CELLULAR BASIS FOR GROWTH AND
TISSUE DIFFERENTIATION PATTERNS IN
LINUM USITATISSIMUM (LINACEAE)
STEMS: THE STEM UNIT

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The stem unit is defined as the smallest portion of a stem that can duplicate the stem in toto through regular rotations and dilations over successive plastochrons. In stems exhibiting \( k(m, n) \) contact parastichy phyllotaxis, the stem unit is delimited vertically and tangentially by the boundaries of four successive leaf primordia along the \( m-, n-, \) and \( (m + n)\)-parastichies and radially by these boundaries extended to the centroid of the stem. With the stem unit concept, node refers only to the region of actual leaf insertion, rather than the entire transverse level of insertion. Many of the conflicting and complicating aspects of the traditional node-internode subdivisions of stems are demonstrated, and the utility of the stem unit in circumventing these is illustrated. The stem unit is proposed as a more useful analytic subunit of the stem with which to examine stem growth and tissue differentiation processes than the more traditional node-internode subdivisions of stems.

We know that both stem and root axes arise through expansion and differentiation of cells that are initially derived from meristematic cells comprising their terminal apical meristems. The kinematics of cellular activity within roots has been well described through very careful studies conducted by Erickson and Goddard (1951) and Goodwin and Avers (1956). From those studies it has been determined that the primary root axis is generated by a temporal displacement of cells through spatially constant domains of cellular activity extending along the vertical axis of the root. A currently unanswered, but very fundamental question is: “Do similar domains of cellular activity exist within the vertical axes of stems?”

Tissues within plant organs are distinguished on the basis of features such as cell size and geometry as well as the histological characteristics of cell walls and cytoplasm. Thus at one level of analysis the patterns of anatomical organization of plant tissues are determined by the interplay between rates and directions of cell expansion and rates and orientations of cell division within the populations of cells that comprise these tissues. Careful analyses of local relative expansion rates and local relative division rates within various tissue categories of roots have revealed that cell size differences between mature tissues are reflected by heterogeneity in rates of cell division within the proximal meristem of the root apex (Jensen and Kavaljian, 1958; Webster, 1980). Similar information on the cellular processes giving rise to tissue differentiation in stems is notably lacking from the literature. Another currently unanswered fundamental question is: “Do similar cell division and expansion rates characterize formation of analogous tissues in stems and roots of plants?”

One of the most obvious differences between stem and root organs of plants is that shoot apical meristems produce leaf primordia at periodic intervals of time, called plastochrons, whereas roots lack these periodic lateral outgrowths in the region of their primary apical meristems. There is good evidence that leaf primordia influence the pattern of tissue differentiation in the stem directly underlying their points of insertion (Young, 1955; Meicenheimer and Larson, 1983; Meicenheimer, 1986, 1987). A currently unanswered and equally fundamental question is: “What influences do leaf primordia have on the cellular activities that give rise to the patterns of tissue differentiation within the vertical axes of stems?”

This is the first part of a series of reports on my investigations into the above questions concerning growth and tissue differentiation processes in Linum usitatissimum L. stems. The basic unit of analysis, the stem unit, is defined and its properties illustrated herein.

Disadvantages of conventional terminology — Plant stems are often subdivided into nodes and internodes. A node is defined as a point on the stem from which one or more leaves arise, whereas an internode is defined as a part of the stem lying between two adjacent nodes (Blackmore and Tootill, 1984). These subunits do not facilitate analyses of the growth and differentiation processes that operate to create stems and their associated internal tissues for the following reasons:

First, there is a conceptual discontinuity between the shoot apical meristem and older portions of the stem with node-internode terminology. As illustrated in Fig. 1, internodes do not exist in the shoot apical meristem because of vertical overlap between nodes. We are thus faced with the fact that while there are obvious regions of stem tissue existing between the regions of primordia insertion within the shoot apical meristem, the accepted terminology of internode cannot be applied to these regions, by definition.

Second, nodes and internodes do not divide the stem into regions that have similar relationships to the leaves on the stem. As illustrated in Figs. 2, 3, the distal portion of an internode in a Linum stem, exhibiting decussate phyllotaxis, is directly underneath a single leaf in one angular view (Fig. 2), but the same internode at equivalent transverse level is tangentially bracketed by the flanks of two leaf primordia, when the stem is rotated 90 degrees.

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from the former angle (Fig. 3). The nearest leaf that is directly above the equivalent transverse level of the latter (Fig. 3) is one internode removed compared to that of the former (Fig. 2). In the former view (Fig. 2) there is a population of small isodiametric cells associated with the nodal region of the stem. In the latter view (Fig. 3) the cells are elongated at the equivalent transverse level of the stem. In a more proximal region of the stem in the former view (Fig. 2) there is a population of elongated cells whose longitudinal dimension is about twice as long as the longitudinal dimension of the elongated cells at an equivalent transverse level in the latter view (Fig. 3). In addition to these differences in the geometry of the epidermal cells on orthogonal sides of the stem, there are noticeable differences in the distribution of the guard cells between the two views. In the former view (Fig. 2) the guard cells are fairly evenly distributed within the epidermis, whereas in the latter (Fig. 3), guard cells are noticeably lacking in the central region of the stem. Thus there is a tangential variation of epidermal cell size and differentiation patterns within any given tangential level of an internode that reflects the variation of the relative proximity of nearest leaves to a particular position in the internode. Similar complications arise in plant stems exhibiting spiral phyllotaxis, but the tangential variation at any given vertical level within an internode is more complex, since at any given angle the proximity to the nearest leaf is more variable.

Third, tissue patterns within node and internode subdivisions of a stem are complex because the vascular cylinder at any vertical level in the stem represents the integral of the spatial and temporal functions for individual leaf traces associated with leaves of different ages that are inserted at unique vertical levels on the stem. This complexity is well illustrated in Fig. 4, which represents the unrolled vascular cylinder of a 13, 5) Linum usitatissimum stem exhibiting a 1(5; 8)/13 leaf trace pattern. This complex spatial pattern arises through the vertical and tangential influence that leaves have on the differentiation of parenchyma, represented by the white regions of the map, within the residual meristem/procambium regions, represented by the shaded regions of the map (Meienheimer, 1986, 1987). Since all leaves are inserted at unique vertical and tangential positions on stems exhibiting spiral phyllotaxis, their relative influences on differentiation processes at any given vertical and tangential position within an internode is unique. Treating transverse segments of internodes as homogeneous bodies of tissue obviously ignores this fundamental aspect of stem anatomy easily seen in any transverse section of a stem.

**Advantages of the stem unit concept**—A new subunit, the stem unit, is proposed to replace the node-internode subunits of stems, which alleviates many of the complicating aspects of these subunits from analyses of stem growth and tissue differentiation. The stem unit is defined as the smallest portion of a stem that through regular rotations and dilations over successive plastochrons can duplicate the stem in toto. In stems exhibiting \( k(m, n) \) contact parastichy phyllotaxis the stem unit is delimited vertically and tangentially by the boundaries of four successive leaf primordia along the \( m \), \( n \), and \( (m + n) \)-

parastichies and radially by these boundaries extended to the centroid of the stem. In the above notation, \( k = \text{jugacity, or number of leaves at the same vertical level on the stem, and } m \leq n \) are integers representing the plastochronic age differences between members of two opposed contact parastichies. With the stem unit concept, node only refers to the region of the stem at which the leaf is actually inserted, rather than the entire transverse level of the insertion.

The stem unit provides a conceptually continuous unit of analysis for the entire stem, beginning within the shoot apical meristem and extending to its most mature portions, as is illustrated in Fig. 1. The entire stem can be subdivided into nodes and subjacent stem units, with each subdivision being of a unique plastochron age and occupying a unique spatial position within the stem as a whole. The sum of all nodes and stem units adequately represents the entire stem, with no confusing spatial overlaps that are inherent to the node-internode concept.

The plastochronic age of the stem unit is defined at the time of formation of the distal boundary primordium since by definition the stem unit is delimited by four boundary primordia and the unit does not exist until the distal boundary primordium is formed. Just prior to the formation of the distal boundary primordium (e.g., 64 in Fig. 1) there is a region of the shoot apical meristem that is delimited by three primordia (61, 59, and 56 in Fig. 1), but this area cannot be considered a stem unit by definition. Rather, this area represents the largest portion of the shoot apical meristem that is unoccupied by leaf primordia. As noted by Meichenheimer and Zagorska-Ma-
rek (1989) the physical boundaries of shoot apical meristem can be defined by the acropetal margins of leaf primordia in a regular manner, but the physical boundaries of the meristem do not form a regular figure of revolution, as is often assumed. Rather there is variation with regard to the longitudinal and tangential boundaries of the shoot apical meristem that is dependent upon the pattern of phyllotaxis. The stem unit (e.g., 64, 61, 59, and 56 in Fig 1) only becomes defined at the time of formation of the distal primordium (64 in Fig 1). Thus the plastochronic age of the stem unit is associated with the distal boundary primordium rather than the more proximal boundary primordia.

The stem unit divides the stem into regions that all have similar relationships with the leaf primordia and leaves borne on the stem. As illustrated by the two decussate Linum stem units that differ in age by six plastochrons (Figs. 3, 5), the distal portion of the stem unit is always directly beneath a leaf or node (L + 1), the proximal portion of the stem unit is always directly above a leaf or node (L - 1), and the central portion of the stem unit is always tangential to two leaves or nodes (L, L'). These relationships hold for stems exhibiting spiral phyllotaxis, as well, although there is vertical separation between the tangential boundary primordia (cf. the m- and n-primordia in Fig. 1).

The stem unit subdivides stem tissues into regions that have similar repeated patterns relative to leaves bounding the stem unit that can be followed over successive plastochrons (Fig. 4). As illustrated by the residual meristem/procambium tissue (shaded) and the parenchymatic tissue (white) of the two Linum usitatissimum stem units (39-36-34-31 and 38-35-33-30), which differ by one plastochron in age, the growth and differentiation processes occurring within the vascular cylinder can be easily followed using the stem unit concept. Note that the leaf gap parenchyma associated with the (m + n) leaves (30, 31) expands acropetally and the interfascicular ray parenchyma associated with the o-leaves (39, 38) expands basipetally within the vascular cylinder as the entire stem unit expands in length. The tangential boundaries of the vascular cylinder are delimited by the junction of interfascicular ray and leaf gap parenchyma associated with the m- (36, 35) and n- (34, 33) leaves within the stem unit. This property of the stem unit can be used to determine the spatial and temporal functions for stem growth and tissue differentiation processes within the longitudinal axis of the stem as a whole.

The stem unit can also facilitate analyses of stems sectioned in the transverse plane (Figs. 4, 6). At any given vertical reference level for a transverse section of a stem there will be k(m + n) stem unit sectors visible. Furthermore, km of these stem units will be composed of sectors that are delimited by the tangential boundaries of the o nodal regions of leaves distal to the reference level (Figs. 4, 6). There will be k(n - m) sectors that correspond to stem unit regions that are delimited by tangential boundaries extending from o to m nodal regions distal to the reference level (Figs. 4, 6). And there will be k(m) sectors that correspond to stem unit regions that are delimited by tangential boundaries extending from m to n nodal regions distal to the reference level (Fig. 4, 6). Thus, if the divergence angles are known between leaves on a stem the internal stem tissues viewed in transverse section can be rapidly subdivided into k(m + n) stem unit sectors that will be equivalent subdivisions regardless of phyllotactic patterns. Of course each of these stem units will be sampled at different relative vertical distances along its total extent such that serial transverse sections are required to examine longitudinal variation within the stem unit. Using the stem unit for analysis ensures that regions of the stem in known proximity to leaves and leaf primordia on the stem are compared in such studies.

A further advantage of the stem unit is that measurements necessary for the quantification of longitudinal stem growth are facilitated since the distal and proximal boundaries of the stem unit lie along steep parastichies that will occur on the same side of the stem. This situation abnegates complications that arise when internode length measurements are attempted between successive reference leaves that are at minimum 90 degrees apart from one another on the stem circumference. Measurement of tangential stem growth is also facilitated since the tangential boundaries of the stem units are well-defined entities, as illustrated in Figs. 3 and 5, which represent the superficial views of two stem units that differ by six plastochrons in age.

By utilizing data gathered from stem units of different ages one can gain insights into the patterns of cellular processes that are contributing to the growth of the stem. For example, a population of small isodiametric cells appears to be maintained beneath the distal node of the stem unit through time (L + 1 in Figs. 3, 5), whereas the more proximal population of elongated cells appears to increase. Within successive stem units the patterns of guard cell differentiation could also be readily assessed.

The stem unit length can be easily utilized to develop a plastochron index that provides an indirect measure of chronologic time for morphological and anatomical events of shoot development. Figure 7 illustrates the temporal functions expressed in plastochrons of stem unit elongation for four individual Linum plants with 57 or more total nodes. The plastochron index was calculated using a 5-mm stem unit index length. Mean coefficient of de-

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Figs. 2, 3, 5. Scanning electron micrographs of epoxy replicas of a decussate Linum shoot. Arrows denote equivalent transverse levels in each view that are orthogonal to one another. Bars = 100 μm. 2. The stem lies directly beneath one of the decussate leaves (L). 3. The stem is rotated 90 degrees from the view in Fig. 2, and the equivalent transverse level of the stem lies tangentially beneath the flanks of the two decussate leaves (L, L'). One complete internode separates the equivalent transverse region from a directly distal leaf (L + 1) in this view compared to Fig. 2. The L + 1 leaf is one plastochron younger than the L, L' leaves. L - 1 represents the axillary region of one of the decussate leaves that is one plastochron older than the L, L' leaves. 5. This stem unit is ca. six plastochrons older than the stem unit illustrated in Fig. 3. The distal portion of the stem unit is always directly beneath a leaf or node (L + 1), the proximal portion is always directly above a leaf or node (L - 1), and the central portion is always tangential to two leaves or nodes (L, L'). The proximal portion of the stem unit has been truncated to preserve resolution of the epidermal cells.
termination for all four exponential regressions on these data was 98.25 (SD = 0.50). This confirms that stem unit elongation was exponential and constant between successive stem units on individual stems. From a previous study (Meicenheimer, 1987) it is known that the Linum plastochron stabilizes at 0.21 days after the initiation of the 35th node. These data verify that the three basic assumptions (Lamoreaux, Chaney, and Brown, 1978) underlying the use of the plastochron index are met in the case of the stem unit. Mean regression coefficients from the four regressions was 0.1197 (SD = 0.0086), indicating that the Linum stem unit expands at a rate of ca. 12% plast−1 or 57% day−1.

**DISCUSSION**

The question of whether similar domains of cellular activity that have been detected in roots exist within the vertical axes of stems has been partially answered by a recent study (Maksymowycz, Maksymowycz, and Orkiszewski, 1985) conducted on the older and more physically accessible parts of the stem. In these studies the classical subdivisions of node and internode were utilized in an elemental analysis of stem elongation. The question still remains unanswered within the relatively inaccessible shoot apical meristem because young leaves surround this region of the stem and prevent direct observation of the living cells. This circumstance is never likely to change, but the concept of the stem unit, for which a developmental index is easily developed, opens up the possibility of using destructive sampling techniques to investigate the cellular processes of stem growth within this region of the shoot.

As pointed out above, internodes do not actually exist in the classical sense of the term, within shoot apical meristems, which further complicates the possibility of
extending quantitative studies of stem growth into this region of the shoot. Previous quantitative studies of shoot apical meristem growth essentially avoided the problem of the nonexistence of internodes in the apical region by defining an “internode” as the vertical distance between two nodal planes (Meichenheimer, 1979, 1981, 1982, 1987). While this definition had the advantage of being conceptually continuous between the shoot apical meristem and more mature regions of the stem, it could only provide quantitative estimates of stem growth in gross dimensional units. The stem unit concept provides a means of analyzing stem growth in terms of the inherent morphological structure at the cellular level of resolution and maintaining conceptual continuity between the shoot apical meristem and more mature regions of the stem.

Progress in understanding the cellular processes of internal stem tissue differentiation has been slow. This is a reflection of the hitherto confounding complexity of stem tissue that can necessarily only be examined in a static state. Some progress has been made along these lines by using methods for kinematic analysis of plant growth developed by Silk and Erickson (1978, 1979) and the plastochron index concept (Erickson and Michelin, 1957) in conjunction with data collected on xylem of individual leaf traces (Meichenheimer and Larson, 1983). The shortcoming with that study was that it only pertained to one particular tissue type within the stem. Integration of the spatial and temporal functions of individual leaf trace functions adequately reflected the behavior of the central trace symphodia at all vertical levels in the stem, but the extension of this analysis to nonvascular tissues was prohibited by the lack of suitable benchmarks to delimit natural boundaries of these tissues within the stem. The stem unit concept provides a definition of such natural boundaries based on the position of leaves within the stem that have long been known to be arranged in well-ordered patterns that are easily analyzed (Erickson, 1983; Meichenheimer and Zagorska-Marek, 1989). Therefore, the stem unit concept opens up the possibility of performing kinematic analysis on all tissues of the stem, not just the vascular tissue. Examination of the cellular differentiation processes of all tissues in developing organs is important because tissue patterns arise through juxtaposition of cells following divergent pathways of differentiation from an initially homogeneous population of cells as illustrated in recent studies performed on leaf trace pattern differentiation through ontogeny (Meichenheimer, 1986, 1987; Meichenheimer and Leonard, 1990).

Finally, the stem unit concept subdivides all stems into component parts that have mathematical relationships with the phylloaxis of the stem. Thus the mathematical rigor of contemporary phylloaxis analysis is easily extended to the stem using the stem unit concept. This provides an equivalent unit of analysis that can be universally applied to the exterior and interior portions of all stems regardless of the phylloaxis. Such a unified unit of analysis opens up the possibility of attaining a new level of understanding of the control processes that are operating to generate the diversity of tissue patterns seen through ontogeny of individual taxa and between different taxa.

In conclusion, it is proposed that the stem unit represents a more useful analytic subunit of the stem with which to examine stem growth and tissue differentiation processes than the more traditional node-internode subdivisions of stems. The stem unit will be used as the unit of analysis in future reports on the cellular basis of Linnm stem growth and tissue differentiation in attempts to answer the fundamental questions posed about tissue differentiation processes in roots and stems.

LITERATURE CITED


——, AND J. M. LEONARD. 1990. Comparison of early lateral vein


