ANALYTICAL STUDY OF ABNORMAL LEAF DEVELOPMENT IN *CYCLOSORUS SUBPUBESCENS* (BL.) CHING

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An anomalous case of leaf development in *Cyclosorus subpubescens* (Bl.) Ching is described and discussed from causal and developmental angles. On one young leaf (whose upper crozier region was damaged due to fungal infection) two small and partially unfolded leaves were found attached in the presumptive sites of pinna and pinnule respectively. Normal leaf morphogenesis in ferns is discussed to suggest that both of these secondarily developed leaves are perhaps an outcome of abnormal development of the lowest lateral leaf segments under changed conditions caused by the death of the crozier of the 'parent' leaf due to fungal infection. Pinnae primordia have been postulated to possess variable potentialities of alternative development exhibited also by various other organs in ferns.

Introduction

Deviations from the normal form of biological entities have always fascinated morphologists. Much descriptive work has been done on the abnormal development of organs in plants (*see* Heslop-Harrison 1952). But such teratomata, if probed into morphogenetically, often yield valuable information about the organized system inherent in the development of the organ in consideration (Sharma *et al.* 1967). The present communication deals in a similar way with a very unusual and unique case of abnormal leaf development in *Cyclosorus subpubescens* (Bl.) Ching. In this fern the authors could discover a peculiar phenomenon, i.e. the repetitive formation of almost normal fronds from the pinna and pinnule primordia of a particular leaf. Such recapitulation in the ontogeny of pinnae and pinnules, to the best of the authors' information, has not been reported so far. A morphogenetic investigation of this behaviour has been made with a view to procure more detailed information about the leaf ontogeny in ferns.

Material and Methods

General leaf morphology of the fern was first studied to reveal its normal structure. Subsequently, the leaf showing the abnormality was removed from the rhizome and fixed in FAA. Further studies were made under a binocular dissecting microscope. The fungal pathogen in the leaf was studied in epidermal scraps of the crozier stained in Lactophenol and Cotton Blue.

OBSERVATIONS

Morphology of the Normal Frond:

The short, prostrate, creeping and perennial rhizome bears twenty to thirty-two fronds and their primordia at a time, which may be grouped into four sets. The first set of leaves is that which unfolded in the current growing season. The second, third and fourth successively younger sets of leaf primordia belong to the future growing seasons in that order. Thus, the apex of the rhizome, at any time, possesses leaves in various stages of their development, belonging to at least four successive growing seasons.

The soft stipes of the mature fronds are 10–30 cm long, dark purplish-brown at the base and tufted downwards with thin brown scales. The fronds are 30–60 cm long, 15–30 cm broad, oblong-lanceolate and once pinnate. The pinnae are 7·5–15 cm long, up to 2·5 cm broad with the lower pairs rather shorter and deflexed. Segments of the pinnae are cut down to a broadly winged rachis, linear-oblong, blunt, entire or very slightly crenate, texture herbaceous and veinlets 6–8 on each side, simple or forked (Fig. 4).

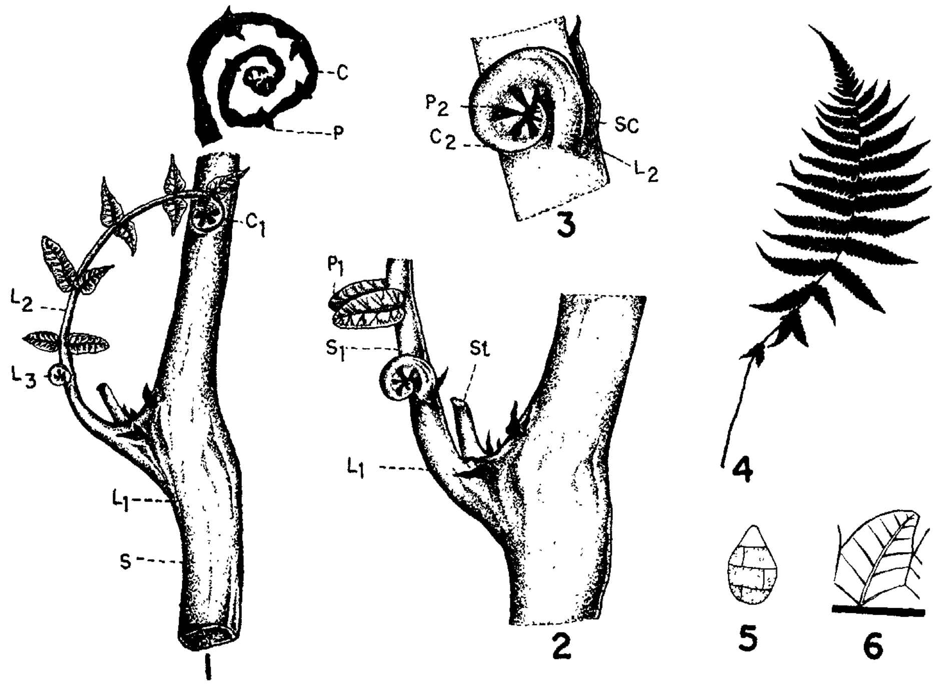
Fungal Infection on the Leaf:

During the normal development of the frond the stipe of the circinately coiled young leaf elongates up to 7–10 cm before the uncoiling of the crozier sets in. However, in many plants it was noticed that instead of unfolding the croziers turned light-brownish and warty and within a couple of days they withered and dried up with a deep-brown appearance. Young leaves of all developmental stages were found to be infected (from very young primordia with tightly closed croziers to elongated unfurling ones). The crozier was the first to be destroyed, followed by the eventual disintegration of the stipe. Such infections were also seen to occur in mature fronds which, as a result, withered and dried up in the same way.

Microscopical examination of the epidermal scraps of the infected portions revealed in them the presence of fungal spores. The spores were many-celled and spindle-shaped, probably of *Alternaria* species.

Abnormalities:

One of the young unfolding leaves was found to exhibit a very unusual phenomenon. This leaf (labelled *L* in Fig. 1), 12 cm in length, had a loose and almost dried-up crozier, which could have continued to unfold in the absence of the fungal infection. The pinnae primordia were also detectable in dried condition on the crozier. The stipe (labelled *S* in Fig. 1) was still green and fleshy. Nearly 4 cm above the base of this stipe a young leaf (labelled L_1 in Fig. 1) was seen emerging out. This portion of the stipe was



FIGS. 1–6. *Cyclosorus subpubescens* (Bl.) Ching. C, C_1 and C_2, croziers of leaves L, L_1 and L_2 respectively; L, parent leaf which gave rise to 'secondary' abnormal leaves; L_1 and L_2, 'secondary' leaves developed abnormally on the stipes of leaves L and L_1 respectively; P, P_1 and P_2, pinnae primordia on the leaves L, L_1 and L_2 respectively; SC, Scales; St, stump-like organ in the vicinity of the site of insertion of the leaf L_1. 1, 'parent' leaf with both the secondary leaves. ×1·6. 2, lower portion of the 'parent' leaf showing the insertion of abnormal leaf L_1 and stump on its stipe. ×3·2. 3, enlarged view of abnormal leaf L_2, showing its attachment along with the associated scale on the stipe of leaf L_1. ×18. 4, a normal leaf showing its once pinnate nature. ×0·18. 5, spore of the pathogenic fungus on the 'parent' leaf. ×267. 6, one pinna lobe enlarged. ×1·6.

conspicuously swollen. The 'daughter' leaf (L_1) was 4·5 cm in length, with five pairs of nearly unfolded pinnae but its apex was still a coiled crozier in which unfurling and the development of younger pairs of pinnae primordia were continuing. The stipe of this 'secondary-daughter' leaf was 1 cm long, soft and slender (Fig. 2). Just by the side of this stipe another similar stump-like organ (labelled *St* in Fig. 2), whose distal portion appeared to be already broken, was found attached with the 'parent' stipe (S). The morphology of this stump was similar to that of the stipe of the 'secondary-daughter' leaf (L_1) to such an extent that it appeared as if it was also a full leaf whose upper portion had been broken accidentally. Few deep brown thin scales were found attached to the bases of these 'daughter' fronds. Thus, a pair of secondary

FIGS. 7A-B. *Cyclosorus subpubescens* (Bl.) Ching—abnormal and normal fronds. 7A, parent frond which gave rise to abnormal leaves. The crozier has been destroyed by the fungal infection and abnormal leaf has come off from the lower portion of the stipe. × 1·2. 7B, a normal young leaf in which uncoiling of the crozier has already started. × 1·2.

fronds (L_1 and St) was seen to emerge, side by side, directly from the stipe of the 'parent' frond (L) whose further development was arrested due to the destruction of the whole of the apical growing region by fungal infection.

Again, a young leaf (labelled L_2 in Fig. 1) was found attached near the base of the stipe of the 'secondary-daughter' frond (L_1). This leaf consisted of a short stipe and a tightly closed crozier, which revealed the formation of pinnae primordia in the normal way (Fig. 3). A large deep brown scale (SC) was also seen arising from the stipe of the 'secondary' leaf in the vicinity of the still younger ('tertiary' leaf) L_2.

A careful examination of the venues of attachment of the secondary leaves (L_1) and stump (St) to the parent stipe (S) did not reveal the presence of any branch bud, in that position, which could have given rise to the new secondary leaves. Both the leaves (L_1 and St) seemed to be attached directly to the rachis in a manner comparable to the placement of pinnae in a normal frond. Similarly, no sign of any branch bud was found at the site of the 'tertiary-daughter' leaf (L_2).

As mentioned earlier the pinnae of mature normal fronds showed segmentation of the blade into deeply cut lobes. No such segmentation was traceable in the unfolded pinnae of the abnormally developed 'secondary' leaf.

Discussion

Critical examination of the material reveals that there has been some kind of abnormal growth or development or both in the basal region of the 'parent' stipe (S) which resulted in the formation of 'secondary-daughter' leaves (L_1 and St). One of them, L_1, showed all the essential features of a normal frond although its early ontogeny was basically different from that of the latter. A 'tertiary-daughter' leaf (L_2) was again formed in the same way on the stipe of the 'secondary' leaf (L_1). Both of these abnormally situated leaves can be regarded as lateral outgrowths of the stipes on which they have occurred. They may have possibly originated in either of the two ways: (i) Branch buds might have occurred on both the 'parent' stipes, which gave rise to the 'secondary' and 'tertiary' leaves in the normal fashion of the shoot apex; (ii) the lowest pinnae primordia of the 'parent' leaves have abnormally developed into 'daughter' fronds. Both of these alternatives will be considered here in detail.

Bower has reported frequent occurrence of lateral buds on the stipes of the leaves of *Dryopteris*, some distance above the leaf bases (Bower 1923, 1928). But in the species of *Cyclosorus* under discussion no such buds or their rudiments could be traced on the normally developing leaves. Also the sites of attachment of the 'secondary' and 'tertiary' abnormal leaves on their respective 'parent' stipes gave no evidence of the presence of buds there. Alternatively, it may be presumed that, in the drying 'parent' leaf (L), the lowest lateral growth centres of the leaf were, by some stimulus, activated to develop as the secondary leaves. Normally, however, the lateral outgrowths of a frond rachis are considered to be of pinna nature. Possibilities are thus raised that the lowest pair of pinnae primordia (on the 'parent' stipes) grew into normal pinnate fronds under abnormal conditions. Before discussing this aspect it will be desirable to make some *a priori* considerations of normal frond development in ferns.

Anatomical studies of leaf development in ferns have already been extensively undertaken. In the past two decades delicate and intricate experimental techniques were employed which yielded valuable results. Wardlaw (1949, 1952) established that the leaf primordium always originates on the apical meristem of the shoot and the former under the regulatory effect of the latter develops as an organ of limited growth and dorsiventral symmetry, i.e. the leaf. Further development of the leaf primordia was studied by several workers. One of them (Sharma 1959) demonstrated that the young leaf primordium possesses two kinds of meristems: first—the distal apical meristem which contributes to the elongation of the rachis, and the second—the marginal meristems, which owe their origin to the former meristem, and which constitute initials of the lateral growth centres on the rachis, i.e. the pinna primordia. These primordia in their turn also have apical and marginal meristems in the same fashion. Comparable observations were also made by Saha (1963). Further aspects of the leaf ontogeny in ferns have received the attention of Wetmore, 1953; Steeves and Briggs, 1958, 1959; Briggs and Steeves, 1959, and others. They have found that in *Osmunda cinnamomea*, the pinnae are initiated in acropetal sequence while apical growth of the leaf is still in progress. More detailed investigations of the inception and development of the pinnae could not be made, as the statement of Steeves and Briggs (1958) goes, 'Because of the coiled condition of the crozier it is extremely difficult to study the initiation and development of the pinnae . . . they appear to develop as marginal outgrowths involving a number of cells rather than single initial. Their subsequent ontogeny has not been investigated in detail.' Briggs and Steeves (1959), Steeves and Briggs (1959) and Voeller (1960) demonstrated experimentally that the pinna primordia, still inside the closed crozier, produce considerable amounts of extractable auxin which regulates the mechanism of crozier uncoiling.

Thus, it appears that the pinna primordium is a lateral growth centre on the developing rachis of the frond in the way as there are lateral growth centres on the growing shoot apex (of course, no attempt is made here to establish homologies between these two conditions). The lateral growth centres borne on the shoot apex of the ferns have been shown to possess alternative paths of development, i.e. being capable of development as a leaf or as a bud. The primordia which would ordinarily develop as leaves can be induced to develop as buds, if they are isolated from the regulatory effect of the shoot apex by the incisions (Wardlaw 1949, 1955; Cutter 1956). Also Steeves (1961) has shown that very young leaf primordia in *Osmunda cinnamomea*, in sterile culture, give rise to leafy shoots which soon form roots and grow on to produce complete plants. Thus in the determination of a primordium of a leaf the early stages are reversible and dependent on a leaf forming stimulus from the parent apex.

Such variable potentialities of alternative developments are not infrequent in ferns. The angle meristems of *Selaginella* under different conditions can develop either as rhizophores or as shoots (Cusick 1954; Williams 1931). Also in some species of *Lycopodium* small vegetative buds known as bulbils are formed probably on leaf-sites (Schoute 1938; Williams 1938). The mechanism of determination of a particular organ during its early developments seems to be a complex gene-controlled biochemical phenomenon occurring in apical meristems. Skoog (1954) and Skoog and Miller (1957) have emphasized the importance of availability of required growth factors in controlling the capacities of tissues for growth and differentiation. Cutter (1958) states, 'Determination, i.e. the fixation of the fate of an organ is most probably controlled by availability of the required growth factors (including nutrients) at a particular time during or preceding the inception or early growth of that organ.'

We can now reconsider the possibility of the development of the lowest pinnae into entire fronds in our abnormal material. It is probable that pinnae (the lateral growth centres on the young, elongating rachis) be looked upon as developing in their organized way under the regulatory, rather 'determinative' effect of the leaf apex. Saha (1963) mentions the predominance of the distal apical meristem of the leaf on the initiation and development of the lateral pinnae. Recently Cutter (1965) has also held the same view, as she writes, '. . . the leaf apex has important function in controlling the development of the subsequent tissues of the leaf. In the writers' view, the leaf apex—not necessarily the apical cell itself—is probably the vital controlling region of the leaf, equivalent to the apical cell group in the shoot apex; in the process of leaf determination, therefore, it is probably what happens to the apex of the primordium that is important.' In our abnormal material the withered and dried crozier of the 'parent' leaf reveals that there existed virtually no regulating or 'determinative' effect of the distal apical meristem of the leaf on the lowest pair of the pinnae primordia which, as it seems, escaped the fungal attack due to partial uncoiling of the crozier and some intercalary elongation of the stipe. These pinnae primordia, under the influence of some particular stimulus, developed as two 'secondary' leaves (L_1 and *St*). The same or similar induction persisted for some time, so that one member of the lowest pair of pinnae primordia on the secondary-daughter leaf (L_1) developed again as a 'tertiary' pinnate frond (L_2), and the other member remained arrested as a large brown scale (perhaps due to the exhaustion of the stimulus or part thereof). Also, the pinnae of the dried parent crozier in their living condition must have produced 'extra' amounts of auxin (for crozier uncoiling) which remained unused due to the death of the crozier. This unused amount of 'extra' auxin in the 'parent' stipe (S), along with a continued supply of nutrients from the persistent and perennial rhizome, might have subscribed to the abnormal developments of both of the lowest pairs of pinnae into entire pinnate fronds.

It is worth mentioning here that the above assumptions are purely hypothetical. Yet, they provide a useful anticipation for further enquiries into the developmental potentialities of pinna primordium in leaves. Developmental studies of the fern frond with special attention to the inception and development of lateral leaf segments (pinna, pinnules and pinnulets, etc.) are likely to furnish valuable information about frond morphogenesis in ferns. Special emphasis is to be laid upon the experimental approaches to these problems, which may contribute not only to the study of the growth of fronds but also to the study of their phylogeny, as Wardlaw (1952) has already emphasized.

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