SHORT COMMUNICATION

Seedling structure in Asteraceae weedy species: considerations on the vasculature system

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Abstract Seedling structure in Asteraceae weedy species: considerations on the vasculature system. The vasculature system of the seedling is of interest since it represents the first mature vascular coordination between shoot and root, which was foreshadowed in the procambial system of the embryo. In this study, seedlings of ten Asteraceae weedy species were analyzed focusing on the vasculature system using a morpho and anatomical approach. The roots were found to be diarch and tetrarch, the species have intermediate or high root/shoot transition, and the cotyledons develop an independent double leaf-trace and a common simple-leaf trace. The transition region of the seedlings is strikingly different when compared with other families, such as, Annonaceae, Cactaceae, Fabaceae, Myrtaceae, Amaranthaceae, Clusiaceae, and Rutaceae. In spite of the uniformity in the vascular arrangement among the Asteraceae seedlings, the conclusion attained from this study is that some essential structural details may be of diagnostic value, such as the stele arrangement, the level of the transition zone, the cotyledon sheath, and the splitting of the phloem. Further, the presence of trilacunar node in the Asteraceae can be the result of a reversal evolutionary process (homoplasy) during the life history of the family.

Introduction

The vasculature system of the seedling is of interest because it represents the first realization of the vascular coordination between shoot and root, which was foreshadowed in the procambial system of the embryo (Esau 1965). The author has also pointed out in the literature that the transition region in seedlings deals with the vascular connection between the shoot and root in the early stage of development, but a full understanding about the development of this connection is not complete yet.

Investigations of the root/shoot transition region of several eudicot families have shown the occurrence of a relatively simple type of region in which, in the leaf traces of the cotyledons, the xylem is reoriented from the exarch condition, and typical in the root, to the endarch one, that is; the arrangement of xylem and phloem changes from alternate to collateral (Esau 1965; Souza 2009). Studies of Asteraceae seedlings (Siler 1931; Thiel 1934; Artschwager 1943; Kellermann 2011) have shown that the root/shoot transition region, which has both independent and common cotyledonary leaf traces, is different from other eudicots (Souza 2009), and it seems to occur in the entire Asteraceae family (Dangeard 1889).

It is worth mentioning that variations in the structure of the transition region depend on the number of leaf traces that continue into the cotyledons and the exact levels that these fundamental changes occur (Esau 1965). Unilacunar nodes with two distinct traces are repeatedly found in the cotyledonary node of several flowering plants (Takhtajan

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1980). However, three traces (one double trace and two lateral traces) have been recorded in some species of Asteraceae (Lee 1914; Siler 1931; Thiel 1934; Artschwager 1943; Kellermann 2011).

In this study, we employed a traditional anatomical approach in a comparative study of seedlings in ten different weedy species, from eight tribes of Asteraceae focusing on the vasculature system of the seedling axis and the cotyledons. In addition, we compared our results concerning the vascular arrangement of the complex root–shoot–cotyledons with other Asteraceae species seedlings available in the literature.

Materials and methods

The ten Asteraceae species from the tribes Astereae, Cichorieae, Heliantheae, Vernonieae, Eupatorieae, and Heleniae (as listed in Table 1) were collected at Maringá, Paraná, and Brazil, between 2011 and 2012; the vouchers were deposited at the Herbário da Universidade Estadual de Maringá (HUEM) (Table 1).

Seeds were washed in sodium hypochlorite solution and distilled water, air-dried and disposed to germinate on moist filter paper in Petri dishes, which were placed in a light- and temperature-controlled Tecnal TE 400 germination chamber. The germinated seeds were planted in a mixture of soil and organic substratum, in equal proportions, in plastic bags, and kept in a greenhouse.

The seedlings were fixed in glutaraldehyde 1 % w/v in a phosphate buffer 0.1 M, pH 7.2 (Karnovsky 1965, modified), and FAA 50 (formalin, acetic acid and 50 % ethyl alcohol in a ratio of 1:1:18) (Johansen 1940) for 5 days. Subsequently, the plant material was dehydrated in a graded ethanol series up to a 70 % ethanol solution, which was used for storage. The seedlings were embedded in historesin and cross sectioned on a rotary microtome according to the standard protocol (Feder and O'Brien 1968). Sections were stained in toluidine blue (O'Brien et al. 1965).

The drawings were made using a light microscope (Willd M20) equipped with a camera lucida. Light microscope photographs were taken using a Leica EZ4D digital camera and subsequently processed using Leica Application Suite version 1.8 software.

Table 1 Species from Asteraceae collected at Maringá, Paraná, Brazil

Species	Habit	Access number	Tribe	Characters			
				Stele	Level of transition	Cotyledon sheath	Splitting of phloem
Subfamily Cichorioideae							
Crepis japonica (L.) Benth	Herb	HUEM 18589	Lactuceae (=Cichorieae)	Diarch	High	Absent	Collet
Sonchus oleraceus L.	Herb	HUEM 578	Lactuceae (=Cichorieae)	Diarch	Intermediate low	Absent	Hypocotyl
Elephantopus mollis Kunth	Herb	HUEM 18590	Vernonieae	Diarch	Intermediate low	Present	Hypocotyl
Subfamily Asteroideae							
Conyza bonariensis (L.) Cronquist	Herb	HUEM 18586	Astereae	Diarch	Intermediate high	Absent	Hypocotyl
Porophyllum ruderale (Jacq.) Cass.[a]	Undershrubby	HUEM 20831	"Helenieae"	Diarch	Intermediate low	Absent	Collet
Cosmos sulphureus Cav.[a]	Shrubby	HUEM 19032	Heliantheae	Tetrarch	Intermediate low	Absent	Do not occurs
Eclipta alba (L.) Hassk	Herb	HUEM 18588	Heliantheae	Diarch	Intermediate low	Absent	Hypocotyl
Galinsoga quadriradiata Ruiz & Pav.	Herb	HUEM 19033	Heliantheae	Diarch	Intermediate low	Present	Hypocotyl
Tridax procumbens L.	Herb	HUEM 18585	Heliantheae	Diarch	Intermediate low	Absent	Hypocotyl
Praxelis clematidea R.M. King & H. Rob.	Herb	HUEM 21181	Eupatorieae	Diarch	Intermediate low	Absent	Hypocotyl

Habit, Access number, tribe and some other characters. The name of the species was based on Bremer (1994). The authors' authorities are according to The International Plant Name Index—IPNI (www.ipni.org)

[a] Species commonly found in wastelands

Results and discussion

The primary root of *Cosmos sulphureus* had a tetrarch radial stele in which the primary phloem groups alternate with the protoxylem poles; whereas all other studied species exhibit diarch roots. Regarding the root system in Asteraceae, Eames (1961) has reported both types of roots in this family. The tetrarchy and diarchy of the stele are common in seedling roots, and the former has been considered the basic type because it is associated with arborescent or woody taxa (Eames 1961; Duke 1969). On the other hand, Lee (1914) has pointed out both tetrarch and diarch roots have probably been interchanged several times during the evolution of angiosperms.

The root/shoot transition in *C. sulphureus* begins a few millimeters below the external collet where the pith begins to appear and the xylem is spread into a continuous tangential ring surrounding the pith, with four projecting protoxylems (Fig. 1c). As the hypocotyl ascends, we can see the xylem ring breaking into four groups (Fig. 1d), each one composed of a median protoxylem and a pair of metaxylems; the phloem strands remain unchanged throughout, never undergoing division. This vascular structure is found throughout the greater part of the hypocotyl. In this arrangement, there are two groups each composed of two metaxylems, one protoxylem and two phloem strands, which constitutes a cotyledonary double trace (Fig. 1e, f), and continue into the cotyledons. Small vascular bundles occur in the upper part of the hypocotyl, between the cotyledonary double traces. These small bundles comprise a ring-like structure, which has simple cotyledonary and epicotyledonary traces (Fig. 1e, f), all of which are endarch. Each simple cotyledonary trace divides into two traces (Fig. 1g), each one supplying the respective cotyledon (Fig. 1h). Thus, the cotyledon receives the independent double trace, which come up the hypocotyl originating in the root, and a common simple trace, which begins in the upper portion of the hypocotyl (trilacunar cotyledonary node) (Fig. 1h). The change from a typical root structure (exarch condition) to the shoot structure (endarch condition) in *C. sulphureus* occurs within the cotyledon and close to the cotyledonary node.

Compared to the tetrarch *C. sulphureus* (Fig. 1), the other diarch Asteraceae species (Fig. 2) have a similar general structure of the root/shoot transition (Fig. 2–4), although they differ in some details. The two root phloem strands break into four strands at the collet (*Crepis japonica* and *Porophyllum ruderale*) or the phloem division occurs along the length of the hypocotyl (remaining species), but in *Elephantopus mollis* the phloem is composed of a common simple trace that originates from two root phloem strands. In *Conyza bonariensis*, the transition usually begins in the middle
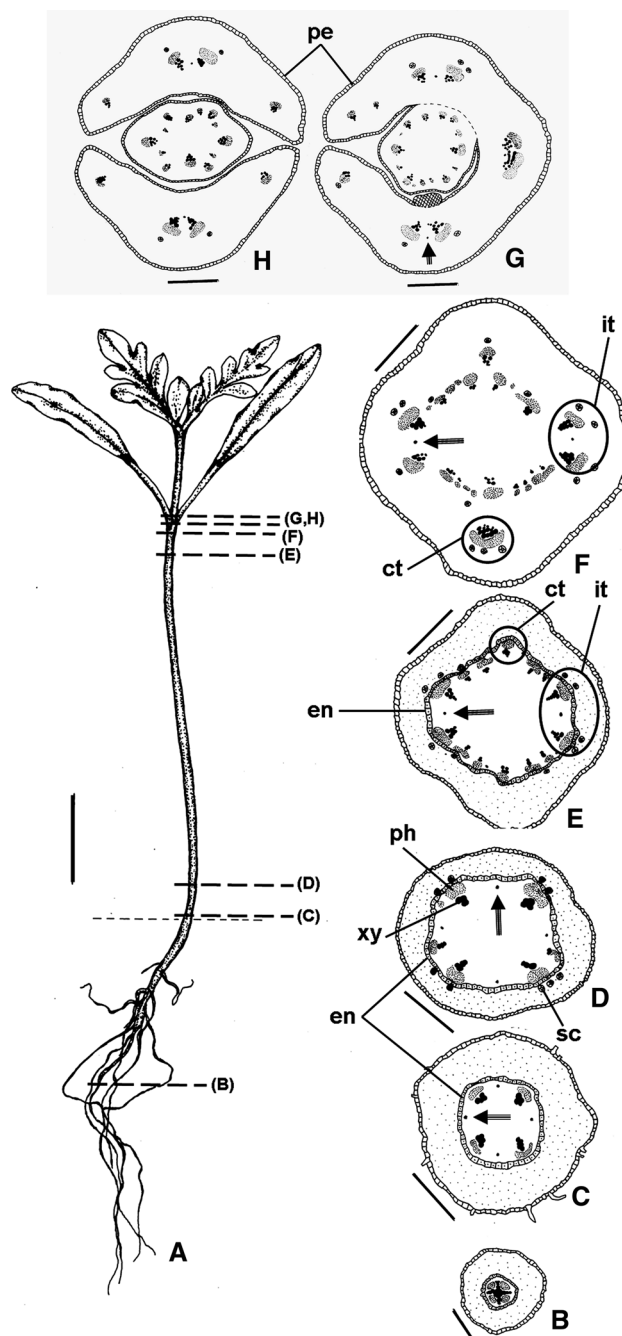

Fig. 1 *Cosmos sulphureus* seedling. **a** 12 day old seedling showing cotyledons and eophylls. The levels indicated by *letters* **b**–**h** correspond to the cross sectional diagrams of successive levels showing vascular connections between epicotyl, cotyledons and root (cross sections). **b** Root. **c** Collet. **d**, **e** Hypocotyl. **f**, **g** Cotyledonary node. **h** Above the cotyledonary node. (*Arrows* indicate tracheary elements of the protoxylem, *ct* common simple trace, *en* endodermis, *it* independent double trace, *pe* cotyledon petiole, *ph* phloem, *sc* secretory canal, *xy* xylem). *Scale bars* 1.5 cm (**a**), 150 μm (**b**), 300 μm (**c**–**h**)

region of the hypocotyl. In *C. japonica*, the parenchymatous pith begins to appear and the xylem breaks just below the cotyledonary node.

Figs. 2–5 Cross sections of the seedlings. **2, 3** *Eclipta alba*. **3, 4** *G. quadriradiata*. **5** *E. mollis*. **2** Diarch root. **3** Base of the hypocotyl. **4** Cotyledonary node. **5** Above the cotyledonary node. (*ct* common simple trace, *dt* cotyledonary simple traces after division of the common trace, *it* independent double trace, *black arrows* indicate protoxylem). *Scale bars* 200 μm
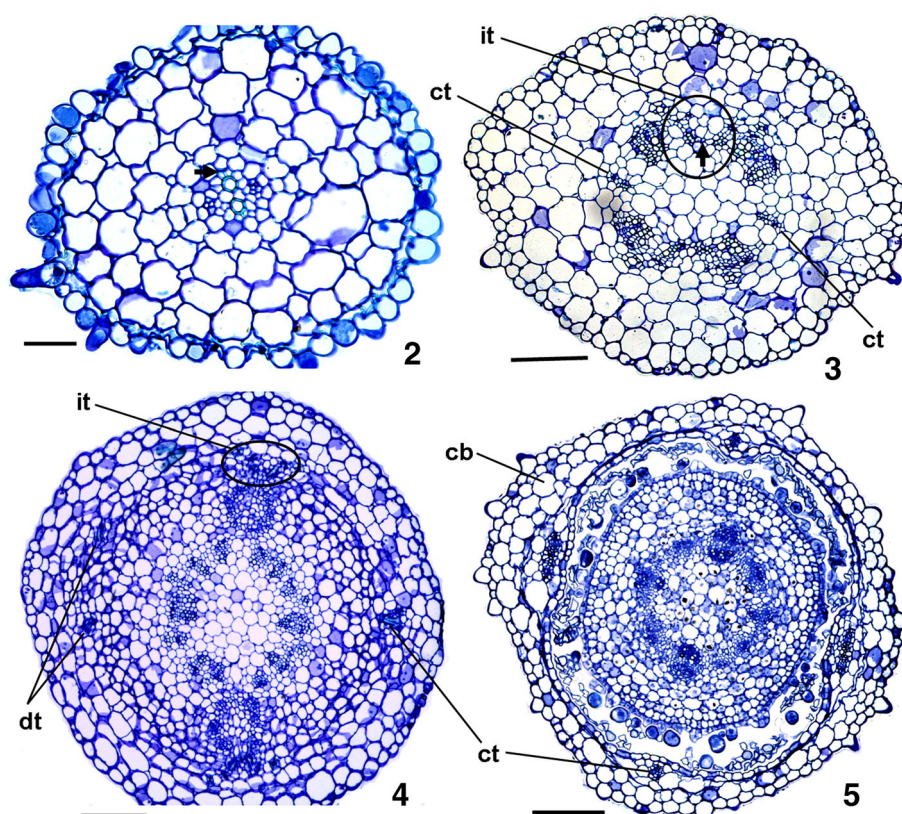
In the diarch *E. mollis* and *Galinsoga quadriradiata*, the transition is incomplete at the cotyledonary node. Each common simple trace undergoes no further division until penetrating into a cotyledonary sheath (Fig. 5), when it undergoes division forming two vascular bundles, one for each cotyledon.

The root/shoot transition zone of the Asteraceae species studied here is similar to other Asteraceae (Compositae) species studied previously by Dangeard (1889), Lee (1914), Siler (1931), Thiel (1934), Artschwager (1943), Kellermann (2011), and it seems to be a common feature for the entire family. Notwithstanding, the variations found here may be precious elements of interest for taxonomical diagnosis, such as: the level of transition (high, low, or intermediate), the formation of the cotyledon sheath, endarchy or tetrarchy, and the splitting of phloem strands.

An attempt to classify the various kinds of transition phenomena in dicot seedlings was made by Compton (1912) in seedlings of Fabaceae species. Thus, applying this classification to the seedlings analyzed here, we note that most species can be classified as intermediate-low transition, with two exceptions: *C. bonariensis*, which shows the intermediate-high type and *C. japonica*, in which the transition is high in the hypocotyl.

The interesting structures of Asteraceae root/shoot transition zone differ in the essential details (Table 1) from some studied families, such as Annonaceae (Mayer et al. 2008), Bignoniaceae (Souza and Oliveira 2004; Souza et al. 2007), Cactaceae (Almeida et al. 2009; Secorun and Souza 2011; Almeida 2013), Fabaceae (Compton 1912; Souza 1982), Myrtaceae (Gogosz et al. 2010), Amaranthaceae, Clusiaceae, and Rutaceae (Esau 1965; Souza 2009).

The cotyledonary nodes of the Asteraceae species analyzed here are trilacunar (Fig. 1f–h), unlike most of the eudicotyledon seedlings that have one or two traces and unilacunar nodes (reviewed in Souza 2009). Takhtajan (1980), discussed the nodal structure of the flowering plants and reported the presence of three different types of nodal structures (unilacunar, trilacunar, and multilacunar), which makes the determination of the evolutionary trends in angiosperms more complex. According to Takhtajan (1980), the unilacunar nodal structure is characteristic of advanced taxa considering that the unilacunar nodal type is secondary in flowering plants and originating from the basic tri-pentalacunar type. Further, he indicated that the anatomy of cotyledonary nodes does not necessarily reflect ancestral conditions in the mature stem, an idea put forward by Benzing (1967).

In fact, in his 1980 work, Takhtajan reported the unilacular node in advanced orders of angiosperms, such as Ericales, Lamiales, Gentianales, Myrtales, and Asterales (the family Campanulaceae), indicating that the families

Asteraceae and Plantaginaceae are the exceptions, and do not have unilacular node. Our results support the presence of a trilacular node in the species comprising the subfamilies "Cichorioideae" (tribes Lactuceae and Vernonieae) and Asteroideae (tribes Astereae, "Helenieae", Heliantheae, and Eupatorieae) (sensu Bremer 1994), which suggests that the trilacunar node is a shared attribute in the entire family, supporting in part, Takhtajan's (1980) findings for Asteraceae. Therefore, assuming that Takhtajan's (1980) hypothesis, i.e., the unilacular node is an advanced character, is correct, then the presence of trilacunar node in the Asteraceae can be explained as result of a reversal evolutionary process (homoplasy) during the life history of the family.

In spite of the uniformity in the vascular arrangement in the Asteraceae seedlings, the conclusion attained from this study is that some essential structural details may be of diagnostic value, such as the stele arrangement, the level of the transition zone, the cotyledonary sheath, and the splitting of the phloem, as shown in Table 1.

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