Chapter 11
Setaria viridis: A Model for Understanding Panicoid Grass Root Systems

Jose Sebastian and José R. Dinneny

Abstract Roots are essential for plant survival on land. Understanding how root traits relate to overall crop yield will be key to sustainably supporting an ever-expanding population. For global food and biomass production, members of the grass family (Poaceae) contribute the lion's share; however, our understanding of grass root biology remains rather poor. Among grasses, Panicoideae subfamily grasses are among the most agronomically important groups of plants. Recently, *Setaria viridis* (Setaria) has emerged as a new genetic model system for Panicoideae grasses. Setaria characteristics such as a relatively small genome, fast life cycle, ease of growing under controlled conditions, and remarkable drought tolerance make it an excellent plant model system to study various aspects of grass biology. Setaria has a typical grass root system architecture composed of a primary, crown and lateral roots, making it feasible to conduct systematic analyses that elucidate general physiological mechanisms of broad relevance. In this chapter, we give an overview of root systems in grasses and Panicoideae grasses and provide a detailed description of the *Setaria viridis* root system highlighting different root types and their internal cellular organization.

Keywords Monocot root systems • Root system biology • Panicoid grasses • *Setaria viridis*

11.1 Introduction

11.1.1 The Importance of Roots

Roots are a multifunctional organ system through which plants obtain most of their water and nutrients required for growth and development. Being sessile organisms, plants depend heavily on their root systems and their adaptive responses to

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J. Sebastian (✉) • J.R. Dinneny
Department of Plant Biology, Carnegie Institution for Science,
260 Panama Street, Stanford, CA 94305, USA
e-mail: jsebastian@carnegiescience.edu; jdinneny@carnegiescience.edu

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optimally extract soil reserves. Important adaptive responses to exogenous stimuli include alterations in root growth rate and branching patterns. This is particularly important when soil resources are either depleted or inaccessible due to a multitude of stresses (Lynch 1995; Grossman and Rice 2012; Postma et al. 2014a). Besides nutrient uptake, roots are also important in anchorage, storage of nutrient reserves, and forming mutually beneficial microbial associations that are often critical for proper plant growth (discussed in Chap. 14, and reviewed by (Bulgarelli et al. 2013)). Considering the rapid increase in the human population and associated nutritional demands, it is of paramount importance to improve our agriculture production through sustainable means. Crop plants with better root traits are predicted to be the next main source of improvement in our agriculture (Hammer et al. 2009; White et al. 2013; Rogers and Benfey 2015). Progress has been made in understanding the biology of roots and root systems; however, most of this is knowledge pertaining to eudicot models, particularly *Arabidopsis thaliana* (reviewed by (Petricka et al. 2012)). While commonalities exist between monocot and eudicot root systems, important differences exist, which require investigation. Knowledge of the genetic networks that drive root growth and response to stress in monocot plants will ultimately help in generating crop plants with superior root systems that are better equipped to grow under resource-limiting agricultural conditions (Kong et al. 2014).

11.1.2 Significance of Panicoideae Grasses

Agronomically, members of the Panicoideae subfamily of grasses are particularly important. There are approximately 3300 species belonging to this subfamily (Grass Phylogeny Working Group (GPWG 2001)) with a truly global presence (Giussani et al. 2001). Many important feed, fodder, and fuel crops such as maize (*Zea mays*), sorghum (*Sorghum bicolor*), foxtail millet (*Setaria italica*), common millet (*Panicum miliaceum*), pearl millet (*Pennisetum glaucum*), sugarcane (*Saccharum officinarum*), Miscanthus (*Miscanthus giganteus*), and switchgrass (*Panicum virgatum*) are all members of this clade. Many of these plants exhibit traits that will be vital in a world facing critical challenges on several fronts including global warming, water scarcity, and degradation of arable lands. For example, crops such as sorghum and millet are capable of growing in arid and semiarid environmental conditions often found in the most economically challenged parts of the globe (Pray and Nagarajan 2002). Miscanthus and switchgrass are fast growing plants capable of cultivation on marginal lands and may assist in supporting the ever-increasing energy demands of the world through biofuel production (Lewandowski et al. 2003; Khanna et al. 2008). As an added advantage, many of the agronomically important Panicoideae grasses have evolved a C_4 photosynthesis system, which is more efficient at converting solar energy to biomass owing to their ability to concentrate CO_2 and reduce photorespiration, thus improving agricultural productivity (Giussani et al. 2001; Zhu et al. 2008; Osborne et al. 2014).

11.1.3 Comparisons Between Eudicot and Grass Root Systems

There are distinct differences between eudicot and grass root system both in terms of root system structure and internal cellular organization. One of the key differences between these two root systems is the presence of additional root types in monocots such as the crown/nodal roots (Fig. 11.1a–c; Table 11.1). These shoot-borne roots together with associated lateral roots form the bulk (90–95 %) of the adult plant root system commonly referred to as a fibrous root system (Metcalfe and Nelson 1985; Hoppe et al. 1986; Hochholdinger et al. 2004a). In contrast, shoot-borne roots are

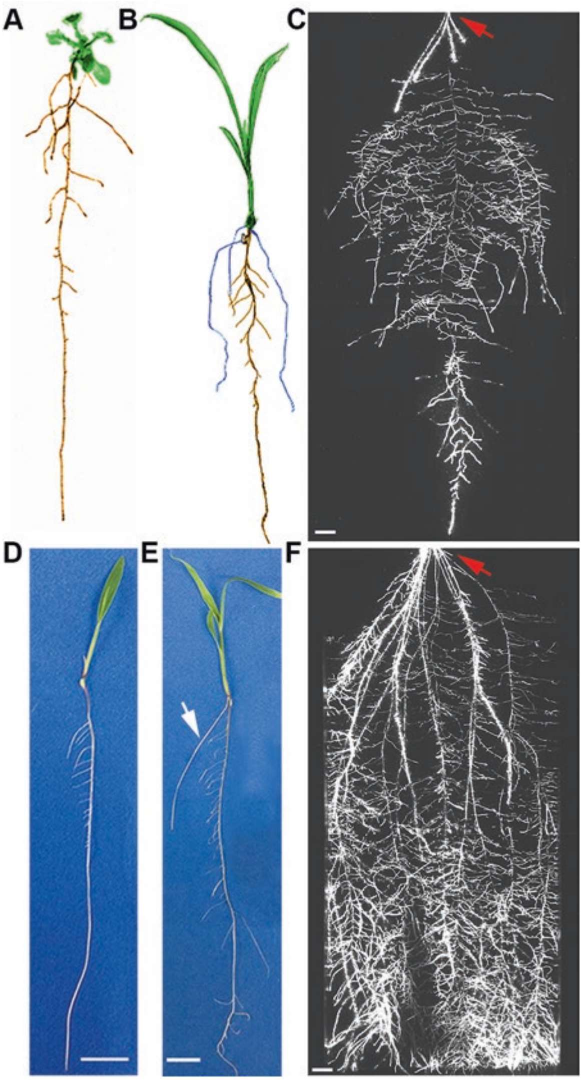

Fig. 11.1 *Setaria viridis* root system. (**a**, **b**) A diagrammatic representation of a eudicot (Arabidopsis) root system and a monocot (Setaria) root system. Embryonic root system is colored in *yellow* and *blue* represents shoot-borne postembryonic root system. (**c**) Luminescence-based image of *S. viridis* root system at 17 days after sowing (DAS). *Arrowhead* indicates the crown region. (**d**) Setaria root system at 4 DAP. Seedlings germinated on agar media. (**e**) Setaria root system at 7 DAP showing the emergence of a crown root from the coleoptilar node. *Arrow head* indicates crown root. (**f**) Luminescence-based image of *S. viridis* root system at 29 DAS. *Arrowhead* indicates the crown region. Scale bars: 1 cm

Table 11.1 Common root types in panicoid grass root systems

Root type	Site of emergence	Time of primordia initiation	Root characteristics
Primary root	Basal pole of embryo	Embryogenesis	Continuation of the radicle. First root to emerge following seed germination
Seminal roots	Scutellar node	Embryogenesis	Emerge few days post seed germination. Exact number per seedling varies. Not every grass species form
Crown roots	Shoot nodes (underground)	Post-embryogenesis	Shoot-borne roots form a major constituent of postembryonic root system in grasses. Exhibit heteroblasty
Brace roots	Shoot nodes (aboveground)	Post-embryogenesis	Shoot-borne roots. Important in lodging resistance. Common in maize
Sett roots	Shoot nodes (underground)	Post-embryogenesis	Highly branched, thin roots found in sugarcane. Crucial in the early establishment of the plant (sett)
Shoot roots	Shoot nodes (underground)	Post-embryogenesis	Thick, less branched roots. Common in sugarcane where they gradually replace sett roots
Buttress roots	Shoot nodes (aboveground)	Post-embryogenesis	Thick roots common in Sorghum. Important in providing lodging resistance
Superficial roots	Shoot nodes (underground)	Post-embryogenesis	Highly branched, thin roots. Common in sugarcane. Usually, grow laterally near the soil surface
Rope roots	Shoot nodes (underground)	Post-embryogenesis	Agglomerations of shoot roots grow vertically into deep soil. Important in nutrient acquisition and anchorage
Lateral roots	Pericycle and endodermis cells of all roots	Post-embryogenesis	Roots formed from other roots. Exhibits a high degree of branching. Important in water and nutrients uptake
Adventitious roots	Mesocotyl	Post-embryogenesis	Usually, not part of the normal root development program. Emerge commonly as a response to external stimuli

typically not a major root type in eudicot root systems, where the primary root and associated several orders of lateral roots constitute the majority of the root system (referred to as a taproot system). The taproot system is adapted to grow deeply into the soil, while the fibrous root system is usually more shallow rooted and suited to exhaustive utilization of water and nutrients in the uppermost levels of the soil. There are also distinct differences in root anatomy between individual grass and dicot roots. There is usually no vascular cambium (a type of lateral meristem) developed in the grass root stele, and therefore these roots exhibit no secondary growth, unlike many eudicot roots (Hochholdinger et al. 2004b; Scarpella and Meijer 2004). There are also differences in xylem vessels with regard to shape and number. The origin of lateral roots also differs; in eudicots they are formed from pericycle cells in all root types, whereas in grasses, cell divisions in both the pericycle and endodermis contribute to lateral root formation (Hochholdinger and Zimmermann 2009; Orman-Ligeza et al. 2013). At a larger scale of organization, however, there are similarities between grass and eudicot roots. In both groups, the root apical meristem, which harbors a stem cell niche at the apex sustains continued root growth by the iterative processes of cell division, followed by cell elongation and differentiation (Bennett and Scheres 2010; Sebastian and Lee 2013). There are two pools of stem cells present at the stem cell niche, the proximal stem cells or vascular initials, (progenitors of vascular tissue) and the distal stem cells or the collumella initials (progenitors of root cap tissue). These stem cells encompass a mitotically inactive group of cells referred to as the quiescent center (QC). Longitudinally, based on cell length, each grass or eudicot root type can be subdivided, starting from the root apex, into three sequentially arranged developmental zones: meristematic, elongation, and differentiation/maturation.

Crown roots emerge from the shoot nodes in a sequential manner beginning from the lower nodes at the shoot base (Onderdonk and Ketcheson 1972). These nodes are collectively referred to as the seedling crown. Although the overall structure and function of different root types in the root system are similar, there is evidence suggesting differences in their biology and responses to external cues (reviewed by (Bellini et al. 2014)). Many studies have reported that the primary, crown, and lateral roots often respond differently to soil/environmental conditions. For example, phosphorus deficiency in rice promotes primary and crown root elongation, while it suppresses lateral root growth (Zhou et al. 2008; Hu et al. 2011; Dai et al. 2012). This is in contrast to most other plants where phosphorus deficiency stimulates both lateral and adventitious root growth while suppressing primary root elongation (Bellini et al. 2014). Similarly, adventitious and lateral root growth is found to be more sensitive to the stress hormone abscisic acid (ABA), while the primary root is less sensitive to ABA (Bellini et al. 2014; De Smet et al. 2003; Duan et al. 2013). Moreover, there are mutants isolated in maize and other species that affect only a particular root type, further highlighting their underlying differences (Hochholdinger et al. 2004b; Bellini et al. 2014).

As mentioned above, most of our understanding of root biology is from Arabidopsis. However, Arabidopsis does not develop an extensive shoot-borne root system as grasses do. Thus, though useful, it is difficult to fully extend knowledge from Arabidopsis to grass species. Therefore, to elucidate grass root biology, it is imperative to develop model systems in the grasses.

11.2 Root Systems in Panicoideae Grasses

The high rate of productivity observed in grasses is, in part, due to their elaborate root systems, which are well adapted for exploring and extracting soil resources and water (Lynch 1995; Aiken and Smucker 1996). Therefore, improvements in our ability to grow panicoid grasses in agriculture will likely require a better understanding of their root systems; but which model will provide the greatest insight? Over the years, considerable efforts have been made to develop rice as a genetic model system, and this has contributed significantly to our understanding of grass root biology

(Itoh et al. 2005; Coudert et al. 2010). However, although the overall root system architecture is similar between rice and panicoid grasses, there is a limit as to how much knowledge we can transfer between the two. Rice is adapted to growth in flooded paddies where hypoxia is an important stress whereas the panicoid grasses are mostly habituated to arid/semiarid soil conditions where water deficit is more likely to be encountered. Moreover, there may be variations in root system growth dynamics due to the differences in C_3 (rice) versus C_4 (many panicoid grasses) photosynthesis. Thus, it is logical to explore the root system of panicoid grasses to get a clearer picture of their growth dynamics, stress responses, and underlying genetic regulatory networks. We will next provide a comparative description of root system characteristics in four different panicoid grasses. Although there is an underlying commonality that involves similarity in root system structure, function, and development, there are distinct root system features unique to each of these grasses. Most of the features illustrated below are species-specific root system adaptations acquired in order to optimize growth and survival in different ecological niches.

11.2.1 Root System Development in Maize

Most of our understanding of root system structure and development in panicoid grasses is from studies of maize. The maize root system can be broadly categorized as embryonic or postembryonic based on the time of root emergence (Hochholdinger et al. 2004b; Feldman 1994; Hochholdinger 2009). Embryonic roots are those that form during embryogenesis through seed germination phases and include a single primary root and seminal roots. Unlike in eudicots, the primary root is formed endogenously from the basal pole of the embryo and thus must penetrate through the embryonic tissues before its emergence near the tip end of the kernel (Hochholdinger et al. 2004b; Feldman 1994). This internal origin of the primary root is a characteristic feature of monocotyledonous plants belonging to the true grass family Poaceae/Gramineae (Hochholdinger 2009). However, the significance of this internal origin is not obvious compared to the site of origin for the eudicot primary root, which is patterned from the outer tissue layers of the embryo (Bennett and Scheres 2010). A varying number of seminal roots are a distinguishing feature of the maize embryonic root system. These are roots formed endogenously from the scutellar node (region of the embryo between the primary root and young shoot) during embryogenesis after the primary root emerges (Feldman 1994). The number of seminal roots can vary widely among different genetic backgrounds, ranging from 0 to 13 per seedling (Feldman 1994; Hochholdinger 2009; Kiesselbach 1949). During the seed germination phase, most of the energy requirement is met by the kernel's nutrient reserves. However, the embryonic root system is crucial in water uptake and any damage to the root system can adversely affect seedling growth and development (Nielsen 2012). In maize, embryonic roots generally form the majority of the rootstock up to 2 weeks post-germination. Subsequently, nodal roots take over as the major constituent of the root system and, together with lateral roots, form the bulk of the adult plant root system (Hoppe et al. 1986).

Nodal roots and lateral roots constitute the postembryonic root system in maize. There are two classes of nodal roots in maize, the crown roots formed below ground and the brace roots formed aboveground. Both classes of shoot-borne roots are initiated in whorls around the stem (Hoppe et al. 1986). An average plant produces six whorls of crown roots and two to three whorls of brace roots, giving rise to approximately 70 nodal roots in total during its life cycle (Hoppe et al. 1986). These roots develop from primordia that originate from ground tissue cells (root cortical cells) opposite to collateral vascular bundles (Martin and Harris 1976). In addition to water and nutrient uptake, they also provide lodging resistance. Lateral roots are those roots formed from other root types (originate from primordia formed from pericycle and endodermis cells) and appear as branches of the main root (thus, also referred as branch roots) from which they originate. These roots are distinguishable from other root types by their relatively short lengths and often determinate apical meristems (Varney and McCully 1991). There can be several orders of lateral roots present in a root system, with the first series of lateral roots termed secondary roots, those borne from them termed tertiary roots, and so forth (Lynch 1995; Esau 1977). Lateral roots greatly increase the absorptive surface area of the root system and act as the main route for water and nutrient uptake (McCully and Canny 1988; Varney and Canny 1993; Postma et al. 2014b).

Different maize root types show comparable radial and longitudinal organization (reviewed by (Hochholdinger 2009)). Radially, root cells are organized in a concentric ring of layers starting from the outermost epidermis to the inner vascular tissue or stele (Hochholdinger et al. 2004b). A single layer of cells constitutes the epidermis. There are two kinds of cells in the epidermis, the root hair-forming trichoblasts and the atrichoblasts that do not form root hairs (Row and Reeder 1957). The ground tissue follows the epidermis and usually contains eight to 15 layers of cortex and a single layer of endodermis characterized by Casparian strips, which are localized lignified thickenings of the secondary cell wall along the medial plane of cells in this tissue (Hochholdinger 2009). The endodermis separates the inner vasculature from the outer root tissues and functions as a barrier to the radial flow of nutrients and water between the vascular cylinder and the outside environment (Dinneny 2014; Robbins et al. 2014). In the mature parts of the root, the outermost cortical cell layer known as the exodermis transforms into an additional barrier layer with a lignified and suberized cell wall and Casparian strips (Feldman 1994; Hose 2001). The innermost vascular tissue is composed of xylem and phloem cells and encircled by a layer of pericycle cells. A mature maize root shows a polyarch organization with a central protostele and many xylem arms (Kiesselbach 1949). The number of xylem vessels varies between root types, for example, when the primary root shows six to ten metaxylem vessels, a nodal root from a higher node may contain up to 48 metaxylem vessels (reviewed by (Hochholdinger 2009)). Phloem strands, which conduct photosynthates, are present in between the xylem vessels (Feldman 1994).

Longitudinally, the maize root can be divided into different zones (Hochholdinger 2009). Starting at the distal end is the root cap composed of up to

10,000 cells (Feldman 1994; Ishikawa and Evans 1993). The root cap cells are involved in a multitude of processes including sensing gravity and moisture (Feldman 1994; Ishikawa and Evans 1993). They also facilitate penetration of the root tip through the soil by secreting mucilage and acting as a cover to protect the meristematic region while the root grows. Proximal to the root cap is the QC composed of approximately 800–1200 cells (Jiang et al. 2003). Proximal to the QC are the rapidly dividing proximal meristem cells and their initials (Hochholdinger et al. 2004b). After several rounds of rapid cell division in the meristematic region, cells enter the elongation zone and undergo anisotropic expansion before they move into the next differentiation/maturation zone, characterized by the presence of root hairs, where cells of all tissues attain final shape and function (Ishikawa and Evans 1993).

11.2.2 Root System Development in Switchgrass

Switchgrass, a perennial grass from the prairies of North America, has recently emerged as a frontrunner for the development of lignocellulosic biofuel crops (Lee 2006; Monti 2012). The embryonic root system consists of a primary root (Newman and Moser 1988). As the seedling develops, additional postembryonic roots emerge. Crown roots form on the lower stem nodes and several orders of lateral roots together form the bulk of the adult rootstock (Metcalfe and Nelson 1985). Switchgrass root systems show an exponential growth spurt in the initial 3 weeks of seedling growth and later gradually slow down (Dalrymple and Dwyer 1967). This active growth phase is thought to be critical for the successful growth and establishment of switchgrass (Zegada-Lizarazu et al. 2010). Another feature of the switchgrass root system is its ability to store nutrient resources in the long-lived root system and rhizomes (underground stems). Switchgrass can mobilize nutrient resources such as carbohydrates and nitrogen from the shoot system to the root system and vice versa depending on the growth season (Zegada-Lizarazu et al. 2010; Vogel 2004; Lemus et al. 2008, 2009; Wayman et al. 2014). This feature could be one of the reasons why switchgrass plants can grow on marginal lands but still maintain productivity (Parrish and Fike 2005). It has been noted that in mature switchgrass plants the upper 1-m of soil contains the bulk of the root system although the roots have been documented to reach a depth of 3 m or more (Ma et al. 2000).

11.2.3 Root System Development in Sugarcane

Contrary to most other members of the Panicoideae grass family, sugarcane is commonly propagated asexually using pieces of the stem referred to as sett. Hence, the whole root system is postembryonic in origin consisting of shoot-borne sett roots, shoot roots, and associated lateral roots (Smith et al. 2005). The first batch of roots formed is the sett roots, usually appearing within 24 h after planting the sett (Venkatraman and Thomas 1922; van Dillewijn 1922; Glover 1967). These fine roots are characterized by their high degree of branching and substitute for embryonic roots in the establishment of the new plant. However, as the plant becomes mature, sett roots gradually disappear (Glover 1967). After about a week post-planting, a new root type starts emerging from the sett base referred to as shoot roots (van Dillewijn 1922; Glover 1967). Shoot roots are thicker and grow more rapidly than sett roots and are the major root type in the sugarcane root system (Smith et al. 2005; van Dillewijn 1922; Glover 1967). Broadly, shoot roots can be categorized into three functional types: (1) Buttress roots are the first group of shoot roots produced, show relatively little branching and are critical in anchorage (initially growing outwards and then downwards into the soil) of the plant (Evans 1935), (2) Superficial roots are finer, highly branched roots produced from higher nodes above the stem base and are key players in water and nutrient uptake, (3) Rope roots are agglomerations of vertical roots that grow vertically into deep soil (often at depths of more than 6 m) and aid in extracting water from deep underwater reserves (Smith et al. 2005; Evans 1936). It has been reported that in modern sugarcane cultivars, the overall root system structure appears to be slightly different, for example, the rope roots are less prominent (Moore 1987). Sugarcane root systems are distinct in their high degree of plasticity, root length density, and distribution pattern in soil (more deeply distributed) from other crops (Smith et al. 2005; Blackburn 1984; Jackson et al. 1996). As with other grasses, the sugarcane root-to-shoot ratio is at its highest during early stages of plant growth and later on gradually declines as the plant matures (Smith et al. 2005).

11.2.4 Root System Development in Sorghum

In Sorghum, the embryonic root system consists of a single primary root produced from the base of the embryo (Singh et al. 2010). The primary root functions throughout the plant life cycle, and, although similar to other grass species, its role is highly diminished after the onset of the postembryonic root system (Ernst 1948). About 1 week after germination, lateral roots start forming and subsequently, at the 4–5 leaf stage, the crown roots develop, which, together, form the postembryonic root system. As the plant grows, crown/nodal roots develop at regular intervals from sequential nodes beginning at the stem base throughout the life cycle (Singh et al. 2010). Nodal roots emerging from higher nodes (above ground) appear thicker and show a more vertical angle with respect to gravity than their counterparts from lower nodes. These roots are commonly referred to as buttress roots and once they enter the soil show a reduction in root diameter (Ernst 1948). It has been reported that, on average, Sorghum plants produce twice as many lateral roots as maize at any given stage of development and is thought to be a contributing factor for this species' high drought tolerance (Miller 1916).

11.3 Setaria as a Model for Panicoid Grass Root Systems

11.3.1 Setaria viridis: A Grass Genetic Model System

Though there are reports describing the morphological characteristics and gene mutants affecting root type development in a few of the panicoid grasses, detailed analyses to dissect the underlying genetic regulatory networks that govern various aspects of root system dynamics/development such as cell-fate decisions and patterning steps are still missing (Bellini et al. 2014; Hochholdinger and Zimmermann 2008; Marcon et al. 2013). This is primarily due to the difficulties in systematically growing and studying these plants due to constraints such as large size, complex genetic makeup, lack of molecular biology tools, long life cycle, and general difficulty of studying root traits in soil. Availability of large numbers of gene mutants and other genetic resources (reporter lines, techniques to alter gene functions, etc.) are essential to characterizing the underlying molecular mechanisms regulating root growth and development in any plant system. In this context, a panicoid grass model species with all the ideal characteristics that one would expect for a genetic model system is of particular significance.

Recently, *Setaria viridis* has emerged as a potential genetic model system to study the panicoid subfamily of grasses. It has a relatively small sequenced genome, short life cycle, robust seed production, ease of growth under controlled conditions, and is transformable (Doust et al. 2009; Brutnell et al. 2010; Van Eck and Swartwood 2015). A method to break the long dormancy of freshly harvested seeds (often up to 4 months) has also been described by our group, thus enabling the full utilization of the rapid life cycle trait (Sebastian et al. 2014). In addition, genetic tools and resources are currently being developed in various labs across the world. As an added advantage, *S. viridis* is closely related to foxtail millet (*S. italica*) and the two species are intercrossable making it feasible to explore crop domestication with this system. Thus, Setaria offers tremendous potential in improving our understanding of root biology in this economically important group of plants.

11.3.2 Root System Development in Setaria

Setaria viridis seeds germinate quickly and the primary root often becomes visible 24 h after imbibition on gel-based media (half-strength MS media, 0.5 % sucrose with 0.6 % Gelrite at 29 °C). The embryonic root system comprises a single primary root emerging from the basal pole of the embryo (Fig. 11.1d). Although occasionally some seedlings produce 1–2 seminal roots, they are mostly absent in Setaria. Around 3–4 days after planting (DAP), lateral roots start to emerge from the primary root (Fig. 11.1d). From seed germination to the emergence of crown roots, the sole primary root and associated lateral roots sustain seedling growth. The first crown roots emerge from the coleoptilar node (first shoot node) around 6–9 DAP and together with lateral roots make up the postembryonic root system in

Setaria (Fig. 11.1e and f). Both primary and crown roots can form several orders of lateral roots as the plant grows (Fig. 11.1c and f). Nadeau and Morrison reported that Setaria roots penetrate soil to a depth of nearly 60 cm with the highest concentration of roots at a depth of around 20–30 cm based on their field experiments (Nadeau and Morrison 1986). As with many other grasses, root system development in Setaria appears to be correlated with the production of leaves and tillers (Watt et al. 2009). For example, the first crown root usually appears when the plant is at the 3-leaf stage.

The primary root is separated from the crown and aerial tissues by the mesocotyl, an internode connecting the scutellar and coleoptilar nodes (Hoshikawa 1969). We have observed that the mesocotyl is more prominent in seeds that are germinated in soil compare to those seedlings that are germinated on tissue culture plates. This appears to be an adaptation to place the coleoptile at or near the soil surface irrespective of the seed planting depth. The mesocotyl can often produce adventitious roots, especially in soil-grown plants. It is remarkable to note that when the shoot system is just a few centimeters in length, the root system can be over three times as large in size; thus demonstrating the incredible rates of root growth these plants are capable of (data not shown). In Setaria, crown roots and lateral roots that are produced from a single plant at different time points often show variations in thickness, a phenomenon known as heteroblasty (Hou and Hill 2002; Zotz et al. 2011).

11.3.3 Cellular Organization of *Setaria viridis* Roots

In seedlings that are germinated on tissue culture plates, the primary root is around 200–250 μm in diameter. It has a highly organized and radially symmetric cellular structure similar to other grass species (Fig. 11.2a–d). Longitudinally, the Setaria primary root can be subdivided into meristematic, elongation, and differentiation/maturation zones. The meristematic zone is characterized by rapidly proliferating meristematic cells. As the root grows, these cells are gradually displaced into the adjacent elongation zone, where they continue to elongate. The meristematic zone also harbors the stem cell niche and the QC. Further work is required to clearly identify the number of QC cells and the organization of the stem cell niche in Setaria roots. The apex of the meristematic zone is covered by the root cap containing >ten layers of collumella/calyptrogen cells (data not shown). Proximal to the elongation zone is the zone of differentiation/maturation, where the cells finally attain their predetermined shape, size, and cell fate and constitute the different tissue types of the root. Epidermal cells in the differentiation zone form root hairs; unicellular extensions of the epidermis which functions in the absorption of nutrients and water by increasing the overall root surface area (reviewed by (Mendrinna and Persson 2015)).

In the Setaria primary root, cells are arranged in a concentric ring of layers starting from the outermost epidermis (Fig. 11.2b and d). Three layers of cortical tissue develop interior to the epidermis with the outermost layer giving rise to the presumptive exodermis. A single layer of endodermis separates the cortex from

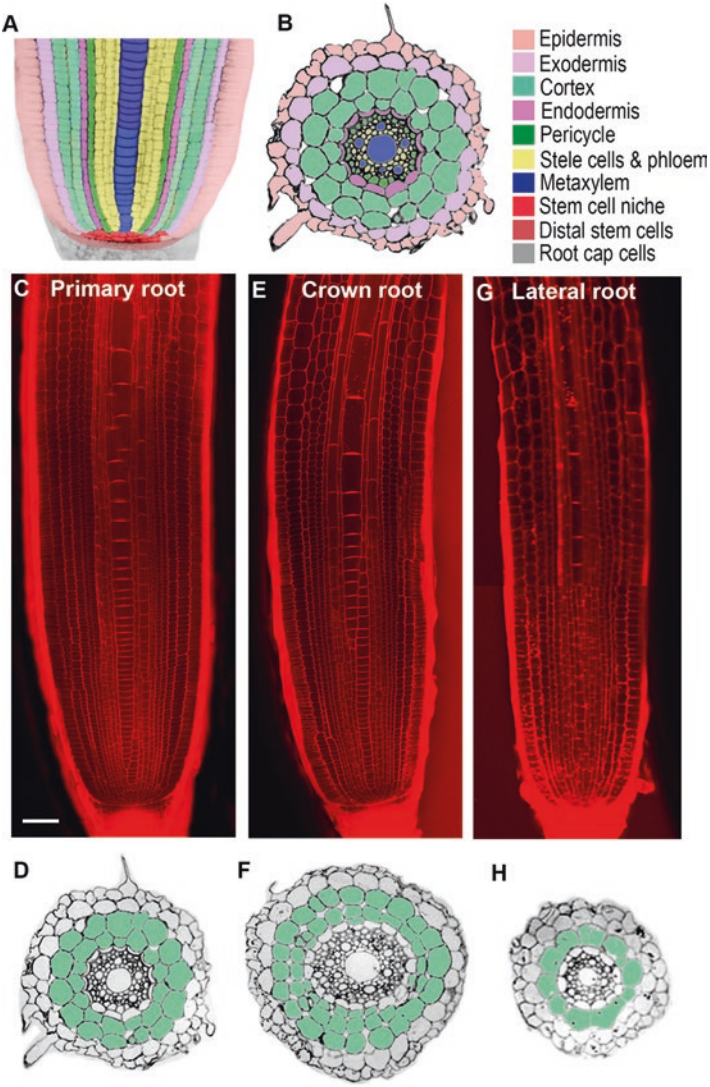

Fig. 11.2 Cellular organization of *Setaria viridis* roots. (**a**, **b**) False-colored longitudinal (**a**) and radial (**b**) cross-section images of a Setaria root tip showing cell/tissue organization. Various colors indicate different cell/tissue types. (**c**, **e**, **g**) Longitudinal optical cross-sections of mPS-PI stained Setaria primary (**c**), crown (**e**), and lateral (**g**) roots. (**d**, **f**, **h**) Radial cross-section images of Setaria primary (**d**), crown (**f**), and lateral (**h**) roots obtained through thin sectioning. Scale bars: 55 µm

the vasculature. The innermost stele, which encompasses the xylem and phloem tissues, is encircled by a layer of pericycle cells (Fig. 11.2b and d). As in other panicoid grasses, the Setaria primary root stele has a polyarch organization, with varying numbers of central metaxylem (or late metaxylem) strands. Soon after emergence, roots have only a single central metaxylem strand, but as the root matures, it may contain four or more strands. Surrounding these central xylem strands are the peripheral xylem strands. Depending on the age of the root, their number can also vary. At 4 DAP, the primary root has one central metaxylem strand and five to six peripheral xylem strands. Compared to the central xylem elements, the peripheral xylem elements are smaller in diameter (Fig. 11.2b and d). Phloem elements are found in between these peripheral xylem strands. As a characteristic feature of grass roots, the parenchymatous cells that are present between the xylem and phloem strands remain as such rather than differentiating into vascular cambium.

In Setaria, the crown roots are usually larger in diameter than the primary roots (250–300 μm) while lateral roots are the narrowest root type in the root system (100–150 μm in diameter). Anatomically, the crown roots and lateral roots are similar to the primary root (Fig. 11.2e–h); however, in certain instances, both crown and lateral roots show variation in the number of cortical cell layers (>two layers in crown roots and ≤2 in lateral roots).

11.4 Conclusions and Prospects

Although grasses have paramount importance to us both directly as a food source and indirectly as animal feed and a valuable source of sustainable clean energy, we are far behind in understanding their biology and growth mechanisms. This is especially true concerning grass root biology, which is particularly understudied and is paradoxically an area likely to hold potential for improvements in the overall productivity of our agriculture systems. This is largely due to the drawbacks grasses possess as model systems amenable to genetic studies and manipulations. However, with the emergence of new model species such as *Setaria viridis*, the tools available to understand grass root biology may enable a more mechanistic and comprehensive understanding of the process. Development of genetic resources such as high-quality fully annotated genomes, availability of genetic mutants, tissue/cell-type reporters, capabilities to edit the genome using the CRISPR-Cas9 system (Ran et al. 2013; Feng et al. 2013), and a rich and diverse germplasm collection with excellent SNP (single nucleotide polymorphism) data are all crucial in this pursuit. Creation of cell-type specific data sets that facilitate thorough characterization of molecular regulatory events at cellular resolution and novel methods to image the root system such as the recently developed GLO-Root imaging system (Rellán-Álvarez et al. 2015), which allows visualization of the root system under physiologically relevant conditions, are also important in dissecting the intricacies of grass root biology and environmental responses.

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