The role of root border cells in plant defense

Martha C. Hawes, Uvini Gunawardena, Susan Miyasaka and Xiaowen Zhao

The survival of a plant depends upon the capacity of root tips to sense and move towards water and other nutrients in the soil. Perhaps because of the root tip’s vital role in plant health, it is ensheathed by large populations of detached somatic cells – root ‘border’ cells – which have the ability to engineer the chemical and physical properties of the external environment. Of particular significance is the production by border cells of specific chemicals that can dramatically alter the behavior of populations of soilborne microflora. Molecular approaches are being used to identify and manipulate the expression of plant genes that control the production and the specialized properties of border cells in transgenic plants. Such plants can be used to test the hypothesis that these unusual cells act as a phalanx of biological ‘goailes’, which neutralize dangers to newly generated root tissue as the root tip makes its way through soil.

Each day, thousands of uniquely differentiated root border cells are synthesized and programmed to separate from the root tips (the region at the apex housing the apical meristem and root cap) of higher plants4. Although they are attached to the root periphery by a water soluble polysaccharide matrix, the middle lamellae of these cells become solubilized by the action of peroxidase enzymes in the cell wall5. By definition, border cells are those cells that disperse into suspension within seconds when root tips are placed into water (Fig. 1). These cells originate from root cap meristematic cells, which give rise to cell layers that progressively differentiate through specialized stages before they finally separate from the cap periphery6. Thus, by the time an individual border cell is delivered to its ultimate destination external to the root, it has already served several functions, such as mucilage secretion, the sensing of gravity and other environmental signals. How many of those functions continue to operate in border cells is unknown. However, the border cells of most species are viable after they detach from the root (Fig. 1), and in culture the cells can divide and differentiate into organized tissue (Fig. 1). Upon separation from the cap, metabolic activity in border cells increases and gene expression undergoes a global switch, such that mRNA and protein profiles are grossly distinct from those of progenitor cells in the root cap7. Border cells then produce specific metabolites, such as anthocyanins and antibiotics7 (Fig. 1), and enzymes including a Rhizobium-induced peroxidase and a low pH galactosidase (C. Jian and M.C. Hawes, unpublished; Fig. 1). We call these unusual cells (previously termed ‘sloughed root cap cells’) root ‘border’ cells to emphasize that they constitute a biotic boundary layer between the root surface and the soil.

In the past, border cells largely have been overlooked as a structural and functional component of root systems. One reason for this oversight is the notion that the cells are a moribund by-product of root-cap turnover. This presumption has prevailed in recent years in spite of the fact that L. Kudriashov demonstrated that it was incorrect in 1919 (Refs 1, 2). Although species in a few families, such as the Compositae, have border cells that can detach from the root cap, in the majority of species examined the border cell populations are ~90% viable8. Border cells of maize and pea grown hydroponically can remain viable for more than three months6, and cells of maize have been reported to survive for a week or more in field soil9. A second reason for the lack of attention paid to border cells is that the cells disperse so efficiently in response to free water that even the gentlest washing of root systems to remove soil before experimental manipulation and examination removes all border cells. However, in the absence of free water the cells cling so tenaciously to the root periphery that their presence is difficult to distinguish even by experienced investigators.

Regulation of border cell production

The number of border cells that can be produced daily by a given root is conserved at the family level, and can range from a dozen for tobacco to >10 000 for cotton and pine. The production of border cell populations for tap roots, lateral and branch roots appears to be similar. Therefore, for complex root systems with hundreds of branches, millions of border cells would be deposited into the soil daily if their production were a constitutively expressed process, which was assumed to be the case for many years5. However, recent studies have established that border cell production in species such as cereals, legumes and cotton, is a tightly regulated process that is controlled by endogenous and environmental signals. In the laboratory, radicles synthesize a number of border cells within 24 h of emergence. As they accumulate on the cap periphery, border cells of pea release an extracellular suppressor that specifically inhibits mitosis in the root-cap meristem, without affecting mitosis in the adjacent apical meristem that gives rise to root growth3. Root-cap turnover ceases and no new border cells are made, but the set of ~4000 cells that has been made remains expressed at the periphery of the cap as the root elongates. If border cells are removed, or if the extracellular suppressor is depleted by dipping the root into water, cell division in the root cap meristem resumes within 5 min, and remains high for 2 h. New cells can be collected from the cap periphery within 1 h after removal of the old ones; after 24 h a new set of 4000 cells is complete and no more cells are made. This process, which can be synchronized experimentally, has been exploited to identify two genes, psugt1 and rcpme1, which play a role at both ends of border cell development – cell division in the root cap meristem and cell separation at the cap periphery, respectively3,5. When the expression of either gene in transgenic roots is inhibited by antisense mRNA mutation, border cell development is blocked. Such mutants are being used under controlled conditions to dissect the molecular and cellular mechanisms by which border cell development is regulated.

How border cell development is regulated is an important topic. Border cells are not detach readily except when they are actually placed into water, a root growing through conditions where the tip never experiences a flush of water, could, theoretically, be expected to retain the same set of border cells throughout its lifetime. Renewed border cell production in response to any exposure to free water, such as during rain or irrigation, would be a logical prediction. However, when roots are grown in a wet semi-solid matrix, such as water agar, where border cells can be seen to be renewed continuously when viewed microscopically, the results are not always found to be...
Suppression of mitosis in root border cells

In Greek mythology, the Sirens’ beautiful songs tempted sailors to linger on their island, and thus distracted them forever from completing their intended journeys. Recent studies suggest that border cells might carry out a similar distraction to pathogenic nematodes on their way to infect root tips (X. Zhao and M.C. Hawes, unpublished). When a pea root, whose border cells have been removed, is placed onto water agar containing a lawn of R. leguminosarum expressing a nod-lacZ reporter gene on solidified culture medium (pH 7.0), intense nod gene expression is detected in bacteria present at the root tip (Fig. 1). This suggests that border cells might play a role in defending the root tip from biotic and abiotic stress.

Border cells attract and immobilize nematodes

In Greek mythology, the Sirens’ beautiful songs tempted sailors to linger on their island, and thus distracted them forever from completing their intended journeys. Recent studies suggest that border cells might carry out a similar distraction to pathogenic nematodes on their way to infect root tips (X. Zhao and M.C. Hawes, unpublished). When a pea root, whose border cells have been removed, is placed onto water agar containing a lawn of root knot nematodes, no sign of recognition is evident at the root tip (Fig. 3). By contrast, when border cells are present nematodes rapidly accumulate around the root tip periphery (Fig. 3).
These roots are lifted from the surface, clumps of border cells left behind reveal high populations of actively motile nematodes (Fig. 3). However, within 30 min, the motility of the nematodes ceases and the worms appear straight and inert, as they do when dead (Fig. 3).

When the immobilization phenomenon is measured using quantitative assays, a >99% loss of mobility develops in response to a single heat-stable, polar fraction of the root exudates (soluble extracellular material). This immobilization effect is reversible – within a few hours to a few days (depending on conditions of the assay) the nematodes resume full mobility. If a similar process occurs in the soil, by the time that the nematodes resume motility, a root tip growing at a rate of 1 mm per hour would be long past being in danger of penetration. This phenomenon might account, in part, for the fact that the normal site of infection by nematodes is behind the root tip, just past the region where border cells are released.

**Mucilage production can repel bacteria and decrease sensitivity to aluminum**

Border cells of legumes and cereals produce a mucilage layer in response to co-cultivation with pathogenic bacteria, but not in response to E. coli. This layer appears to repel the bacteria (Fig. 4), but its mechanism of action is unknown. Recent studies have revealed that co-cultivation of border cells with aluminum results in the production of a mucilage layer similar to that which occurs in response to pathogenic bacteria (S. Miyasaka and M.C. Hawes, unpublished). Within 2 h of exposure to aluminum, a layer of mucilage around individual border cells increases in a dosage-dependent manner, from being barely detectable (Fig. 4) to being as wide (Fig. 4) or wider than the cell’s diameter (Fig. 4). Interestingly, the development of the layer is correlated with a near-total cessation of aluminum-induced border cell death. For the first 2 h of exposure to aluminum, a dosage-dependent linear rate of border cell death proceeds. After 2 h, when the mucilage layer is in place, the rate of cell death drops precipitously, such that the viability of the cell population remains largely static for the next 20 h (S. Miyasaka and M.C. Hawes, unpublished). The results are consistent with the hypothesis that border cells have the capacity to synthesize an indissoluble extracellular structure that interferes with the ability of aluminum to cause further cellular damage. The mechanism by which this occurs is unknown, but one possibility is that the charged aluminum molecule becomes immobilized by binding to the polysaccharide layer. If this is the case, the capacity of thousands of detached border cells to immobilize aluminum ions could have a profound effect on the ability of aluminum to damage the root tip, the primary site of aluminum’s toxic effects.

**Border cells act as a decoy for fungal infection**

When pea seedlings are inoculated uniformly with spores of pathogenic fungi, nearly all develop visible lesions in the region just behind the root tip within 24 h (U. Gunawardena and M.C. Hawes, unpublished). A small proportion of seedlings also develop a visible lesion at the root tip. When excised and placed onto culture medium, fungal mycelium emerges from these infected tips, confirming that the root tip is not inherently resistant to infection (Fig. 5). In the majority of seedlings (>90%), the root tip remains white, and to the naked eye appears to be infection free. However, microscopic observation reveals that the tip is actually covered in fungal hyphae (Fig. 5).
When the tip is placed into water and agitated gently, this ‘mantle’ detaches and can be seen when grabbed by would-be tacklers, leaving the runner free to keep moving down the field.

A similar mechanism, mediated by border cell detachment, could, in part, account for the observation that root tips generally escape infection and colonization in the field.

Model

Taken together, the observed responses of border cells to aluminum, nematodes and pathogenic fungi are consistent with a two-step model in which the root tip is protected by an unusual and complex process. First, the root cap meristem actively produces a ‘front’ consisting of thousands of border cells that can engineer the environment to render a threat temporarily harmless. Elongating cells generated by the apical meristem then push the root tip into and through the newly ‘engineered’ environment.

The primary mechanism by which the threat is rendered harmless by the border cells might vary and could be constitutive (i.e. the secretion of a chemical that anesthetizes nematodes) or inducible in response to a particular signal (i.e. the production of a mucilage layer that immobilizes aluminum).

It is important to note again that only in the presence of a flush of free water, such as after rain or irrigation, would the ensemble release tear-away jerseys that were designed to detach when grabbed by would-be tacklers, leaving the runner free to keep moving down the field.

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When the tip is placed into water and agitated gently, this ‘mantle’ detaches and can be seen microscopically to consist of thousands of digested border cells held together by actively growing fungal mycelium. Even under extreme conditions, which include high concentrations of spores and heat-shock temperatures, the root tip remains sterile for days (Fig. 5), indistinguishable from un inoculated controls (Fig. 5) in spite of having been covered by a sheath of necrotic fungi. In these plants, root growth and development are comparable to that of the controls, indicating that the apical meristems are fully functional. In the 1950s, running backs (American football players) were outfitted with
This newly synthesized tissue comprises the same terminal few millimeters of tissue that detects and responds to environmental stimuli to condition movement toward nutrients and away from danger. Not surprisingly, such newly synthesized tissue is also especially vulnerable to abiotic and biotic disease. For humans, this is the functional equivalent of having to rely on newborn babies to explore and search a predator-filled wilderness for supplies to send back to base camp. This biological dilemma might help to account for the fact that in most species the vulnerable but essential root meristems are surrounded by an array of border cell ‘foot soldiers’.2

Other defensive strategies might not work for the tip

We have proposed that a fail-safe defense mechanism is needed to protect the root tip because of its importance to the overall health of the plant. However, it is well established that plants already have complex defense pathways that are activated rapidly in response to external signals. Within moments of recognition of biotic and abiotic stresses, plants can alter their surface structures, generate toxic mituens, and activate the expression of genes for the synthesis of dozens of antimicrobial proteins and other metabolites3. Why would an elaborate alternative method involving the separation of masses of detached cells be needed by plants that already are well equipped to defend their cells? One explanation for the defensive strategies in plants often involve the so-called ‘hypersensitive reaction’, in which the rapid necrosis of a few plant cells is thought to inhibit the spread of a pathogen.4 In most tissues, including the region behind the root tip where most root–microbe relationships are initiated, the sacrifice of a few cells may contain the infection with little or no deleterious effect on plant health. However, in the root tip, there is no margin for the loss of a few cells in the interest of containing infection when those few cells comprise the apical meristem.5

"...in the root tip, there is no margin for the loss of a few cells in the interest of containing infection when those few cells comprise the apical meristem.”

at the tip, but in the region of elongation, just behind the tip where the production of border cells does not occur. We suspect that the main reason that the effect of border cells on root infection has never been examined, is its effectiveness: so few pathogens and symbionts have been reported to initiate infection or colonization at the root tip that there has been little reason to focus on its role in infection. In the availability of plants with altered root cell production means that the most obvious prediction of our model can now be tested. If the presence of border cells is the reason root tips are largely impermeable to infection, then inhibiting their production and/or release will result in a change in the site and/or nature of infection. If this is the case, the study of how border cells can defend the tip so successfully might elucidate novel defense strategies that can be applied to tissues that do not come equipped with their own cellular ‘goals.’ It might not be feasible to program other tissues to produce and separate populations of border cells. However, genetic engineering approaches can be used to harness the cellular and extra-cellular mechanisms by which border cells neutralize dangerous molecules or organisms and express them in tissues that are not as well protected as the root tip.

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References


In Figure 2, dihydroflavonol (not flavone) is acted on by flavonol synthase (FLS) to form flavonol. In Figure 4, the structure of the 2-hydroxyisoflavanone intermediate was depicted incorrectly. Corrected versions of these portions of the figures are printed below.

**Fig. 2.**

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**Fig. 4.**

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**Erratum**

In the December 1999 issue of Trends in Plant Science, we published a research news article by Albrecht G. von Arnim (1999) Phytochrome in the limelight. Trends Plant Sci. 4, 465–466. In Figure 1, top left it should read phyAr and not phyBr. A corrected version of this portion of the figure is printed left.

We apologize to the authors and to our readers for these errors. A corrected version of these articles can be viewed in HTML format at plants.trends.com

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