JOUENAL OF THE TENNESSEE ACADEMY OF SCIENCE
VOLUME 63, NUMBER 1, JANUARY 1988

MORPHOGENESIS IN THREE CULTIVARS OF BOSTON FERN. I. SOME ASPECTS OF LEAF AND STEM ANATOMY*

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ABSTRACT

Certain aspects of leaf, stolon, and rhizome anatomy of three cultivars of Boston fern, Nephrolepis exaltata, were examined in section in order to study anatomical similarities and differences among these cultivars and to become familiar with certain aspects of the anatomy. Such information would be useful in later studies of induction, proliferation, and differentiation of callus and free cells derived from each of the three cultivars. Examination of leaf, stolon, and rhizome sections revealed that all three cultivars contain the usual anatomical arrangement of cells and tissues common to the Nephrolepis species. Moreover, the anatomical features studied were similar in all three cultivars.

INTRODUCTION

The Boston series of ferns have been popular ornamental plants for many years. These ferns are sterile except for Nephrolepis exaltata, c.v. 'bostoniensis' "var.fertilis." Non–fertile cultivars of Boston ferns are propagated in the greenhouse by simple layering of runners (stolons) which originate from the base of the petioles, take root, and form new plants (Morton, 1958). Recent advances in sterile tissue culture methods have made possible the production of large numbers of plantlets by inducing stolon tip multiplication in Boston fern (Harper, 1976) and in Fishtail fern (Beck and Caponetti, 1983). Many nurseries are now producing large numbers of Boston fern in tissue culture from excised stolon tips rather than by greenhouse propagation of layered stolon tips.

The capacity for rapid propagation and general amenability of Boston and Fishtail ferns in sterile tissue culture has led to the idea that Nephrolepis species and cultivars could serve as a model system for beginning studies of morphogenesis in these pteridophytes. A study of the induction, proliferation, and differentiation of callus and free cells of Nephrolepis exaltata and some of its cultivars would serve to answer some basic questions in the morphogenesis of ferns. Complete differentiation of whole plants from undifferentiated cells and tissues has not been accomplished yet with pteridophytes. In addition, these basic studies could eventually supply ornamental horticulturists and the nursery trade with new and improved methods for the rapid propagation of the Boston fern group which is popular as ornamental plants.

Before conducting morphogenetic studies, some aspects of leaf and stem anatomy should be examined in order to determine anatomical similarities and differences among cultivars, and to report on those anatomical aspects deemed important for later morphogenetic studies of callus and free cell induction. Such is the purpose of the present investigation.

MATERIALS AND METHODS

The experimental plants were Boston fern, *Nephrolepis exaltata* c.v. 'bostoniensis' and two of its dwarf cultivars, *N. exaltata,* c.v. 'scotti' and *N. exaltata,* c.v. 'dwarf boston.' The Boston ferns were available from the departmental greenhouse collection obtained from North Carolina Farms, Indian Train, North Carolina. The dwarf cultivars were obtained from the New York Botanical Garden, Bronx, New York, courtesy of Drs. Bruce McAlpin and John Mickel.

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* This publication is a portion of a dissertation submitted by Thomas E. Byrne in partial fulfillment of the requirements for the Ph.D. degree at The University of Tennessee, Knoxville.

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Leaf blade (pinna), rhizome, and stolon tip segments were killed and fixed in three different fixatives, namely CRAF, gluteraldehyde, and FAA. The CRAF was composed of 3.0 ml of 1% chromium oxide, 2.0 ml of 10% acetic acid, 1.0 ml of 20% formaldehyde, and 4.0 ml of distilled water. The gluteraldehyde consisted of 5% glut-eralde– hyde in distilled water. The FAA was composed of 90.0 ml of 50% ethanol, 5.0 ml of glacial acetic acid, and 5.0 ml of 40% formalin. Fixative formulae were modified from Jensen (1962). All three fixative solutions were allowed 24 hours contact time with all tissues.

The CRAF- and gluteraldehyde-fixed tissues were dehydrated with ethanol at concentrations of 5%, 10%, 20%, 30%, and 40%. Alcohol changes were made at 30–minute intervals. After completion of the ethanol series, the tissues were further dehydrated with tertiary butyl alcohol at concentrations of 50%, 60%, 70%, 80%, 90%, and three changes of 100%. These alcohol changes were made at 30–minute intervals also. The FAA–fixed tissues were dehydrated with tertiary butyl alcohol at concentrations of 50%, 60%, 70%, 80%, 90%, and three changes of 100% at 30–minute intervals. Alcohol schedules were modified from Jensen (1962).

All dehydrated tissues were infiltrated and embedded with Fisher Tissue Prep. All sections were cut on a rotary microtome at a thickness of 10 microns, mounted on slides, and stained withhematoxylin dye and iron alum as a mordant (Jensen, 1962). Permanent slides were examined with a Leitz compound microscope equipped with an automatic photographic system.

RESULTS AND DISCUSSION

Leaf and stem tissues were killed and fixed in three different fixatives, namely CRAF, 5% gluteraldehyde, and FAA, in order to determine which fixative would yield the best sections for anatomical study. Preliminary experiments demonstrated that CRAF provided the best fixation with the least damage to the tissues.

Gluteraldehyde induced fractures and gaps between cell walls which were attributed to improper hardening action, causing the tissues to crack when being sectioned on the microtome. The FAA induced cellular shrinkage due to uneven dehydration with the alcohol series. Therefore, the results reported here are based on leaf and stem tissues killed and fixed with CRAF.

The anatomy of leaf and stem tissues was essentially the same in all three cultivars. Photomicrographs which display the morphology of the tissues are presented as typical examples for the cultivars under study. A median longitudinal section of a stolon apex displays several tissues.

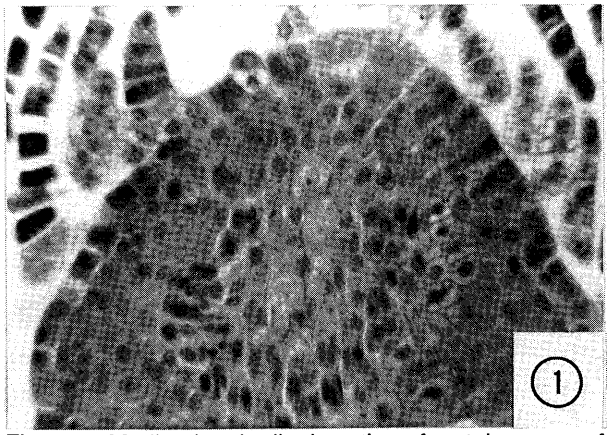

Figure 1. Median longitudinal section of a stolon apex of *Nephrolepis exaltata* c. v. 'dwarf boston' (200×).

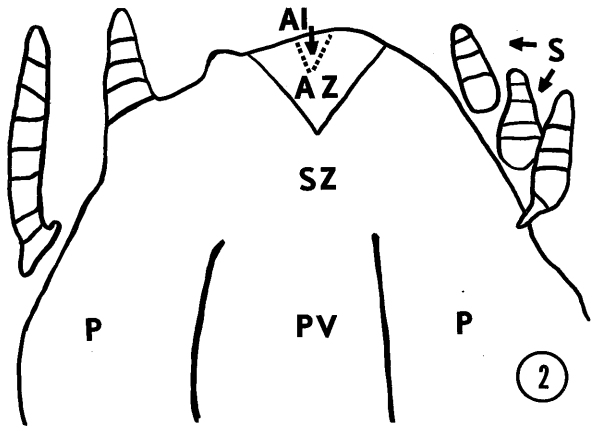

Figure 2. Schematic representation of the stolon apex, apical initials, AI; apical zone, AZ; subapical zone, SZ; parenchyma tissue, P; provascular tissue, PV; scales, A.

Figures 1 and 2 demonstrate the generally observed position of the apical initials, apical zone, subapical zone, parenchyma tissue, provascular tissue and scales. Espagnac (1973) observed similar correlations in his morphological studies of *Nephrolepis biserrata* (Sw.) Schott., and we have followed his terminology. These tissues were consistent in the stolons of all three cultivars. Longitudinal sections of stolon tips showed the presence of a terminal and two lateral bud primordia as the common situation in all three cultivars (Figures 3 and 4).

The anatomy of the leaf blade in cross section was simple in structure, with an upper and lower epidermis enclosing uniform mesophyll tissue with centrally located vascular tissue of the main and lateral veins. This was observed in all three cultivars (Figures 5 and 6). Each vein in cross section consists of primary xylem overlaying primary phloem enclosed by several layers of schlerechyma and surrounded by a vein sheath of parenchyma.

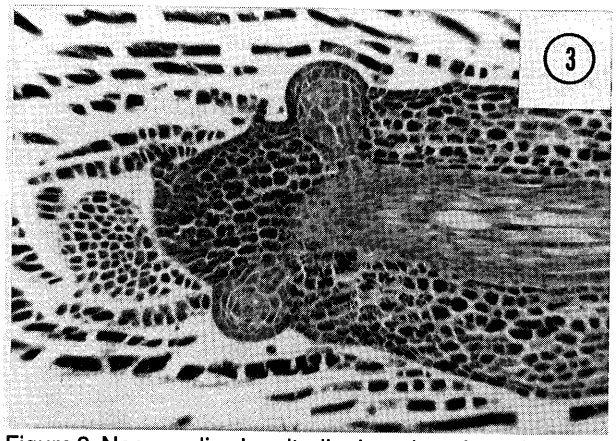

Figure 3. Near median longitudinal section of a stolon tip of *Nephrolepis exaltata* c.v. 'scotti' demonstrating the positions of the terminal and the two lateral bud primordia (80×).

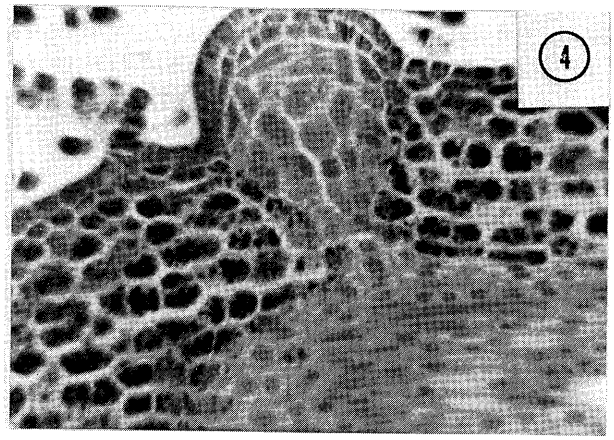

Figure 4. Enlarged view from Figure 3 showing detail of a lateral bud primordium (200×).

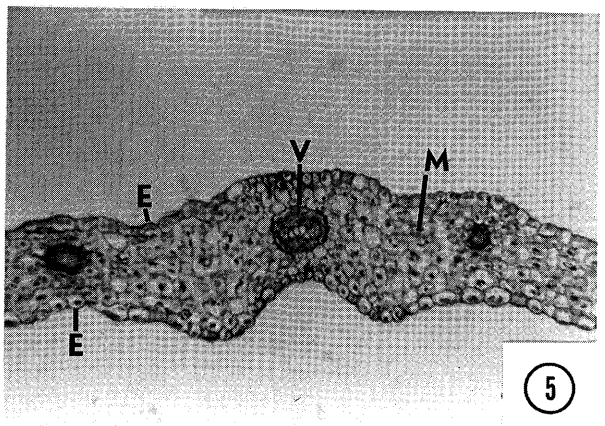

Figure 5. Cross section of a leaf blade of *N exaltata* c.v. 'bostoniensis' showing the upper and lower epidermis, E; mesophyll tissue, M; and vascular tissue, V (80×).

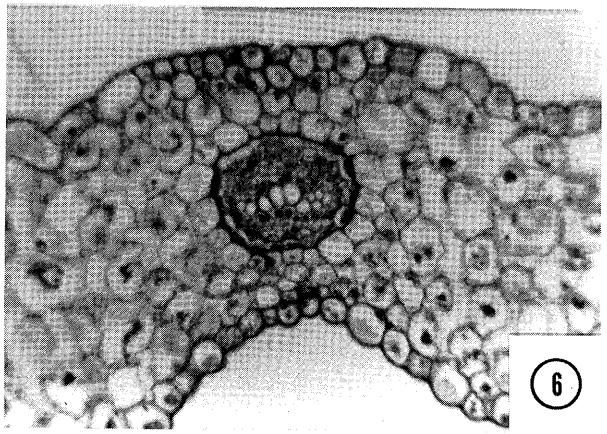

Figure 6. Enlarged view from Figure 5 showing detail of the upper and lower epidermis, mesophyll, and vascular tissue in the main vein (200×).

The anatomy of the rhizome in cross section was also similar in all three cultivars. The vascular tissue was in the form of a dictyostele with leaf traces consisting usually of three strands as shown in Figures 7 and 8. A detailed view of a vascular bundle (Figure 8) displays the anatomy observed throughout the study. The endodermis and pericycle are clearly observable despite separation due to the effects of fixation. In the central area of the vascular bundle, the primary xylem is clearly evident and is surrounded by primary phloem. A ground tissue of parenchyma surrounds each of the vascular strands, and is bordered on the outside of the tissue by the usual epidermis. This description corresponds to that of Haupt (1953) and Bierhorst (1971)on general fern rhizome anatomy.

Now that certain aspects of leaf and stem anatomy of three cultivars of Boston fern are understood, the next step is to attempt the induction of callus on specific leaf and stem tissues. Such is the subject of the next publication in this series.

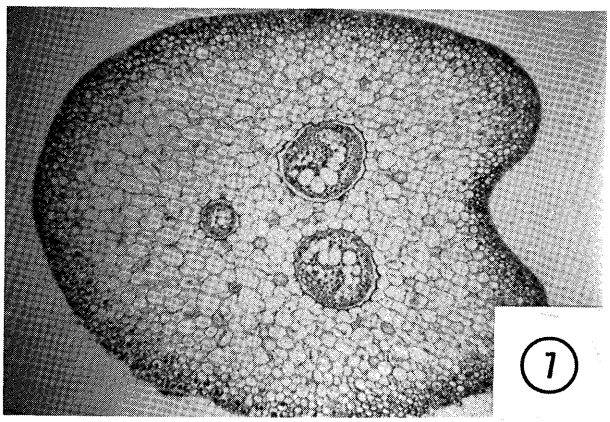

Figure 7. Cross section of a rhizome of *N exaltata* c.v. 'dwarf boston' showing 3 strands of vascular tissue (80×).

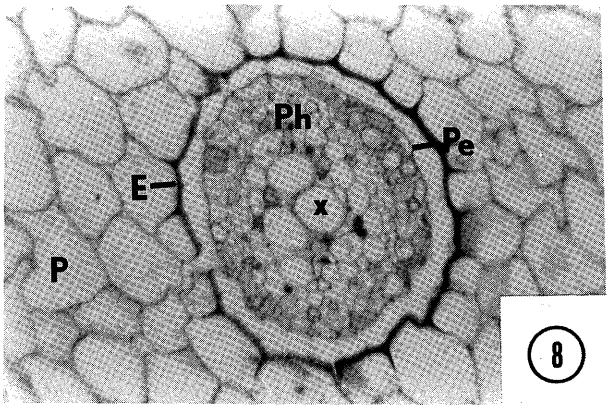

Figure 8. Cross section of rhizome of *N exaltata* c.v. 'bostoniensis' demonstrating position of xylem, X; phloem, Ph; pericycle, Pe; endodermis, E; and parenchyma tissue, P (200×).

ACKNOWLEDGMENTS

We thank the owners of North Carolina Farms for donating initial specimens of cultivar 'bostoniensis', and Drs. Bruce McAlpin and John Mickel for supplying specimens of the dwarf cultivars.

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