

DOI: 10.5586/asbp.3515

Publication history

Received: 2016-06-10

Accepted: 2016-09-23

Published: 2016-09-30

Handling editor

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Authors' contributions

EMG: idea of the study, planned and performed experiments; EMG and JAJ: discussed and wrote the manuscript

Funding

This project was supported by the University of Wrocław (grant No. 1068/S/IBE/16 to EG).

Competing interestsJAJ: member of the *Acta Societatis Botanicorum Poloniae* editorial council; EMG: no competing interests**Copyright notice**© The Author(s) 2016. This is an Open Access article distributed under the terms of the [Creative Commons Attribution License](#), which permits redistribution, commercial and non-commercial, provided that the article is properly cited.**Citation**Gola EM, Jernstedt JA. Vascular structure contributes to shoot sectoriality in *Selaginella kraussiana*. *Acta Soc Bot Pol.* 2016;85(3):3515. <http://dx.doi.org/10.5586/asbp.3515>**Digital signature**

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ORIGINAL RESEARCH PAPER

Vascular structure contributes to shoot sectoriality in *Selaginella kraussiana*

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Selaginella species are characterized by regular anisotomous dichotomous divisions of the shoot apical meristem, giving rise to two new axes (branches) which differ in size. A vital process is the formation of vascular connections, which enables continuous communication and consequent functional and developmental integration of a plant during branching. Here, we present the sequence of developmental changes in the vascular system of *Selaginella kraussiana* related to dichotomous branching. Stem vasculature in *Selaginella kraussiana* consists of two meristeles which change in arrangement during shoot development. Using dye tracers, we documented developmental functional isolation of meristeles associated with the specific structure of the stelar system, which results in a spatiotemporal sectoriality of the shoot. We discuss sectoriality in terms of possible significance for shoot development.

Keywordsanisotomy; dichotomous branching; meristele; *Selaginella kraussiana*; shoot sectoriality; vascular system**Introduction**

Formation of a regular pattern of branches resulting in modular plant body construction is one of the vital roles of shoot apical meristem (SAM) activity. In a majority of vascular plants, branching is associated with a repetitive formation of axillary buds and their subsequent development typically some distance from the SAM [1–3]. Alternatively, in the lycophytes *Selaginella* and *Lycopodium* s. l., new branches are the outcomes of a terminal split of the actively functioning meristem, i.e., a SAM dichotomy. During dichotomy, the apex and the vascular system supplying it are completely reorganized and divided into two twin branches. When the size of both resultant apices is similar, they form equal (= isotomous) branches; alternatively, due to uneven (= anisotomous) terminal division of the meristem and/or unequal outgrowth, two branches differing in size develop [4–6]. The plane of dichotomy and underlying developmental events appear to be strictly controlled and conserved in plant ontogeny, defining regular plant body construction and the characteristic appearance of most lycophytes.

Branch system formation in the genus *Selaginella*, where dichotomous divisions of the shoot occur only in one plane and are morphologically unequal with resultant axes maintaining their initial size discrepancy during subsequent growth, is of special interest. Repetition and alternation of such anisotomous “minor” and “major” axes during successive divisions produce a strikingly regular branching pattern [7–9].

Shoot architecture is even more complex, as at each branching point one or two detached meristems (called “angle meristems” [8,10]) are present, which give rise to the rhizophores, the unique root-bearing axial organ of *Selaginella* (for review see [11,12]).

The mechanisms responsible for the development of the regular and complex shoot branch system in *Selaginella* are not fully known. Some explanations stress, however, the role of vascular tissue [13–15]. The vasculature of *Selaginella* has been generalized to be a protostele with meristeles, with the number and arrangement of the meristeles species- and organ- dependent. Based on a literature survey ([16,17] and references therein), the overall stelar structure in *Selaginella* can be classified into several categories, although stelar type and vascular tissue arrangement can change during stem development. The simplest vascular system contains a single, usually dorsiventrally flattened stele (protostele), e.g., in *S. martensii* [9,17], *S. rupestris* [18], and *S. apoda* [19,20]. In some species, shoot vasculature is composed of two meristeles (i.e., two protosteles, each enclosed within its own endodermis) running parallel to each other in the stem (*S. kraussiana*) [8,17] or of three or more meristeles [*S. willdenovii* [7,13], *S. lyallii*, and *S. uliginosa* [17]]. The stele in the shoot can also form a complex network, resembling an actino-plectostele as in the giant *S. exaltata* [16]. Regardless of the number of meristeles, they are located in a central air chamber (lacuna) and supported by short endodermal cells (trabeculae). Trabeculae have well-developed Casparian strips at the anticlinal cell walls [8,17,21] and additionally, cell walls undergo cuticularization forming a so-called trabecular ring [22]. Casparian strips, which are thickenings of the endodermal cell walls containing suberin, together with the trabecular ring with deposited cutin, are the barrier for water-soluble substances transported in the apoplast. In contrast to shoots, the vascular system in rhizophores and roots is much simpler, usually composed of only one central protostele, and devoid of an air chamber [8,9,17].

Development of the stelar system in *Selaginella* during dichotomy as well as its possible relationship to formation of the complex shoot branch system are not fully elucidated despite general knowledge of the species diversity. It has been suggested that the regular alterations of major and minor axes may result from physical or mechanical constraints (e.g., counter-balancing organ or axis placement), thus assuring the maintenance of plant structural integrity (*S. martensii*) [14]. Alternatively, regular alternations of major and minor axes may be determined by the number of steles entering each axis (two out of three vs. all three meristeles as in *S. willdenovii*) [13], or the asymmetrical distribution of procambium in the subdistal part of the apex could control the regular pattern of anisotomous branching in *Selaginella* [15].

As members of the earliest diverging vascular plant clade, *Selaginella* species have become model organisms for studying molecular genetic regulatory mechanisms in both developmental and evolutionary contexts [23–26]. In spite of this increasing interest, it is surprising how little is known about the developmental changes of the vascular system of *Selaginella* spp. despite its indisputable role in the functioning and integration of the plant. For example, only the vasculature of older fully mature shoots and branches has been described in *S. kraussiana* [8], and no functional analysis of this system has been reported. Thus, the main goal of this research was a structural and functional analysis of the vascular system in relation to anisotomous division of *Selaginella* shoots. Understanding vascular system developmental properties such as relative timing, position of initiation and maturation, and growth rate can lead to increased understanding of the anatomical events involved in dichotomous branching. Furthermore, the structural separation of meristeles in *Selaginella* stems by Casparian strips suggests the possibility of independent functioning, e.g., in differential transport of nutrients and other substances.

Therefore, this research focused on three main questions: (i) how does the vascular system develop in relation to anisotomous dichotomous branching of *Selaginella* shoots; (ii) does anatomical separation of the meristeles by Casparian strips result in their functional isolation; and (iii) if so, does functional independence of meristeles contribute to or reflect the specification of identifiable shoot sectors, as recognized, e.g., by differential transport of substances? Answers to these questions are discussed in light of the intimate relationship between shoot morphology and vascular anatomy as anisotomous branching proceeds in *Selaginella* shoots.

Material and methods

Material

Selaginella kraussiana (Kunze) A. Braun (Selaginellaceae) plants were provided by the Botanical Garden of the University of Wrocław, Poland. They were grown at 24°C, under long photoperiod (16 h light / 8 h dark).

Anatomical analyses

Shoot tips including two consecutive macroscopically visible branching points were collected and fixed in a 5% formyl : 50% ethanol : 5% acetic acid mixture (FAA). Plant material was dehydrated in an increasing series of tertiary alcohols (50, 70, 90, 100%, three changes in the latter), embedded in Paraplast X-tra (Sigma-Aldrich, USA) [27] and cut as 5–7- μ m-thick transverse sections on a rotary microtome (Leica RM2135, Leica Instruments, Germany). Sections were stained with a 1% Alcian blue-safranin O mixture (2:1) [27], or according to a modified PAS method [28]. For the latter, series of dewaxed and hydrated sections were treated with 1% periodic acid for 20 min in a water bath (40°C), thoroughly rinsed with distilled water, and stained with Schiff's reagent (fuchsin-sulfite reagent, Sigma-Aldrich, USA) for one hour in the dark. Sections then were quickly passed through 0.5% sodium bisulfite solution (three times, 2 min in each change), dehydrated in an increasing ethanol series (up to 100%), and mounted in euparal (Carl Roth GmbH, Germany).

Additionally, to visualize the branching point structure in toto, fresh shoot fragments were stained with 0.1% basic fuchsin for 30 min followed by clearing in 5% potassium hydroxide (overnight, in 60°C). The material then was washed thoroughly with water and dehydrated in an ethanol series (10, 30, 50, 70%) as described by Webster and Steeves [8]. In addition, the older 4th or 5th macroscopically visible branching points were cleared in potassium hydroxide as described above, stained with propidium iodide (PI) in a working concentration of 100 μ g/mL, and examined with an epi-fluorescence microscope.

Dye transport assay

Stems with two consecutive macroscopically visible dichotomies were selected for the dye transport assay. Aqueous solutions of 1% acid fuchsin and the apoplasmic tracers Lucifer Yellow or Texas Red, in 1 mg/mL working concentrations, were separately applied to the individual dissected meristemes. Dye distribution in the entire branching system was examined using bright field illumination or with fluorescent light.

All analyses were performed with an Olympus BX 50 microscope (Olympus Optical, Poland) with bright field optics and illumination for epi-fluorescence, using blue (470–490 nm, for Lucifer Yellow) or green (530–550 nm, for Texas Red and propidium iodide) excitation filters (Olympus Optical, Poland). Images were recorded with a digital camera (Olympus DP70, Olympus Optical, Poland), using the Cell[^]B software (Olympus Optical, Poland) and processed in Fireworks MX 2004 (Macromedia, USA).

Results

Architecture of the shoot system

General organography of *Selaginella kraussiana* shoots is well established and will be only briefly summarized here. The shoot system of *S. kraussiana* is composed of slender stems, bearing pairs of dimorphic microphylls. Smaller leaves occur in two alternating ranks on the dorsal side; opposite or nearly opposite these are the larger leaves, also in two alternating ranks, on or toward the ventral side of the slightly dorsiventrally

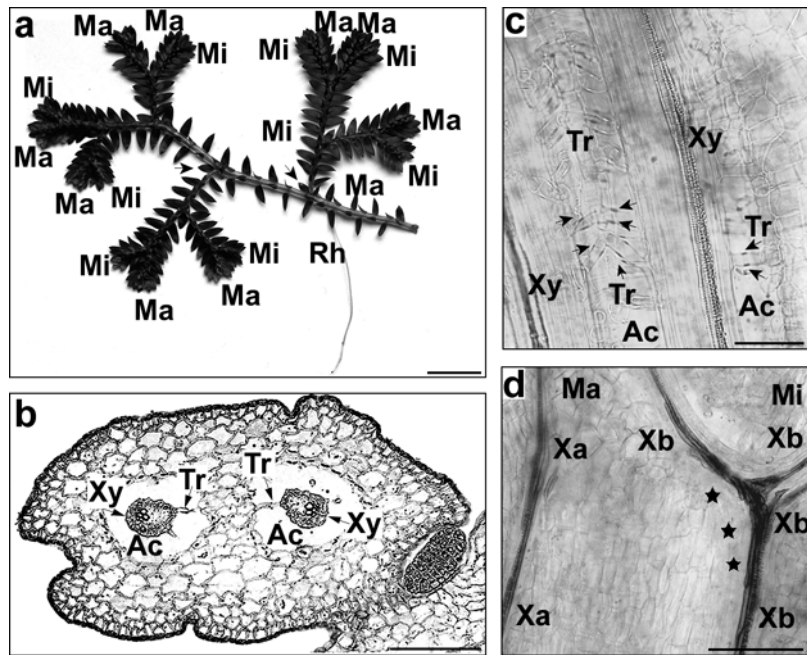


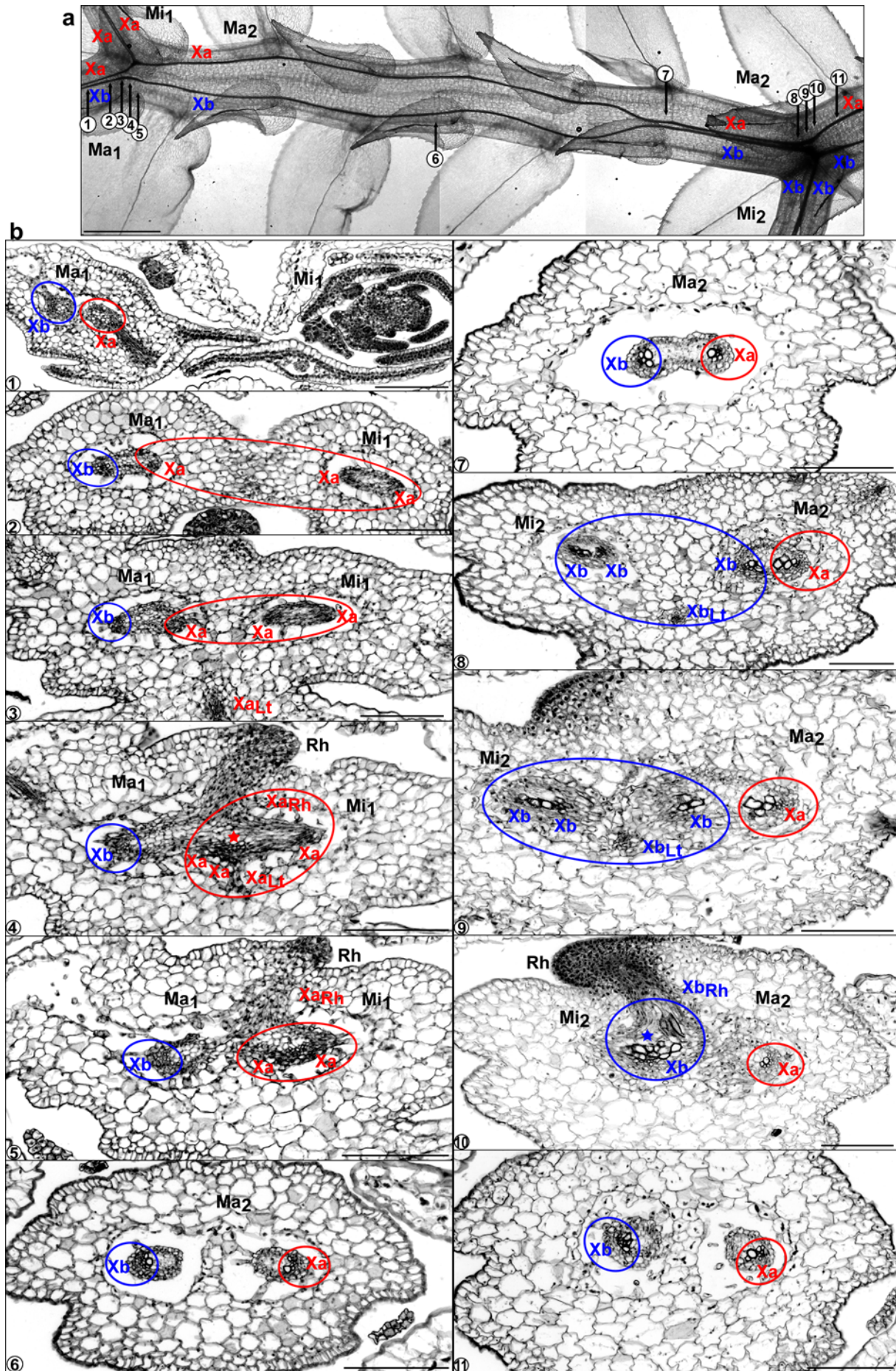
Fig. 1 General morphological and anatomical features of *Selaginella kraussiana*. **a** Regular shoot dichotomous branching, with alternating major and minor branches and a developed rhizophore at an older branching point. Angle leaves (microphylls) in two successive branching points are indicated by arrows. **b** Stem cross-section view (**c**) of the cleared stem segment presenting two monarch meristemes (each with one protoxylem pole), anchored in the air chamber by short endodermal cells (trabeculae). **d** Cleared 3rd shoot branching point, showing the xylem strand differentiating into three parts (right side), which form two meristemes of the minor branch and an extension of the meristeme xylem in the major axis. The increased number of xylem elements in the meristeme at the side of the minor branch is marked with black stars. Abbreviations: Ac – air chamber (lacuna); Ma – major branch; Mi – minor branch; Rh – rhizophore; Tr – trabecula; Xy – xylem; Xa – xylem strand which remains unchanged in the branching point; Xb – xylem strand which splits into three parts at the branching point, giving rise to the vasculature of the minor branch and a continuation of the existing meristeme of the major branch; black stars mark the mass of xylem in the meristeme at the side of the minor branch. Scale bars: **a** 1 cm; **b–d** 100 μ m.

flattened stems (Fig. 1a). Shoot apices divide unequally and regularly, forming the system of alternating major and minor dichotomous branches (Fig. 1a). On the dorsal side of each branching point, an angle meristem is subtended by the so-called angle leaf; the angle meristem is the site of origin of the rhizophore. On the ventral side, the angle leaf is inserted but in this species no angle meristem is present.

Structure of the vascular system

To characterize the vascular system in relation to repetitive anisotomous branching, anatomical changes in vascular structure through two consecutive macroscopically visible branching points were analyzed in basipetal sequence (from younger to older branches) in accordance with developmental stages. In total, the vascular structure of 20 *Selaginella* shoots was analyzed in serial transverse sections, additionally in 20 cleared apical fragments of shoots and 12 older (the 4th or 5th) cleared branching points.

The vascular system in *S. kraussiana* shoots originated from the primary meristematic tissue, procambium, which occupied the central area in the subdistal region of the apex. Initially, there was a single core of undifferentiated cells, which at a distance of ca. 90–110 μ m from the apex became recognizable as two discrete vascular strands, the meristemes (“1” in Fig. 2b). The meristemes were circular in cross sectional profile at this level and eventually run parallel to each other for most of the stem between



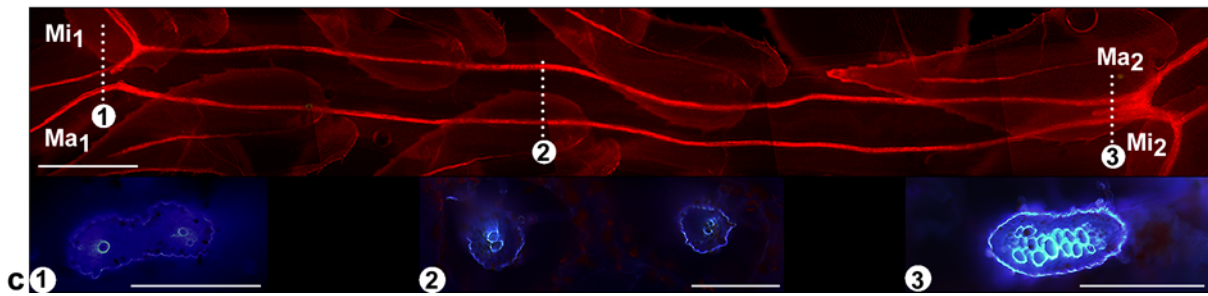


Fig. 2 Structure of the vascular system of *Selaginella kraussiana* shoots through points of dichotomous branching. **a** Cleared shoot segment showing two consecutive young (2nd and 3rd) branch points. **b** Series of transverse sections through the shoot, showing a sequence of developmental changes in the vasculature during two consecutive dichotomies. Sections are presented in a basipetal sequence (from younger to older branches); numbers in circles correspond to sections from positions 1 to 11, indicating the level of the section in panel (a). Upon dichotomous branching, the relative arrangement of both meristemes changes; although they fuse and split, their xylem strands remain separate. Each separate xylem strand and the vascular bundles related to it (including a rhizophore and angle leaves) is labeled and encircled with a different color (red or blue). In the meristeme, at the side of the current minor branch, the xylem mass is larger (stars) as vascular bundles of the rhizophore and the angle microphyll connect with this meristeme. **c** Cleared shoot segment with two consecutive dichotomies stained with propidium iodide and observed using green filter (530–550 nm), and corresponding hand cross sections (autofluorescence in UV light). The vascular system in the relatively younger (3rd) branching point (at left, labeled with the number 1) is still open (with separate xylem strands), whereas in the older (4th) branching point (at right, at the level labeled 3) additional xylem elements occur in the stele connecting both xylem strands. Between branch points, two typical meristemes are present (position and cross section labeled 2). Abbreviations: Ma – major branch; Mi – minor branch; Rh – rhizophore; Xa and Xb – two separate xylem strands, and connected to them vascular bundles of a rhizophore (Xa_{Rh} or Xb_{Rh}) or an angle leaf (microphyll; Xa_{Lt} or Xb_{Lt}). All vascular strands related to xylem strand Xa are labeled or encircled in red; those related to the xylem Xb are in blue. Red or blue stars mark the increased amount of xylem in the corresponding meristeme at the side of the minor branch. Scale bars: **a** and upper panel in **c**: 1 cm; **b** and lower panels in **c**: 100 μ m.

successive branching points (Fig. 1b, Fig. 2a,c). Each meristeme was enclosed in its own endodermis, the short trabeculae with Casparian strips on transverse and radial cell walls (Fig. 1b,c), and situated in an air chamber (lacuna; Fig. 1b,c, Fig. 2). The meristemes were monarch, i.e., each possessed only one protoxylem pole (Fig. 1b, Fig. 2b). In longitudinal view, the first protoxylem elements were recognizable at a distance of ca. 130–150 μ m below the apex, at the level of the first macroscopically visible dichotomy. Importantly, new protoxylem elements differentiated in the acropetal direction typically as the extensions of fully matured xylem strands. Vascular bundles of microphylls located on one side of the stem (in one dorsal and one ventral row) connected in a regular pattern to only one respective meristeme (Fig. 2a). As a consequence, microphylls of one pair, each located on opposite sides of the stem, connected to different meristemes.

At subsequent branching points, there was a change in the structure of the vascular system. What had been two separate but similarly sized meristemes each with one protoxylem pole above the level of branching points (“1” and “6” in Fig. 2b) were succeeded basipetally by one slightly flattened protostele with two xylem poles, separated by parenchyma (“2”, “3”, and “7” in Fig. 2b, protostele of the major axis Ma_1 or Ma_2 with two xylem poles: Xa encircled in red and Xb in blue). The xylem pole located at the side of the minor branch was larger, consisting of a greater number of cells than the opposite xylem pole (Fig. 1d, “4”, “5” – xylem strand Xa and “8”–“10” – xylem strand Xb in Fig. 2b). At the level of branching, this distal mass of xylem (“4”, “5” – xylem Xa and “8”, “10” – xylem Xb in Fig. 2b) of the major axis meristeme differentiates as three xylem strands, differing in their ultimate destinations. Two of these strands formed the two meristemes of the minor branch of this dichotomy, whereas the third one continued into the major branch as the extension of the xylem (and meristeme) that was present in the major axis stem before the dichotomy under consideration (e.g., distal branching point in Fig. 2a – xylem Xa, Fig. 1d, Fig. 2a, “2”–“6” in Fig. 2b – xylem strand Xa encircled in red, “8”–“11” in Fig. 2b – xylem strand Xb encircled in blue). In addition, this particular (third) vascular strand was ultimately connected to a newly developing rhizophore and a dorsal angle microphyll (“3”–“5” in Fig. 2b – xylem Xa, “8”–“10” in Fig. 2b – xylem Xb). At the same time, the opposite meristeme continued in the major branch without changes in either the number of xylem elements or in

xylem arrangement (e.g., Fig. 2a – Xb xylem strand in the distal branching point). Concomitantly with the connection of vascular bundles for the rhizophore and angle microphyll, the number of tracheary elements in the xylem strand at the side of the minor branch (e.g., Ma₁ Xa in the distal branching point in Fig. 2a and Xb in the basal branching point) increased from ca. 5–6 cells to ca. 8–9 cells in cross-sectional view (Fig. 1d and “4” and “10” in Fig. 2b; xylem mass marked with stars). At the same time, the number of xylem elements in the opposite meristele at the major axis remained the same (on average, 5–6 elements).

Below the branching point, the protostele again divided into two discrete meristeles (“6” and “11” in Fig. 2b). At the next branching point, the pattern of the vascular connections was a mirror image of the previous branch point (and the next older), involving the fusion and subsequent splitting of the opposite meristele (Fig. 2a,b). This pattern was repeated over the length of a shoot. Although the meristeles were not distinctly separate at each branch point, their xylem elements did not directly contact at any point of their development. Separation of xylem poles at the branching point was observed in shoots with typically two or three macroscopically visible dichotomies. In older branching points (starting from the 4th), the additional xylem elements matured in the stele, connecting both xylem poles (Fig. 2c).

Dye loading assay

Meristeles in *S. kraussiana* stems were mostly separated in between dichotomies, and in the young branching points even while fused, the xylem poles remained separate (Fig. 2). To test the hypothesis of functional isolation of xylem strands, both non-fluorescent and fluorescent apoplasmic dyes, which are transported as water, were directly loaded into the vascular system through one dissected exposed meristele, either at the side of a minor or a major branch. In total, the distribution of each tracer was analyzed in 15 young stem fragments with two macroscopically visible branching points. There were no differences in dye spread and distribution pattern in the vascular system between the tracers (non-fluorescent vs. fluorescent; Fig. 3, Fig. 4). As a consequence, only acid fuchsin was loaded in comparable experiments with older shoots, i.e., the 4th or 5th subsequent branching points (10 samples). In young branching points, the signal of the tracer was detected only in the meristele to which the dye was applied and its distal splits and in the vascular bundles of all microphylls supplied by this particular meristele (one dorsal and one ventral row of microphylls; Fig. 3a,b,d,e). If the dye was loaded at the side of the major branch, the signal was detected only in half of the major branch vasculature (Fig. 3b,e, Fig. 4b,c). If a minor branch was formed at the side loaded, the dye signal was present in the entire vasculature of the minor branch, half of the vasculature of the major branch, and in the angle microphylls (Fig. 3a,d, Fig. 4a). At the same time, no signal was observed in the other meristele or in microphylls supplied by it, even in the branching point. Thus, the signal was distributed in only one sagittal sector of the shoot (Fig. 3a,b,d,e). In contrast, in the older branching point, regardless of the meristele to which the dye was loaded, the signal was detected in the entire vascular system, including both meristeles of the major and minor branches and all microphyll vascular bundles (Fig. 3c,f). This indicates that in older branching segments, isolated shoot sectors are not present.

Discussion

The interrelationship between the highly regular pattern of the vascular system and the equally regular shoot architectural pattern in dichotomously branching *Selaginella* is poorly understood even after a century of studies (e.g., [29]). Here, we describe anatomical changes in the vascular system of *Selaginella kraussiana* shoots through successive dichotomies. In addition, we present evidence for developmentally regulated structural and functional isolation of the vascular strands, leading to transient shoot sectoriality. Together, our findings contribute to better understanding of the integrative function of the vascular system in *Selaginella* shoots.

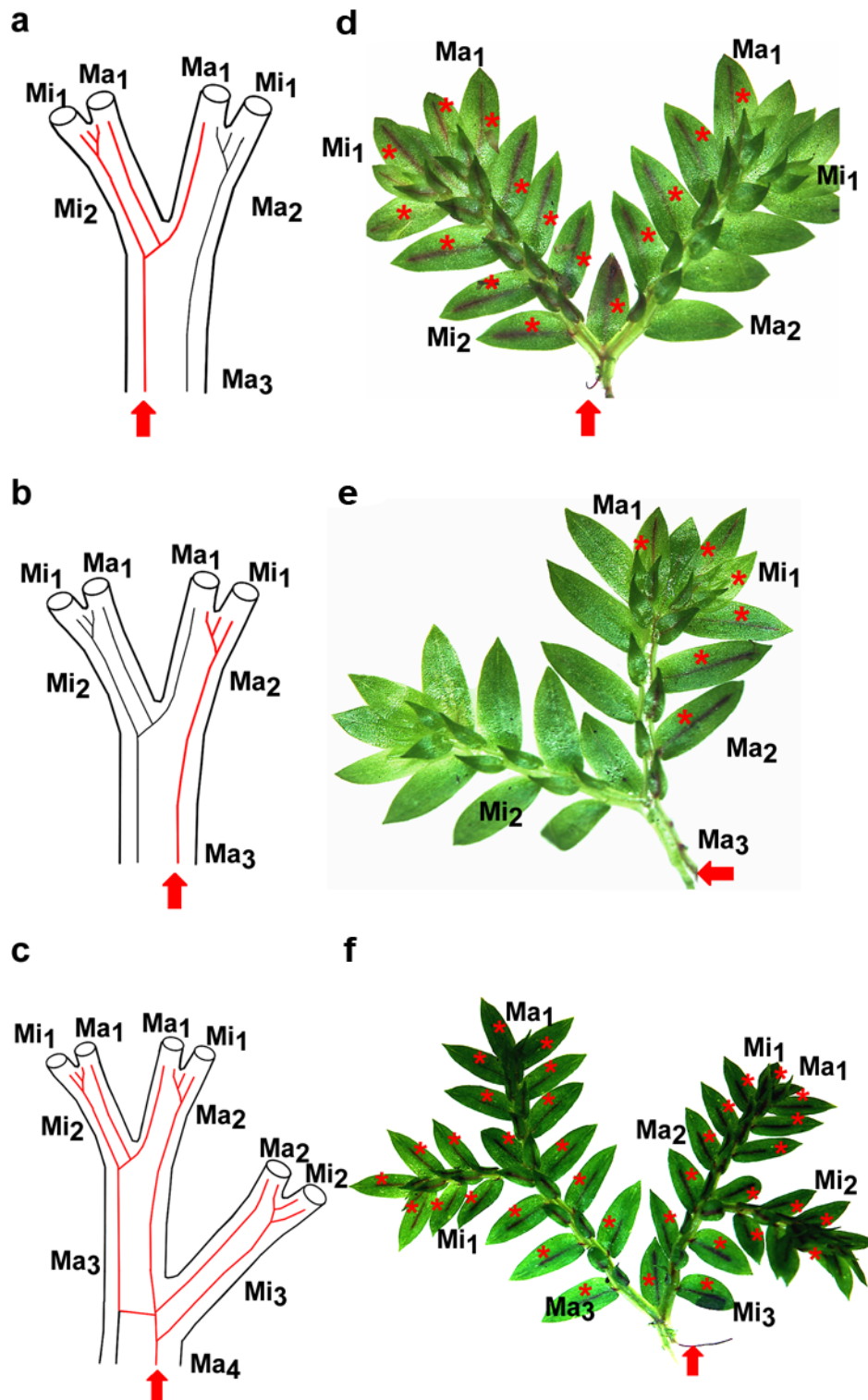


Fig. 3 Shoot sectoriality in *Selaginella kraussiana* as related to vascular structure. Diagrams (a–c) of shoot branch points and vascular system showing the meristele into which dye was loaded (red block arrow), and the resulting dye distribution (red meristemes). Corresponding shoot segments (d–f) show dye distribution; red asterisks indicate presence of dye in microphyll vascular bundles. **a, d** Dye loaded into meristele at the side of the distal minor branch is detected in the vasculature of the entire minor axis and in one lateral sector of the major branch. **b, e** Dye loaded into the meristele which continues unchanged through the branching point is distributed only in one lateral sector of the major branch. **c, f** Dye loaded to meristele at the side of the minor branch (similar to **a** and **d**), but in an older branching point. Due to the xylem connection between meristemes at this level (see “3” in Fig. 2c), dye spreads into the entire shoot system. The same pattern of dye distribution occurs when the dye is loaded into the other meristele (not shown). Abbreviations: Ma – major branch; Mi – minor branch; subscripts indicate the hierarchy of branching with subscript 1 for the youngest first macroscopically visible dichotomy (closest to the shoot tip); red lines show dye distribution in the vascular system; red block arrows point to the meristele into which the dye was loaded; red asterisks mark microphyll vascular bundles with detectable dye.



Fig. 4 Dye distribution in the shoot system of *Selaginella kraussiana*. **a** Distribution of acid fuchsin loaded into the meristele at the side of the minor branch. Red color is visible in the vasculature of the entire minor axis and in one sagittal sector of the major branch. **b,c** Fluorescent apoplasmic dyes Lucifer Yellow (**b**) and Texas Red (**c**) loaded into the meristele which continues unchanged through the branch point. Fluorescent signal is detectable only in one half (one sagittal sector) of the major axis. Abbreviations: Ma – major branch; Mi – minor branch; subscripts indicate the hierarchy of branching with subscript 1 for the youngest first macroscopically visible dichotomy (closest to the shoot tip).

The shoot system in *Selaginella kraussiana* is formed by unequal dichotomous divisions of the shoot apex. The resultant axes maintain their initial size discrepancy during subsequent growth such that a regular pattern of alternating “major” and “minor” branches is evident. In *S. kraussiana*, the vascular system is relatively simple for most of the stem length, consisting of two distinct meristeles, each supplying microphylls at one side of the shoot. However, during development, there is a rearrangement of meristeles at every branch point. While both meristeles connect in the branch junction, forming a transient flattened protostele as that in, e.g., *S. apoda* [20] or *S. martensii* [9], their xylem poles remain discrete and show differentiation in specific relation to the branching pattern. The xylem pole at the side of the minor branch differentiates into a complex vascular network, contributing half of the vasculature of the next (distal) major branch and forming the entire vasculature of the next minor axis. In addition, the minor branch xylem pole makes a vascular connection to a rhizophore. At the same time, the other (opposite) xylem pole remains unchanged until the next dichotomy. This rearrangement of vascular strands may be a common process upon dichotomous branching; a similar situation with consistent splitting and fusing of specific meristeles or xylem strands was shown during branching in other *Selaginella* species (*S. willdenovii*, *S. exaltata*) [7,13,16].

It has been long surmised that the presence of Casparian strips [17], additionally encircled by trabecular rings [22], efficiently restricts apoplasmic transport in *Selaginella* stems. Our dye tracer results are consistent with such a role of these anatomical features, that is, maintaining structural separation of meristeles along the prevailing length of the stem and functional isolation of meristeles in young shoots. Dyes spread only in the meristele into which they were loaded, and into all organs supplied by it. For a time during early shoot development, i.e., within one or two visible dichotomies of the apex, distinct sagittal shoot sectors exist. Interestingly, this transport isolation is maintained even upon meristele fusion distal to the branch point. A lack of free tracer diffusion or leakage across the stele may reflect the absence of tracheid to tracheid connection or could indicate that transfer between both xylem strands is actively blocked. Importantly, this vascular isolation is abolished with ageing of the branches. In older branching points, additional xylem elements differentiate in the central part of the protostele, connecting heretofore separate xylem strands. The universality of these observations is not yet known and it would be very informative to examine those *Selaginella* species with more complex stelar systems. However, for the majority of the genus, including the model species *S. moellendorffii* and intended-to-be-model species *S. apoda* [20], there are only single cross-sections of the stem published, without developmental analyses and reconstructions of the entire system. Since we know meristele arrangement is changeable, the lack of such data makes it difficult to generalize.

Dye loading experiments, although not directly showing xylem or phloem transport, are treated as a reliable method to visualize the structure and functionality of vascular connections [30–32]. This method was successfully used, e.g., to show the sympodial network in the primary vascular system of clonal species [32] and the continuity of particular vessels in trees (e.g., [33–35]). In our case, dye spreading has established the sectoriality of the shoot and the developmentally regulated independence of the vascular strands. These two opposite conditions, sectoriality (independence) and integration of the entire system, are interpreted as complementary stages in the life of a plant [31,36], enabling proper initiation and completion of diverse developmental programs. In this respect, the change in the structure and connectivity of the vascular strands in *S. kraussiana* shoots could potentially be associated with the two roles played by the vascular system during shoot development. Closing of the vasculature by physical connection in older parts of the *Selaginella* shoot can be understood in terms of integrating the entire plant and enabling effective transport, as it is generally accepted that a closed vascular system minimizes or prevents interruptions in water and nutrient supply (e.g., [37] and references therein). However, the role or significance of the earlier developmental isolation of the vascular strands, at the time of their maturation, in younger parts of the shoot, is not so obvious. As the temporal sectoriality of the vasculature is limited to the apical part of the shoot, and only at a certain stage of growth, this suggests the possibility that functional isolation of meristemes at a critical stage could be important for initiating, facilitating, or completing one or more developmental pathways.

The vascular system, which enables long-distance transport of nutrients and other substances, could be a potential source of or conduit for morphogenetic signals involved in determination of the regular patterns of dichotomous branching. Sectorial differences in morphogen allocation, especially within the apical (young) part of the shoot, could potentially be related to specification of the minor vs. major dichotomous branch. This hypothesis is supported by the fact that preferential transport in selected vascular bundles has been shown to be closely related to the metabolic requirements of the developing sinks [31,32], e.g., in our case, new shoot apices.

The nature of any such signal remains to be determined; however, regulation of dichotomous branching of roots by hormonal balance has been suggested [38]. In *S. kraussiana* roots, the antagonistic interaction between cytokinin and auxin is involved in branching control. In shoots, the ultimate combination of hormones awaits discovery [38]. The presence of auxin has been confirmed in *Selaginella* (e.g., [13,39,40]), as well as its key role in morphogenesis, i.e., in determining developmental identity or fate of angle meristemes and/or rhizophores [10,39,41] and in microphyll formation [38]. However, the direct effect of auxin alone on regulation of dichotomy has been rejected [38], despite its well-documented role in branching [1,2,42] and vascular differentiation (e.g., [43]) in seed plants.

Acknowledgments

We thank the three anonymous reviewers for their helpful comments and suggestions for improving the manuscript. EMG thanks her colleagues in the Department of Plant Developmental Biology for critical reading and commenting on the manuscript.

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