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Review

Extracellular Trapping of Soil Contaminants by Root Border Cells: New Insights into Plant Defense

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Abstract: Soil and water pollution by metals and other toxic chemicals is difficult to measure and control, and, as such, presents an ongoing global threat to sustainable agriculture and human health. Efforts to remove contaminants by plant-mediated pathways, or "phytoremediation", though widely studied, have failed to yield consistent, predictable removal of biological and chemical contaminants. Emerging research has revealed that one major limitation to using plants to clean up the environment is that plants are programmed to protect themselves: Like white blood cells in animals, border cells released from plant root tips carry out an extracellular trapping process to neutralize threats and prevent injury to the host. Variability in border cell trapping has been found to be correlated with variation in sensitivity of roots to aluminum, and removal of border cell results in increased Al uptake into the root tip. Studies now have implicated border cells in responses of diverse plant roots to a range of heavy metals, including arsenic, copper, cadmium, lead, mercury, iron, and zinc. A better understanding of border cell extracellular traps and their role in preventing toxin uptake may facilitate efforts to use plants as a nondestructive approach to neutralize environmental threats.

Keywords: root border cells; extracellular DNA; neutrophil extracellular traps; rhizofiltration; heavy metals

1. Root Border Cells

Most plant species synthesize cell populations that are programmed to disperse into the external environment surrounding the root tip in response to free water or abrasion (Figure 1). For many years, these so-called "sloughed root cap cell" populations were thought to be a product of tissue disintegration based on the logical presumption that cells falling from the root surface must be dead. This was despite the observation in 1919 [1] that "sloughed root cap cells" from pea and corn could remain 100% viable for months in hydroponic culture. Long-term survival of the detached cells in culture eventually was confirmed, but the presumption remained that these cells expressed the phenotypes of the whole plant with regard to pathogen recognition and response [2].

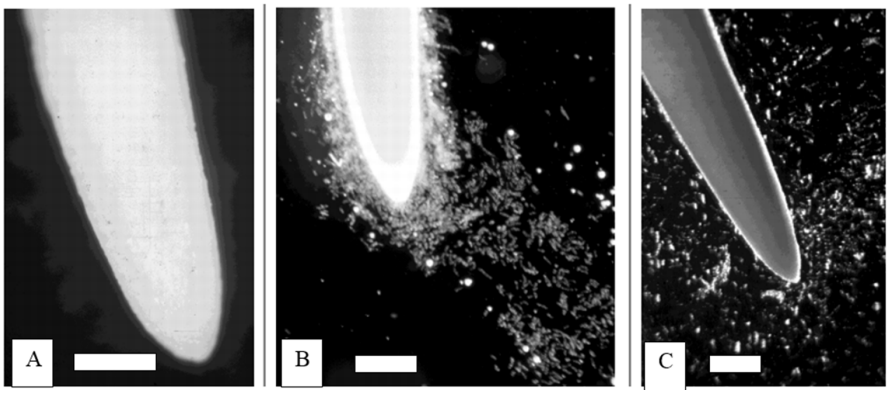

Figure 1. Dynamics of border cell dispersal upon immersion into water. (**A**) When roots are maintained at >98% humidity, border cells remain tightly appressed to the surface and invisible; (**B**) Upon immersion of the root tip into water, the root cap mucilage absorbs water instantaneously, and cells begin to disperse within seconds; (**C**) Within minutes, all border cells disperse into suspension, leaving the root tip surface free of cells. Scale bars: 1 mm.

Direct tests revealed instead that protein profiles and gene expression patterns in the detached cells are markedly distinct even from progenitor root cap cells [3]. Therefore, the term "border cells" was introduced as a new alternative to "sloughed root cap" cells to emphasize that these cell populations comprise a cellular interface that does not function biochemically in the same manner as cells within the root cap [4,5]. Despite observations that border cells synthesize and export a slimy matrix that immobilizes diverse plant pathogens, the actual function of the cells remained obscure until parallels with newly described immune responses in animals were discovered [6].

2. Extracellular Traps in Animals and Plants

In 2004, a previously overlooked foundation of mammalian defense was reported for the first time: In response to stress signals, neutrophils within the blood system export a slimy matrix that immobilizes diverse pathogens [7]. These "neutrophil extracellular traps" or "NETs" are comprised of proteins including histone, actin, and enzymes involved in reactive oxygen species (ROS) pathways, together with extracellular DNA (exDNA) [8]. Pathogens such as Group A Streptococcus produce extracellular enzymes with DNase activity (exDNase) that facilitate release from NETs and allow systemic spread of the bacteria [9]. The importance of exDNase as a survival mechanism has been validated *in vitro*, as knockout mutations of the exDNases result in loss of pathogen virulence [10].

The discovery of NETs in animals finally provided insight into why plants invest so much energy in producing thousands of healthy cells destined to disperse from root tips into the soil: A parallel extracellular trapping process operates in plants [6]. In response to pathogens and other stress signals, viable border cells rapidly synthesize and export an extracellular complex comprised of DNA together with >100 proteins including histone, actin and ROS enzymes [11,12]. When root tips are treated with DNase I, resistance to pathogen invasion is abolished [6,12]. As in animal pathogens such as Group A Streptococcus, knockout mutations of exDNase in the bacterial plant pathogen, *Ralstonia solanacaerum*, result in reduced virulence and loss of ability of the pathogen to move systemically through the plant [13].

Like the defense pathway-inducing signals from pathogens, metals including lead, copper, mercury, silver and cadmium also activate ROS pathways in mammalian cells [14,15]. A recent survey of human neutrophils now has implicated NETs in the systemic localization patterns, or trapping, of metals within human blood [16]. Given the remarkable parallels between exDNA-based immune responses in animals and plants, this observation may help to explain a series of studies, summarized below, suggesting that root border cells also play a role in trapping and localization of metals.

3. Border Cell Trapping of Aluminum

Aluminum toxicity is a limiting factor in crop production in acid soils, which facilitate solubilization of the metal [17]. Genotypic variation in plant sensitivity has been well documented, but mechanisms for resistance remain under investigation [18,19]. Roots are an important target for Al-induced damage, and inhibition of root growth occurs rapidly in response to exposure of the root tip to aluminum [17,20]. The hypothesis that border cells play a role in avoidance of Al uptake was tested directly using roots of pea (*Pisum sativum* L.) and snapbean (*Phaseolus vulgaris* L.) from the Fabaceae family [21,22]. Seedling roots with and without border cells were immersed into liquid containing Al [22]. Even though border cells disperse from the root tip within minutes upon immersion into liquid (Figure 1), there was an obvious increase in Al staining within the root whose border cells were gone at the time of immersion (Figure 2B) compared with those whose border cells were present (Figure 2C).

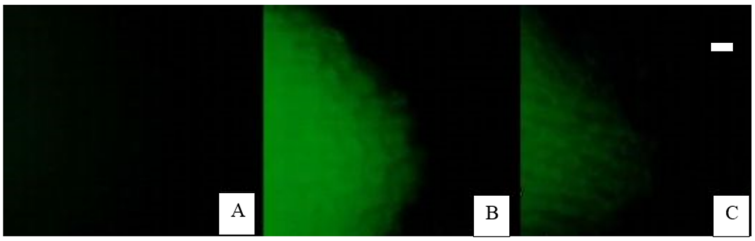

Figure 2. Border cell inhibition of aluminum uptake into the root cap detected by lumogallion staining. (**A**) Control roots incubated for 30 min at pH 5.2, in the absence of Al reveal no fluorescence; (**B**) Intense staining occurs in root tips whose border cells were dispersed prior to immersion of the root into 200 μM Al for 30 min; (**C**) Reduced uptake of aluminum into root tips whose border cells were present on the root cap periphery at the time the roots were immersed into 200 μM Al for 30 min [22]. Scale bar: 30 microns.

Border cells from an Al-sensitive snapbean cultivar incubated with Al in a simple salt solution were killed more rapidly than cells from a resistant cultivar, suggesting that whole-plant tolerance mechanisms are expressed in the border cell populations [21]. Of particular interest was the finding that individual cells from the resistant cultivar produced larger mucilage layers (now called "extracellular traps") [11] in response to Al than cells from the sensitive cultivar (Figure 3). The mechanisms underlying Al-border cell interactions remain to be defined. However, Al is known to complex with DNA, so the discovery that DNA is an integral component of border cell extracellular traps may yield new hypotheses to be explored [23].

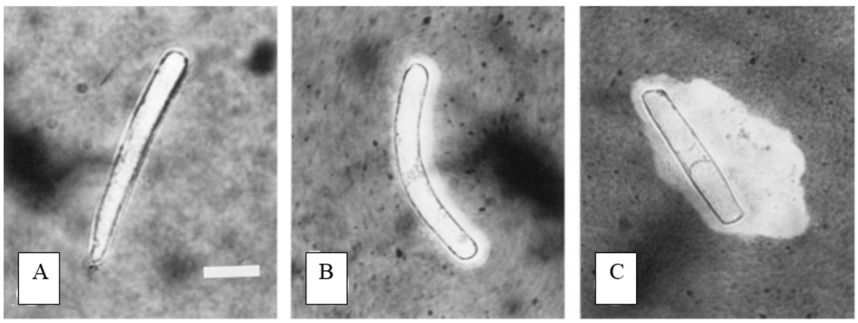

Figure 3. Dosage dependent induction of extracellular trap formation in border cells in response to aluminum. Extracellular trap formation was visualized using India ink, which does not penetrate the trap. (**A**) Border cells from snapbean border cells in water have little or no visible extracellular trap. Within 1 h of immersion in 50 micromoles aluminum (**B**) or 100 micromoles (**C**), increased trap formation is evident. Trap dimensions at the higher level was significantly greater (p = 0.0001) than the lower level. [Figure reproduced with permission from reference 21]. Scale bar: 20 microns.

4. Border Cell Trapping of Other Soil Contaminants

Dynamic interactions in response to copper, cadmium, boron, lead, mercury, iron, and arsenic as well as aluminum have now been described for border cells of cereals, legumes, cotton, coyotillo and fern (Table 1) [21,22,24–47]. Efforts to define underlying mechanisms are in early stages of discovery, but results suggest that signals controlling border cell production and trapping responses in the field may yield new approaches to plant protection [48–51].

Table 1. Border cells and metals: publications from 2001–2015.

Date	Metal	Plant	Reference
2001	aluminum	snapbean	[21]
2003	aluminum	pea	[22]
2003	aluminum	wheat	[24]
2003	copper	Silene	[25]
2003	cadmium	coyotillo	[26]
2004	aluminum	barley	[27]
2005	aluminum	barley	[28]
2006	aluminum	pea	[29]
2007	aluminum+boron	pea	[30]
2008	aluminum	cowpea	[31]
2008	iron	rice	[32]
2008	lead, mercury	mung bean	[33]
2009	aluminum	pea	[34]
2011	aluminum	rice	[35]
2011	aluminum	soybean	[36]
2011	copper, nickel, zinc	cowpea	[37]
2011	iron	rice	[38]
2012	iron	rice	[39]
2012	arsenic	cowpea	[40]
2012	iron, aluminum	rice	[41]
2012	aluminum	oats	[42]
2012	arsenic	fern	[43]
2013	boron, aluminum	pea	[44]
2013	aluminum	soybean	[45]
2013	copper	cotton	[46]
2014	cadmium	fern	[47]

5. Border Cell Number *vs.* Arsenic Uptake into Edible Plants

Two studies with arsenic (Table 1), in cowpea (*Vigna unguiculata*) and fern (*Pteris vittata*) [40,43], are of particular interest in view of a recent *in vivo* study of arsenic taken from the environment into plants under diverse growth conditions [52]. A significant inverse correlation was found between number of border cells produced by the species of interest and uptake of arsenic into the plant (Figure 4). Thus, for example, members of the Brassica family do not produce populations of viable dispersed border cells, whereas legumes produce several thousand per day [53,54]. It will be of interest to explore the possibility that there is a direct relationship between the production and viability of border cells and the sensitivity of plants to toxins in the soil.

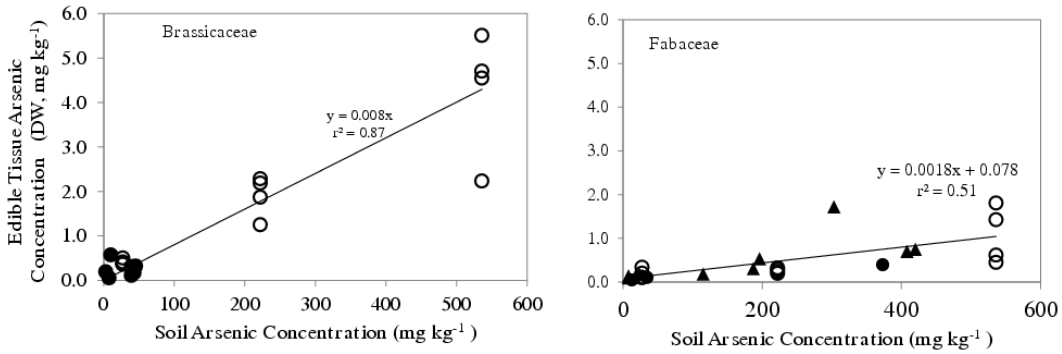

Figure 4. Arsenic concentration in the edible portion of Brassicaceae (**left**, no border cell production) and Fabaceae (**right**, 3000–4000 border cells produced per root per day) as a function of soil arsenic concentration. Values were compiled from reference [52]. Open symbols (○) represent vegetables grown in the greenhouse, closed symbols (●) represent vegetables grown in home gardens, and the closed triangles (▲) represent values from the literature.

6. Rhizofiltration *vs*. Rhizoprotection

Multiple research studies have focused on phytotechnologies (detection, degradation, removal or contaminant of soil, groundwater, surface water, sediments, or air), but these studies have suffered from the inability to show consistent, predictable removal of biological and chemical contaminants [55,56]. However, emerging research on extracellular trapping by border cells of plant roots (Table 1), highlights the potential for utilization of plants in bioremediation of contaminated water and soil and may help to explain variability with divergent species. "Rhizofiltration" is a category of phytoremediation that focuses on using plant root systems to remove contaminants from soil and water [57]. Rhizofiltration has been researched as a remediation tool for nearly fifty years, but despite continued efforts, use of this approach has been hampered by unexplained variability in uptake of pathogens and metals by plants and lack of efficacy in removal of contaminants [58–65]. The discovery that border cells trap metals suggests that plants have mechanisms to prevent uptake into plant tissue, while at the same time sequestering contaminants. Because contaminant removal models rely on kinetic constants based on root uptake, this recent finding could easily account for lack of agreement between modeled and measured plant "uptake".

Border cells naturally disperse into liquid and accumulate into a visible mass at the bottom of the vessel as new border cells are produced to replace the detached populations [1]. It will be of interest in future studies to test directly the amount of metals and other contaminants that are trapped by border cells in their role as "neutrophils" protecting the plant from danger [66,67], and to explore the use of this simple approach to remove hazardous chemicals from soil and water under diverse conditions. Considering the key role metals can play in the metabolism of microorganisms as well as plants and animals [16], such information may also yield new insights into potential relationships between metal trapping and microbial growth, development, and establishment of the rhizosphere "microbiome" [68,69]. Studies reporting variation in border cell production and properties among different species will be important tools for defining mechanisms and consequences of metal trapping [70–75].

7. Conclusions

Phytotechnologies may be used to prevent contaminant exposure and, in effect, be a tool for primary prevention in environmental public health [76]. Of particular importance will be studies to determine if the same mechanisms which have been implicated in metal trapping within roots also operate in border cell populations [77]. An improved understanding of border cell extracellular traps and their role in preventing toxin uptake may facilitate efforts to further utilize plants as a

nondestructive approach to reduce environmental threats. Data thus far indicate the promise of phytotechnologies, and border cell extracellular traps may be the key to take this remediation strategy to the next level.

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