

A Developmental Genetic Basis for Defining Root Classes

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ABSTRACT

Root class nomenclature has not been standardized across species and groups (families). This has led to a lack of transferability of conclusions from one species to another. The International Society for Root Research (ISRR) has approved a four class nomenclature for roots (Zobel and Waisel, 2010). A review of literature that has not been routinely cited by plant anatomists suggests that there are at least four classes and eight subclasses of root, based on developmental genetic characteristics across eudicots and monocots. The use of this classification scheme to develop research on specific classes of root may suggest a far more simple genetic basis than that suggested by current molecular and proteomic research.

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Abbreviations: ISRR, International Society for Root Research.

THE INTERNATIONAL SOCIETY FOR ROOT RESEARCH (ISRR) has approved a nomenclature for roots (Zobel and Waisel, 2010). This nomenclature was first promulgated as a result of a cumulative 250+ years of root research by the nomenclature committee members. Several members of the committee added genetic and functional evidence in support of the divisions chosen (Zobel, 1975; Waisel and Eshel, 1991). There is a critical need for such a nomenclature: for instance, out of thousands of root genetic, physiological, and biochemical papers, going back more than two hundred years, only a couple of handfuls have carefully delineated exactly which roots were being studied, while the majority pooled all the roots of their plants (mostly seedlings) into a single sample. If there are important functional differences between classes of root, pooling the classes can only lead to a lack of clarity and, ultimately, to misinterpretation.

Because of the morphological and anatomical similarity of roots from different parts of the plant, it is a logical assumption that form follows function, and, therefore, all roots are functionally identical. This is not the case (Bushamuka and Zobel, 1998a, b; Waisel and Eshel, 2002). Root function can be described at many different levels, from the ecological (Fitter, 2002), to the physiological (e.g., Zobel et al., 1992), to the molecular (e.g., Wang et al., 2007). Although root classification can be based on function (e.g., ecological function; Fitter, 2002), it has historically been based on anatomy (Fahn, 1982). Root function and development are ultimately controlled by

Published in *Crop Sci.* 51:1410–1413 (2011).

doi: 10.2135/cropsci2010.11.0652

Published online 10 May 2011.

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a combination of genetics and environment. As Scheres et al. (1996) noted, “Regardless of how much faith one has in anatomical definitions, they should not be taken as more than a means of communication before subsequent genetic analysis.” In a discussion of classification schemes, Kirchoff et al. (2008) identified developmental genetics as one basis for classification in plants. A review of the literature on heritable root characteristics and genetic mutations that affect root system morphology (e.g., low level developmental genetics) reveals a potential root structural ontology that is more extensive than that devised by the ISRR.

Embryonic plants have two distinct parts: the shoot and the root (Esau, 1965). New roots may arise from either of these two parts, that is, shoot-borne roots and root-borne roots. The discovery by Jenkins (1930) of the *rt* (rootless) mutant of maize (*Zea mays* L.) demonstrated that shoot-borne roots are not developmentally genetically equivalent to root-borne roots. Thus began the developmental genetic classification of roots. Zobel (1972a, b) demonstrated that, in the diageotropica (*dgt*) mutant of tomato (*Solanum lycopersicum* L.) (a point mutation; Zobel, 1972a), lateral root initiation is inhibited in both shoot-borne roots and the tap root. In a subsequent study, Zobel (1975) hybridized *dgt* to *ro* (rosette), a tomato genotype lacking shoot-borne roots (generally, in eudicots, these shoot-borne roots arise off the internodes as well as the nodes). The double homozygote plant (*dgt/dgt, ro/ro*) had branch roots from the base of the hypocotyl, and these hypocotyl roots had no lateral roots (Zobel, 1975). Zobel (1975) termed these roots *basal roots* based on their location at the base of the hypocotyl and above the base of the tap root. The tap root is the only root that is a continuation of the initial axis of the embryo rather than being lateral to that axis. These results suggest four classes of root: tap root, basal root, shoot-borne root, and lateral root and thus provides one of the bases for the ISRR classification framework (Zobel and Waisel, 2010).

It is significant that the terms *hypocotyl* (meaning below the cotyledons) and *mesocotyl* (meaning between [the shoot and root]) have a long history of scientific discussion (see Esau, 1965, and Fahn, 1982) with little agreement other than that the organ is neither shoot nor root. To avoid confusion and forestall extensive discussion, this study uses the definition of hypocotyl put forward by Avery (1930), who classifies the tissue between the coleoptyle or cotyledons and the tap root as the hypocotyl, regardless of whether it is physically above or below the cotyledon(s). Based on the ISRR terminology (Zobel and Waisel, 2010), the term *basal root* will also be used exclusively. Weinhold (1966) demonstrated, with both eudicots and monocots, that there are two types of basal root. The *first stage basal roots* (*Grenzwürzeln* in the original German text) were always at the same morphological site, but *second stage basal roots* can emerge at different locations on the hypocotyl in different species. Weinhold’s (1966) work

was based on only five species but is supported by later work by Hoshikawa (1969).

Hoshikawa (1969), using 219 different grass species, identified and classified subfamilies based on differences in both first stage (*transitory node roots*, in his terms) and second stage basal root (*mesocotylar roots*) development. Hoshikawa’s (1969) research introduced a technique that allows the observation of phenomena that had previously been obscured. If you bury grass seeds, in sand, to a depth “(a) large seeds, for example *Zea* and *Coix* were in 7 to 10 cm; (b) medium-sized seeds, such as *Oryza* and *Triticum* in 5 cm; (c) small seeds, like those of *Panicum* in 3 cm; and (d) very small seeds, as found in *Eragrostis*, in 1.5 cm.” (Hoshikawa, 1969) hypocotyl elongation is usually stimulated and first vs. second order basal roots can be differentiated. After doing this, Hoshikawa found that only subfamily *Festucoideae* species regularly had first order basal roots; only the *Bambusoideae* and some of the *Triticeae* did not have an elongated hypocotyl, and only the *Bambusoideae*, *Oryzoideae*, and *Festucoideae* did not develop roots on the hypocotyl (i.e., the *Eragrostoideae* and *Panicoideae* routinely had roots on the hypocotyl). In addition, Hoshikawa’s data show that the *Oryzoideae*, *Festucoideae*, and *Eragrostoideae* have robust root growth from the coleoptile node (*cotyledonary node*) but the others do not. In summary, Hoshikawa found that grasses had or did not have first order basal roots, second order basal roots, and coleoptile node roots, dependent on their subfamily, a developmental genetic difference between these classes of root. We have confirmed Hoshikawa’s (1969) results with a number of representative species (R.W. Zobel, unpublished data, 2010). To summarize, this data confirms a genetic distinction between Weinhold’s first order and second order basal roots and suggests an additional class of root: coleoptilar node roots.

In the meantime, researchers in Hochholdinger’s group have dramatically expanded the mutation-based definition of different root types using corn root mutants with biochemical and molecular techniques. These results have been recently reviewed (Hochholdinger et al., 2004b) and are summarized here:

1. They restated Jenkins’ (1930) research, which demonstrates that shoot-borne roots are genetically distinct from earlier developed roots.
2. They demonstrated that in corn (*Zea mays* L.), the tap root and its lateral roots were not affected by the mutant *rtcs*, which eliminates all roots other than these two classes. This demonstrates that the tap root and its lateral roots are distinct from all other corn roots, including basal and shoot-borne roots.
3. On the other hand, only the coleoptile node roots and the lateral roots of the tap and basal roots were affected by *lrt1*. This is a demonstration of the uniqueness of three more classes:
 - a. Coleoptile node roots are distinct from

Table 1. Eight root classes as established by the International Society for Root Research and the literature review presented here. Abbreviations were selected for descriptive simplicity and size to fit easily into the plant ontology.

ISRR framework classes	Subclass	Abbreviation
Tap root first root to emerge)		TRT
Basal root (develops from the hypocotyl)	First stage (at the base of the taproot)	BRT
	Second stage (on the hypocotyl above the tap root)	HRT
Shoot-borne root (develops from shoot tissues)	Coleoptile node	CNRT
	Upper nodes (most prevalent in monocots)	SNRT
	Internodes (most prevalent in eudicots)	SIRT
Lateral root (branches from another root)	On tap or basal root	Prefix main root abbreviation with an L: that is, LTRT or LHRT (a lateral branching from another lateral = LTRT2 or LTRT3 dependent on branching level)
	On shoot-borne root	Begins with LSB: LSBNRT(similar to above)

other shoot-borne, tap, and basal roots (point 2 and point 3a), and

- b. Tap and basal lateral roots are unique from
- c. shoot-borne lateral roots and
- d. the tap root.

Thus six classes of root have been defined by corn root mutation analysis: tap root, basal root, lateral root (basal and tap), lateral root (shoot-borne), coleoptile node roots, and shoot-borne (nodal) roots.

Where does all this fit in the ISRR framework? Referring to Table 1, the first column contains the root classes of the ISRR framework. In the second column are the subclasses, as discussed above.

- The first ISRR class is the tap root, (leaving aside discussions about a seminal root replacing the radicle in some Poaceae), with, as yet, no subclasses.
- The second ISRR class is basal root. This class can currently be considered to have two subclasses based on the research of Weinhold (1966) and Hoshikawa (1969): basal root (first stage) and hypocotyl root (second stage basal root).
- The third class is the shoot-borne root with three subclasses: coleoptile node roots, stem nodal roots, and stem internodal roots. The first two are defined by the Jenkins (1930) and Hochholdinger et al. (2004b) research and the third by Zobel (1975).
- The fourth ISRR class is the lateral root. Based on the Hochholdinger et al. (2004b) data, there are lateral roots from the tap or basal roots, and a distinct class that arises from shoot-borne roots.

A primary intent of the ISRR for development of the framework was to assist in the communication between researchers about exactly which roots they were studying. With a few qualifications, this expanded framework accomplishes that desire with the data currently available. The framework is based on easily recognized morphological structures. One caveat is that in many shallow planted monocots the hypocotyl normally does not elongate, making it difficult to assess the basal roots. As Hoshikawa (1969) demonstrated, this can be accomplished by planting the seed deep in soil, or other opaque media, forcing the elongation of the hypocotyl.

Kirchoff et al. (2008) specify five different approaches to characterize morphological nomenclature. The discussion here has been an aspect of their second approach, that is, “developmental genetics with mutants or other genetic bases to separate different characteristics.” Since the four primary classes hold up with both eudicots and monocots, it is currently assumed that the rest also do.

Functional distinction implies differential gene activation patterns and/or epigenetic controls resulting in distinct proteomes. Tap root, basal root, and lateral root classes have been demonstrated to be functionally distinct in tomato, corn, and soybean [*Glycine max* (L.) Merr.] (Zobel et al., 1992; Bushamuka and Zobel, 1998a, b). Hochholdinger’s group is currently expanding the functional differentiation of some of these eight classes with maize (Hochholdinger et al., 2004a, b; Sauer et al., 2006; Hochholdinger, 2009). Lynch’s bean (*Phaseolus vulgaris* L.) adventitious roots are second order basal roots (HRT in Table 1) and develop in response to phosphorus levels (Lynch and Brown, 2001).

Future genomic, proteomic, and epigenetic research should be able to clarify the functional differences of these eight root classes through studies of contrasting root classes. Proteomic comparisons of the same root classes on eudicots vs. monocots should give evidence for (or against) the evolutionary stability of the root genome. Since most root research has been conducted without reference to specific root classes, it is premature to use that research to conclude multigenetic or multi-epigenetic control over a given root system morphological or functional characteristic. By restricting research to a single class it may be demonstrated that a given functional characteristic is simply inherited or has a relatively easily understood molecular pattern. The ability to do this without reference to the framework provided here is problematic. Zheng et al. (2003) attempted to study quantitative trait loci (QTL) under flooding and nonflooding conditions for three root classes in rice (*Oryza sativa* L.). Their growth and identification conditions compromised their results: They measured the longest seminal root on rice plants planted less than 3 cm deep; the seminal roots on these plants were a mixture of the tap root (the single seminal root) and coleoptile roots (see Hoshikawa [1969], who

demonstrated that there are no basal or hypocotyl roots in oryza species, but many strong coleoptile roots. The hypocotyl does not elongate when seeds are germinated on a surface, thus clumping the two classes of root. The measured lateral root number and length are also equivalently compromised since the parent roots were a mixture of shoot-borne (coleoptile roots) and tap (seminal) root.

Obviously, further research to define the classes of root needs to be performed, that is, are coleoptile lateral roots shoot-borne laterals or are they primary (tap, basal, or hypocotyl) root type laterals? Also, the Hoshikawa (1969) data concludes that maize has no basal (roots), and yet extensive detailed anatomy (Avery, 1930) clearly shows both basal (classic seminal roots) and hypocotyl roots on this species. The Hochholdinger group has known about the Hoshikawa (1969) data for less than a year (Hochholdinger, personal communication, 2010) and has yet to publish their observations and mutant data relative to this dichotomy.

In conclusion, there is still much research needed to confirm and further refine the presented framework and its relevance to different species. The information presented suggests at least eight developmental genetic classes of root. If the early results of Zobel's lab and Waisel's lab hold up with these additional classes of root, eight functional classes of root are suggested. A conservative assessment of the situation, however, would suggest that some functional redundancy between classes will be found. Perhaps gene activation studies, during root class initiation, will turn up genetic redundancies as well. The most rapid approach to confirm or refine these conclusions would be for scientists currently conducting root research to routinely separate their roots into the proposed classes and to compare the responses among the different classes. In our lab, we have initiated a proteomics comparison of the different root classes on grass plants grown in aeroponics to identify functional differences and/or similarities.

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