PROL.
VEG. LB.
6A
6B
6C
6D
6E

FIGS. 1–6E.

probe into the causal factors which could account for such abnormal shape and form of the fern. The vegetative and fertile components of the leaves have undergone considerable modifications to look curious. At the time of collection the plant had three unfolded leaves. The leaf lamina had become unusually thick and leathery, its margins became wavy and the laminar segments assumed a wing-like form. The midribs of the leaf segments became very conspicuous but the lateral veins almost disappeared. The fronds at the two ends were more or less equal in height and the middle one was comparatively smaller (Fig. 3A). All the three leaves possessed their respective fertile components which exhibited varying degrees of flattening (lamination) and sterilization.



FIGS. 7–10. *Helminthostachys zeylanica* (L.) Hook. 7, an abnormal plant showing luxuriantly developed frond with pinnation in leaf lobes. × $\frac{1}{12}$. 8, a plant with abnormally bifurcated fertile spike. × $\frac{1}{9}$. 9, a globular fertile spike, enlarged. × $3\frac{3}{4}$. 10, photograph of the plant in Fig. 3A, showing details as expressed in its figure description. × $\frac{1}{3}$.

(iv) *Finely lobed and oblong lamina.*— One of the plants in the Kusmi forest (Gorakhpur) revealed very luxuriant growth accompanied with the formation of very abnormal mature leaves. The leaf segments were oblong with rounded apices. The lamina of each leaf lobe was pinnately dissected to give it the appearance of a deeply cut feathery structure (Fig. 7). Each of these leaves, however, had a very prominent and luxuriantly fertile spike which was normal. This laminar deviation in *Helminthostachys zeylanica* bears striking resemblance with corresponding teratological aberrations in the leaves of some genetically disturbed members of *Scolopendrium vulgare* as reported by Anderson-Kottö (1929). The marked deviation in the form of the leaf blade may be attributed to some change brought about in the genome of this plant. Cytological investigations of this plant are in progress to find out whether or not polyploidy has been instrumental in bringing about profound changes in the leaf form of this individual plant.

Abnormalities in the Fertile Spike:

(i) *Bifurcation of the spike.*—Accessory branchings of the spike, as reported by Bower (1926), have also been repeatedly recorded in the forests mentioned earlier. Several such specimens were collected by the authors from Kusmi and Tilkonia forests respectively. In one case the bifurcation occurred roughly in the middle of the spike (Fig. 8), whereas in the other the forking was instituted just below the apex. In both cases the apical regions of the fertile spikes were not bare but bore sporangia all over. The developmental pattern of the sporangiophores and sporangia was normal. In another specimen the tip of the normal fertile spike had somehow become damaged due to some biotic or physical factors resulting in the formation of a small lateral spikelet-like fertile branch at the base of the parent one. The lateral branching of the fertile spike is not without interest, keeping in mind the more frequent occurrence of forking (dichotomy) in it.

(ii) *Suppressed, globular spikes.*—Two plants of *Helminthostachys* have been collected in which extreme suppression of the linear dimension of the spike has set in. The elongated spike has been replaced by a ball-like globular fertile organ (receptacle) bearing sporangia around its surface (Figs. 4, 5, 9). The sporangia are, in fact, attached separately and directly to the inflated receptacle. The sporangiophores are either lacking or simply constructed (devoid of any vegetative laciniae at their tip). The thick and massive wall of many sporangia proliferated into finger-like projections of various shapes and sizes (Figs. 6A–6E). These are structures of special interest. In one of the plants the fertile spike was almost devoid of a stalk and looked as good as sessile. In the other specimen it (stipe) was only a few millimetres long. The sporangia on these spikes were abnormally large and few in number.

(iii) *Sterilized spikes.*—The most interesting modification of the fertile spike was expressed in terms of their progressive sterilization accompanied by the flattening and lamination of the spike axis. In one instance, the dorsiventrally compressed (laminate) sterile spike looked arrow-shaped with bulgings on its surface, in another it merely assumed a semilunar appearance. In still another case the flattened spike forked into two branches with a conspicuous papillate enation situated in the angle of the branching (Figs. 3B, 3C).

Discussion and Conclusion

Abnormalities in the sporogenous members of the Ophioglossales have occasionally been described, but teratisms in the vegetative structures in this group have not drawn enough attention. Such abnormalities in the vegetative structures probably generally arise due to several external and internal factors, such as environmental changes, nutritional disturbances, wounds and injuries and other mechanical stimuli, etc. The abnormalities like bifurcation

of the stipe of the leaf and pinnation in the laminar lobes might, perhaps, have resulted in response to higher metabolic status of the plant, which was growing in soil, made rich by humus made available by a thick cover of decaying Sal leaves. On the contrary, reduced and condensed laminar size in several plants is perhaps the consequence of malnutrition obtained by the plants in habitats of poor food supply.

The Ophioglossaceae are noteworthy in having a very high number of chromosomes (Manton 1950). Such a magnitude of genetic material offers more chances for aberrations in its organization and subsequent somatic manifestation of the same. Similar causes are likely to be responsible for such abnormalities as the pinnation in the lamina lobe and bifurcation of the fertile spike. Another interesting deviation in the fertile spike, i.e. its reduction to small globular structure, again, seems to be caused by the poor metabolic status of the plant.

Most interesting of all these abnormalities were those shown by the plant in Fig. 3A. The reduction in the leaf size is so much prominent that it warrants the presence of factors, other than the nutritional ones, responsible for the same. These reduced leaves bore fertile spikes which were totally sterile and laminate in shape. Microscopical examination of the cross-section passing through these spikes show no sign of sporogenous tissue in them which, perhaps, were parenchymatized in their early ontogeny. This plant, it would seem, possessed inherent aberration in its genetic complex which, as mentioned earlier, is not impossible to occur in the members of the Ophioglossales having a vast number of chromosomes.

Abnormalities in the fertile spikes of *Ophioglossum* have occasionally been used by several workers as a substrate for explaining the morphological nature of the same organ. But it is always better and safer as well to treat these teratomata on causal grounds instead of using the data for phylogenetic and other similar considerations as Arber (1950) points: 'While the study of teratism yields interesting informations about the potentialities of the plants, which may throw light on normal forms, such data must not be considered on phylogenetic basis.' Further studies of the cytology of the abnormal materials are in progress and their outcome has been reserved for a future publication. The results of such investigations are expected to illucidate the phenomena, hitherto described, more clearly and successfully.

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