https://doi.org/10.56093/ijas.v90i10.107884

Indian Journal of Agricultural Sciences **90** (10): 1850–5, October 2020/Review Article

Root border cells: A pioneer's of plant defence in rhizosphere

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Received: 16 April 2020; Accepted: 17 July 2020

ABSTRACT

The environment around the root in rhizosphere is a complex region where multiple interactions take place among soil, plant and microorganisms. Plant growth and architecture of entire plant, in fact depends upon the roots which provide sufficient amount of nutrients and water. Root tips while sensing the rhizosphere for availability nutrients counteract with array of harmful microorganisms in the soil. In order to protect from these stresses plant roots have evolved specialized cells known as root border cells which act as front line defence mechanism in rhizosphere. Border cells are individual or bunch of programmed viable cells released from root tip which forms protective sheath between root and external environment. Border cells secret hydrated mucilage that contains antimicrobial compounds and extracellular DNA which governs the behaviour of microbiome in the soil. Production of border cell is regulated by number of factors such as phytohormones, PME enzyme and transcription factor NLP7. Plant breeding and genetic engineering could be used to exploit border cells defence mechanism as a new avenue for disease control. This review summarizes about the importance, properties of border cells, mechanism, and regulation of border cells production and role of RETs as element of plant defence.

Key words: ExDNase, Plant defence, Rhizosphere, Root border cells, Root extracellular traps (RETs), Root exudates,

The rhizosphere of plant is a complex region where plant, soil and microbes are interacting with one another. Though several management practices have been developed to control the pathogen. The extensive use of synthetic fungicides is increasingly being restricted in order to reduce their risks to human health and environmental pollution (Darshan *et al*. 2018; Darshan *et al*. 2019).The plant root is persistently exposed to a large number of natural enemies inhabiting in this specialized region (Aggarwal *et al*. 2004). Root will change its growth pattern in response to various edaphic conditions (Fitter and Strickland 1992). Generally, approximately 5-21% of total fixed carbon is being transported into the rhizosphere through secretion of wide range of compounds as root exudates (Derrien *et al.* 2004). The root cap is well established as vital source of root exudate in which root border cells are diagnosed as a principal carbon component. In that around 98 percent of root exudates contains this specialized cell known as border cells and associated components (Griffin *et al*. 1976; Hamamoto *et al*. 2006). All these compounds are having profound effect on shaping the soil ecosystem and also influence the soil microbial communities in positive and negative manner. In order to overcome these negative interactions, plant roots employ the front-line defence by producing specialized cells called root border cells (BCs) and also subsequently produces antimicrobial compounds to avoid negative interaction. Each day, lot of unambiguously differentiated root BCs are synthesized and scheduled to release from the root tips (the vicinity at the apex lodging the root cap and apical meristem) of higher plants including cereals, pulses, cucurbits (Curlango-Rivera *et al.* 2013). Inspite they are firmly stick to the periphery of the root by a water-soluble matrix containing polysaccharide, the middle lamellae between the adjacent cells is dissolved by the array of pectolytic enzymes. The originated border cells give birth to the cell layers that gradually transformed through discrete stages before they disperse from the periphery of root cap. As per BCs definition they are not component of the root cap so it's not good to call it as root cap cells and they are originally called "sloughed root cap" cells as the term 'sloughed' means death and decay, but later Hawes (1983) significantly discovered that these cells from corn and oats root caps act like barriers to the host-specific toxins from *Cochliobolus heterostrophus* and *Cochliobolus victoriae* respectively (Hawes and wheeler 1982; 1984).

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This led to the origin of specialised living cells called root border cells. BCs are defined as "Programmed living cells detached from the root cap into the external environment as individual cells, small aggregates, or as a group of attached cells" (Hawes and Pueppke 1986; Hamamoto *et al*. 2006; Driouich *et al*. 2010). Interestingly, plant roots liberate a massive range of BCs into the rhizosphere which performs a key role in governing the root-microbe interactions and also form a boundary layer between the root surfaces and the soil environment (Jones *et al.* 2009; Aggarwal *et al*. 2007, 2014). These are physiologically viable cells that can sustain as far as they are supplemented with sufficient nutrients and are shielded from biotic and abiotic stress. This review summarizes the crucial information about the properties, production and regulation of the BCs and their role in plant defence.

Importance of root border cells in the rhizosphere

Whenever selection pressure exists on plants undoubtedly it has to use substantial amount of energy to carry out normal physiological processes, even though it releases large number of healthy cells from the root periphery in a regulated manner (Hawes *et al*. 1998; 2000). It suggests a critical role for these cells in the rhizosphere in response to biotic and abiotic environmental stimuli such as metal toxicity, heat shock and fungal challenges (Zhao *et al*. 2000a; Cannesan *et al.* 2011). The amount of BCs produced is positively correlated with the existence of mycorrhizal associations in the plant rhizosphere. Plant species with larger number of BCs have a higher mycorrhizal association and vice-versa (Niemira *et al*. 1996; Nagahashi and Doud, 2004). It is reported that BCs control the growth and gene expression in symbiotic bacteria. BCs secrete antimicrobial proteins, phytoalexins and pectinolytic enzymes within the extracellular matrix around the root cap (Wen *et al.* 2007; Aggarwal *et al*. 2007).

BCs can attract or repel pathogenic organisms like bacteria, fungus and nematodes. For example, root knot nematode *Meloidogyne incognita* the host specific chemotaxis to border cells yields aggregation of nematodes and no further infection and motility was observed and also there was long quiescence stage (Zhao *et al*. 2000b; Hubbard *et al.* 2005). Similarly, in case of instantaneous disclosure of pea roots to *Nectriahaemato coca* fungi leads to the formation of a mantle of mucilage compounds, BCs and hyphae that cover the root tip, results in drastic reduction in the population of pathogens in the soil (Gunawardena *et al.* 2005; Rogers *et al.* 2000). In case zoospores of *Pythium dissotocum* are favourably attracted to BCs by recognising chemical signals secreted by cotton root BCs or root tips (Goldberg *et al.* 1989).The conidia of *Fusarium solani f* sp. *pisi* showed fungistasis action when exposed to border cells of pea and resulted in no further fungal infection (Gunawardena and Hawes 2002). Recent studies has discovered that root BCs secrete exDNA along with various proteins to form RETs to trap the phytopathogens which act in similar way as the NETs 'neutrophil extracellular traps' in mammals published by Brinkmann *et al.* (2004).

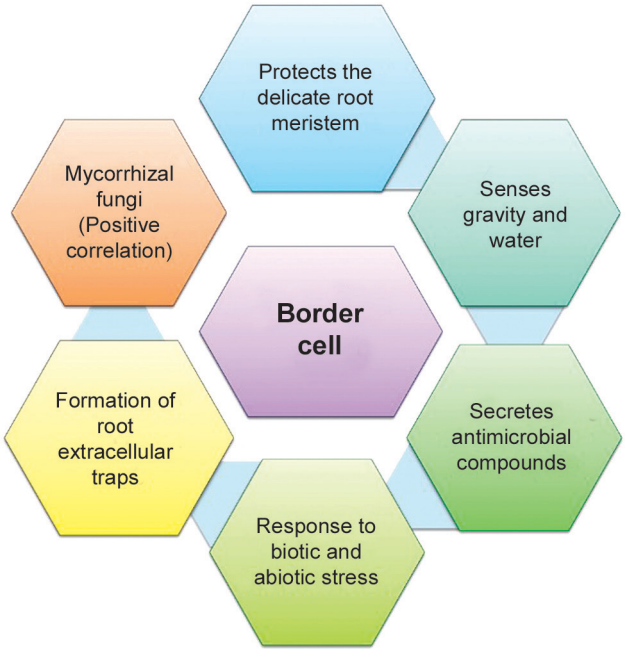

Fig 1 Importance of root border cells in the rhizosphere.

Properties of root border cells

- *BCs require water to disperse from the root tip*: In moisture deprivation, BCs remain adhere to the root periphery but disperse away from the root tip like an expulsion when immersed in water.The cells are entangled in a mucilage layers that can hold 1000 times of its weight in water (Bengough and McKenzie 1997).
- *Release of BCs primarily depends on pectin degrading enzyme complexes*: It consists of pectin methyl esterase, pectatelyase and polygalacturanse (Stephenson and Hawes 1994; Wen *et al.* 1999).
- *The detached BCs are metabolically active and also viable:* It could be revealed by a vital stain, fluorescein diacetate (Hawes and Brigham 1992).
- *These cells are distinct from normal root cells:* These cells display distinct traits which are not present in other normal cells, even not with their immediate progenitors (Brigham *et al*. 1995).
- *BCs are capable of undergoing cell division and dedifferentiation***:** The formation of callus in tissue culture medium amended with growth regulator or with root exudates indicates the totipotency nature of BCs. BCs originate from root cap meristematic cells; thus their production is positively correlated with rate of mitosis in the root cap meristem (Driouich *et al.* 2007).
- *The production of BCs in plants is preserved at the family level:* The specific number of BCs produced per day is determined by the genotype of the plant (Table 1).
- *BCs produce chemical signals recognized by microorganisms:* These chemical signals can act as decoys that attract dangerous soil-borne organisms and also it

Table 1 The number of BCs produced by different plant families

Family	Number of border cell produced in 24 h
Apiaceace	2300- 2500
Asteraceae	2000- 2500
Brassicaceae	1000- 1200
Cucurbitaceae	2000- 2500
Euphorbiaceae	1600-2700
Fabaceace	4000- 5000
Graminaceae	1500- 2000
Liliaceae	1000- 1500
Malvaceae	8000- 10000
Pinaceae	8000- 11000
Solanaceae	<100

provides the valid model to investigate infection process at the cellular level (Driouich *et al.* 2013; Wen *et al.* 2009).

- *BCs effectively respond to both biotic and abiotic stresses:* In case of biotic stress, there is development of mucilage layer that protects the plant roots from pathogen infection (Vicre *et al.* 2005). Similarly, it also responds to abiotic stress by directional changes in the movement of roots in response to metals like aluminium the roots began to grow away and thereby it protects the plant from toxicity (Zhao *et al*. 2000a; Wen *et al*. 2009; Hawes *et al*. 2012).

Mechanism of border cell production

Root BCs which were traditionally considered as dead cells, are now well established as living cell. As normal living cell, BCs also consists of numerous golgi stacks, mitochondria, and secretory vesicles (Hawes *et al*. 2000; Singh *et al.* 2018). During BCs production, cell division takes place in the meristematic cells of root cap that displaces cell tiers in the direction of the periphery of the root cap. Afterwards cell tiers exhibit morphologically and physiologically specialized functions in the columella region (Dolan *et al.* 1993; Wenzel and Rost 2001). As each tier sequentially separated, function of previous tier terminates with a concomitant switch in expression of related genes and later new functions are inaugurated in the differentiated cells (Rogers *et al.* 2000).The excision of BCs from the root cap by action of rain or irrigation water begins the production of a new set of BCs.The number of root cap remains constant in size; hence cell division should occur within the meristematic tissue to replace the departed cells. After removal of border cells there is significant change in mRNAs level of starch synthase, starch branching enzyme, pectin methyl-esterase and histone.

Intact BCs need to be separated from root tip. Their separation process involves the action of hydrolytic enzymes and corresponding pectolytic enzyme on the middle lamella (Fischer and Bennet 1991; Hadfield and Bennet 1998). Wen *et al.* (1999) showed the positive correlation between border cells development and root cap localised PME activity. Cell walls of BCs contain significant amounts of pectic polysaccharides, PMEs which catalyses pectin dimethyl esterification which helps in BC separation (Micheli 2001). In the study conducted in pea root caps, it was confirmed that PME activity and concentration of soluble de-esterified pectin's dramatically increases in the cap during separation of BCs (Hawes and Brigham 1992). Similar results were found in the root caps of sunflower (*Helianthus annuus*), alfalfa (*Medicago sativa*) and corn (*Zea mays*) plants (Stephenson and Hawes 1994). More interestingly, it was reported that PME gene knockdowns blocks the separation of BCs in transgenic pea (Wen *et al.* 1999). In these transformed plants, instead, BCs assembled at the root tip as clusters and unable to disassociate from the cap and disperse extracellularly.

Regulation of border cell production

Border cell production switch can be geared on and off independently of root growth and development (Iijima *et al*. 2003; 2004). Mitosis in the root cap meristem (but not the apical meristem) is initiated in response to the excision of border cells within 15 min. and followed by activation of genes throughout the cap. The regulation of cell division border cells production is specifically localized to the root cap meristem. During root cap development array of genes are involved and for example *rcpme1*, a gene encoding PME which is required for border cell separation, is expressed tissue-specifically in peripheral root cap cells (Hawes *et al.* 2000). In transgenic plants with silenced *rcpme1* gene, border cell separation is inhibited and accumulates in a form of clump and do not disperse into water like normal border cells. The regulation of border cell production will take place by targeting the mitotic events in the root cap meristem. When once the border cell reaches threshold level it blocks the cell division by producing special signal compounds called Factor B repressor. That's why the production of border cell is self-limiting events (Wen *et al.* 1999).

Upon border cell removal there is drastic changes in gene expression throughout the cap. Karve *et al.* (2016) reported that transcription factor *Nin-Like Protein 7* (*NLP7*), which maintains the levels of cellulose and pectin, is needed for the proper release of BCs in Arabidopsis. Two genes *psugt1* and *rcpme1* are involved in cell division, cell separation at the cap periphery and play an important role in border cell development (Hawes *et al.* 1998). Expression of *rcpme1* in the root cap is correlated with BCs separation in pea root development as observed by Wen *et al*. (1999). The previous studies have established that the external environmental signals can override the control of BCs development using endogenous signals (Zhao *et al.* 2000a). For example, when pea is exposed to higher CO_2 levels, there is two-fold increase in the number of BCs production than normal conditions.

Influence of phytohormones in release of BCs

The release of BCs is mainly regulated by the

phytohormones such as auxin and ethylene, which also control root cap size, shape and differentiation (Ponce *et al*. 2005; Gilroy and Jones 2000). Pre-incubation of root with auxin significantly increases the shedding of root BCs. In contrast, BCs release is significantly reduced when roots are treated to the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (Driouich *et al.* 2007). Thus, BCs detachment appears to be regulated by complex interactions between ethylene and auxin that controls the cell differentiation in the root cap. At present the detail of molecular perception and action mechanism of these phytohormones is not known and needs deep investigation in near future.

Root extracellular traps (RETs) as element of plant defense

Recent studies have highlighted the role of extracellular factors in regulating mammalian defense (Braun and Whallon 1954; Rovira 1956; Streitfeld *et al*. 1962;Tran *et al.* 2016). These studies reveal that cells including eosinophils, neutrophils and mast cells forms 'neutrophils extracellular traps' (NETs) comprising histones (H4), exDNA and antimicrobial proteins (Brinkmann *et al*. 2004; Wang *et al.* 2015). The pathogens get entrapped and killed when exposed to these specialized complexes called NETs. When the exDNA component of NETs is depleted, trapping is inhibited and resistance level is gradually reduced. Analogously in plants, exDNA is necessary component for executing defense at root tip by forming RETs (Cannesan *et al*. 2011; Hawes *et al.* 1998; 2012). Synthesis and export of exDNA from the root tip has been confirmed by series of direct test. It is revealed that when endonuclease DNase 1 degrade the exDNA, root tip become highly susceptible to fungal infection; however, when extracellular DNA of RETs is destroyed slowly by the exonuclease BAL31, thereby it delays the loss of resistance to fungal infection (Wen *et al*. 2007; 2009). Therefore, results indicate that root border cells may have function similar to that occurs in mammalian cells. Mutation in exDNases encoding genes of microorganisms result in the loss of virulence of the pathogen and gives the idea that exDNases are virulence factors that opens a new route for designing disease management strategies. Holloman and Holliday (1973) first time reported the production of nuclease enzyme by a fungal pathogen *Ustilago maydis* inciting smut of maize. Subsequently series of study reported the production of exDNases by number of fungal pathogens such as *Helminthosporium solani, Alternaria limicola, Phytophthora infestans, Ascochita rabei, Colletotrichum coccode, Pythium irregulare, Gaeumannomyces graminis* and *Puccinia striiformis* (Curlango-Rivera *et al*. 2013; Hadwiger *et al.* 2013). But not much information is available on the extracellular nucleases secreted by phyto-pathogenic bacteria. The exDNases *NucM* is produced by *Dickeyada dantii* which causes soft-rot in tuber crops (Moulard *et al.* 1993; Sumby *et al.* 2005). Hawes *et al.* (2012) reported the trapping of *Agrobacterium tumefaciens* and *Ralstonia solanacearum* in extracellular environment of pea root by the border cells. The pattern of corn border cell production is significantly altered when exposed to wild type (WT) of *R. solanacearum* compared with mutants *(ΔnucA, ΔnucB, and ΔnucA/B* nuclease). The presence of immobilized layer of bacteria at the edge of the BCs trap indicates the evidence of trapping (Tran *et al.* 2016).

Conclusion and future prospective

It is vitally important to maintain the healthy root in the rhizosphere for proper plant growth, development and defences (Jones *et al*. 2009; Larkin 2015; Aggarwal et al. 2011, 2014). Border cells and border-like cells are launched from the root tip as discrete cells and small aggregates, or as a bunch of connected cells (Hawes *et al.* 2012). Border cells are viable element of the root system that regulates the root interplay with dwelling microbes of the rhizosphere. As they release from root tip, the cells begin to produce and secrete hydrated mucilage extracellularly that consists of secondary metabolites, polysaccharides, extracellular DNA (exDNA) and antimicrobial proteins (Huskey 2013). The further study of root border cells defense might decipher novel defense strategies that can be utilised to the tissues which are not furnished with their own cellular 'goalies'. It would not be possible to program different tissues to produce and separate the populations of border cells. However, biotechnological approach could be utilized to exploit the extracellular and cellular mechanisms by which border cells counter balance the dangerous molecules or pathogens and express them in tissues that are not equipped with BCs. Resistance against the pathogens present in rhizosphere could be achieved via conventional plant breeding techniques by selecting for novel root architect that efficiently produce the BCs (Singh *et al.* 2019; Singh *et al.* 2020). On the other hand, genetically engineering the rhizospheric microorganisms that secrete extracellular nuclease inhibitors might provide a completely unique sort of biocontrol for soil borne pathogens (Biswas *et al*. 2000; Ahammed *et al*. 2008a, 2012). The identification of genes sequences that encode Ex DNAases in pathogens by advanced genomic tools and quenching their expression can provide a novel way for effective disease management of soil borne pathogens.

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