POINTS OF VIEW

On divides

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Are there growing divides between research directions in the plant sciences? — As recent technological and methodological discoveries are incorporated in research, significant steps are made toward a deeper understanding of the biology of plants. The need to master these rapidly accumulating and fast evolving new concepts and techniques leads to increasing professional specialization of individuals and, sometimes, of institutions. A shortcoming of such in-depth specialization is the resulting segmentation of research interests and activities, whereby different research directions are explored by distinct groups of scientists. This trend is bound to lead to compartmentalization of knowledge between such groups with different interests. Given that all these different scientific endeavors ultimately converge on the plant, a unitary entity whose development and functioning are the results of complex interactions, such compartmentalization cannot be profitable in the long run. Nevertheless, alarming signs are out that it is already happening, leaving open gaps between different disciplines in the plant sciences. One of the trends we see in plant biology today is a disjunction between the rapidly evolving and broadly encompassing applications of molecular biology, and the more traditional study of anatomy and morphology. When molecular biology tools are used outside the framework provided by classic knowledge of developmental plant anatomy, the consequences can be serious.

To illustrate the disjunction between molecular biology and traditional anatomy, and the pitfalls associated with it, I will briefly discuss here two studies of vascular tissue differentiation. The differentiation of vascular tissues has been for a long time one of the focal directions of developmental studies in plants. Work with model systems such as *Zinnia elegans* (mesophyll cell cultures) and *Arabidopsis thaliana* has brought light into many aspects of vascular tissue development, including the differentiation of tracheary elements and secondary cell wall synthesis. For a better understanding of the examples discussed below I will first provide a brief review of the relevant anatomical aspects of primary xylem development.

Tracheary element development and secondary wall thickening patterns. — Timing of the maturation of primary xylem tracheary elements (TEs) has been used to differentiate protoxylem, comprising the earliest differentiated TEs, from metaxylem, consisting of TEs differentiated later in development (Fahn, 1990). More specifically, protoxylem is defined as the TEs differentiated in elongating parts of the plant body, as opposed to metaxylem, defined as TEs differentiated in parts of the plant body that do not undergo elongation, that is, after elongation has ceased (Esau, 1977). TEs that differentiate in elongating parts of the plant body (protoxylem TEs) lay down secondary wall thickenings in annular or helical patterns, whereas TEs that differentiate after elongation has ceased (metaxylem TEs) will deposit secondary walls with scalariform, reticulate, or pitted patterns.

The distribution of secondary wall thickening patterns in the plant body has a simple explanation in the structure and function of TEs. Functional TEs are conduits devoid of cell contents and need secondary wall material to reinforce the thin primary walls and prevent their collapse. Protoxylem TEs differentiate and become functional while the surrounding tissues are still elongating. In order to stay functional these TEs have to be built to allow for stretching without breaking. This poses a mechanical problem because secondary wall material lacks the elasticity that characterizes primary walls. Thus, while primary walls can stretch, secondary walls are prone to breakage during elongation, which would lead to TE collapse and loss of functionality. The solution to this problem is the production, in protoxylem TEs, of secondary wall thickenings with patterns that allow for stretching—annular and helical (Esau, 1965). In metaxylem, TEs do not undergo any elongation, so secondary walls are laid down in patterns that ensure more complete coverage of the primary walls (scalariform, reticulate, pitted). These confer increased rigidity allowing for development of wider TEs that conduct more efficiently.

In the most general sense, the type of TEs formed in a given vascular strand depends on the timing of TE differentiation with respect to the timing of the cessation of elongation in that part of the plant body. TEs are differentiated continuously during and after elongation in the primary xylem of cauline bundles in most seed plants, so the complete series of secondary wall thickening patterns from protoxylem to metaxylem (i.e., annular-helical-scalariform-reticulate-pitted) is present in those bundles. However, if all TEs in a vascular strand are formed exclu-

sively during elongation, that strand will consist only of TEs with annular and helical thickenings (protoxylem). Not surprisingly etiolation which promotes prolonged elongation results in vascular strands with higher proportions of annular and helical TEs (Goodwin, 1942; Stafford, 1948). Conversely, primary xylem can consist exclusively of scalariform/reticulate/pitted TEs (metaxylem) in vascular strands differentiated in parts of plants that undergo very little elongation (e.g., *Botrychium* and marattialean fern rhizomes) or grow very slowly (e.g., cycad stems). Even in systems where TE precursor cells are not incorporated in tissues (i.e., *Zinnia* mesophyll cultures), the postponing of TE differentiation until *after* expansion of most cells is completed leads to increased numbers of reticulate patterned TEs (Roberts & Haigler, 1994).

Dead cells don't transcribe and secondary walls aren't all about lignin. — An in situ hybridization study of *Zinnia elegans* stems (Dahiya & al., 2006) documented the transcription patterns of *ZeFLA11*, a fasciclin-domain-containing gene isolated by cDNA-AFLP analysis of differentiating TEs (Milioni & al., 2002). The finds of the study were interpreted in terms of selective transcription of *ZeFLA11* in TEs with reticulate secondary wall thickening (metaxylem), and not in TEs with helical thickenings (protoxylem). This conclusion was used to suggest that *ZeFLA11* is required during deposition of secondary walls exclusively in metaxylem TEs. When considered more carefully in light of the characteristics of xylem differentiation discussed above, the conclusions of the study are not warranted by the results, and the experimental design proves to be inappropriate for the question addressed.

The study checked for transcription of *ZeFLA11* in the *mature* zone of *Zinnia* stems and found it only in actively differentiating metaxylem TEs (stages of differentiation 2 and 3; Dahiya & al., 2006). This is not surprising since both cauline bundles (consisting of protoxylem and metaxylem in *Zinnia*) and foliar bundles (leaf traces, consisting of protoxylem) are functional in mature stem segments. In other words, except for actively differentiating metaxylem, all TEs formed earlier in those bundles (during and after elongation, that is, protoxylem and older metaxylem, respectively) were mature and conducting in the analyzed stem segments. Functional, conducting TEs are formed by programmed death of cells that leaves behind only their walls. Being dead, functional TEs could not have been actively transcribing anything at the time of observations. That is why in situ hybridization did not detect any *ZeFLA11* transcript in these functional TEs, whether they were protoxylem or metaxylem. In brief, the transcript was absent from those cells because they were dead, and not because they were protoxylem or early metaxylem. Since the study did not test for *ZeFLA11* transcript in actively differentiating protoxylem (absent in mature stem segments), the conclusion that *ZeFLA11* transcription characterizes exclusively metaxylem elements has no empirical support.

Failure to integrate the results of molecular methods with basic facts of plant anatomy in Dahiya & al.'s study leaves unanswered the question of whether *ZeFLA11* is expressed only in metaxylem and not in protoxylem TEs as they differentiate. The in situ hybridization data of that study contribute only a confirmation of Milioni & al.'s (2002) earlier finding that the product of *ZeFLA11* expression has a role in secondary cell wall formation.

This case of a molecular biology study disconnected from the reality of plant anatomy is not singular. In a more dramatic example, Lev-Yadun & al. (2005) reinterpreted the anatomy of *Arabidopsis thaliana revoluta/interfascicular fiberless1* mutants (Talbert & al., 1995; Zhong & al., 1997; Ratcliffe & al., 2000) based on anatomical observations in polarized light. Zhong & al. (1997) had previously isolated and named the *ifl1* mutants based on what they interpreted as a lack of interfascicular fibers in their inflorescence stems. Their interpretation of the phenotypic effects of the mutation was based on poor histochemical staining targeting lignin. Lev-Yadun & al.'s (2005) observations in polarized light revealed that mutant plants do, in fact, produce fibers, but those fibers have poorly lignified secondary walls. Thus, where histochemical staining had failed to reveal the presence of fibers, use of a different method of secondary wall identification—polarized light microscopy—succeeded by identifying the characteristic birefringence of crystalline cellulose.

Grow your own anatomist. — The two examples discussed above show that a divide is growing in the study of plant development between molecular approaches and classic anatomical knowledge, with alarming effects. Powerful molecular tools can be dangerous double-edged swords when used outside the conceptual framework provided by developmental plant anatomy. Losing sight of this invaluable body of knowledge accumulated over more than a century of careful observations can lead to erroneous interpretations with potentially drastic consequences. If in the case of Dahiya & al.'s (2006) study the consequences of misinterpretations are not immediately apparent, Lev-Yadun & al.'s (2005) work points to much more serious consequences. In the latter, a mutation initially believed to downregulate the production of fibers, proved to promote production of fibers with poorly lignified walls, a trait highly sought after in the paper industry and very profitable if transformed into species harvested commercially.

If we can recognize such divides before they become unbridgeable, we will be able to take measures to prevent the segmentation of knowledge and avoid its consequences. The onus of such measures is primarily on the

researchers. One obvious way to avoid mishaps in our era of increasing specialization is to initiate partnerships with plant anatomists. Journal editors can contribute too, by enlisting the help of plant anatomists. However, in a world where molecular biology is making tremendous strides toward explaining more and more of the life of plants, classic plant anatomy seems to be fast becoming a lost art. As I have attempted to show above, this is already producing consequences, and if we genuinely want to make full use of the discovery potential of molecular biology, we have to base that kind of work on solid plant anatomy foundations. In conclusion, to those doing research in the molecular field who don't have the time to master anatomical knowledge but who understand the dangers of the situation, as well as to journal editors in the trade, I say: "Grow your own anatomist!"

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