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The Role of Seed-Vectored Endophytes in Seedling Development and Establishment

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Abstract

Seed-vectored endophytes internally colonize plant seeds and remain without eliciting disease symptoms. Microbes are carried by seeds generation after generation to benefit the host during and following seed germination. Seed-vectored endophytes have been poorly investigated and research targeted at understanding their biology will have immense applications in agriculture and horticulture. Some reports are available on the roles of seed endophytes in plant growth promotion via nutrient acquisition and biocontrol of soil borne diseases, but mechanisms of interaction at the endophyte-host interface, especially during seed germination and seedling establishment, have not been explored adequately. The present paper is intended to review the role of seed vectored endophytes in seed germination, seedlings development and in developing the rhizosphere community. The mechanisms of interaction and movement of seed inhabiting endophytic bacteria are explained with the help of pictorial models.

Key words: Seed endophytes, Seedling development, Rhizophagy cycle, Bio-control, Plant growth

Introduction

All plants live in close association with microbes or they have been evolved with microorganisms present in their surroundings. These microbes, whether living on surface of plant roots, stems or leaves, or

within their tissues, together constitute the 'plant microbiome' (Hardoim et al. 2015). Endophytes are microbes living within plant tissues during part, or all, of their life cycles without causing obvious negative impacts to the host plant (Wilson 1995). These microbes exist in a balanced state of symbiosis, although a beneficial symbiosis (mutualism), may turn in to commensalism or parasitism depending on circumstances (e.g., under nutrient stress). Some endophytes are perhaps just neutral inhabitants of plants. Microbial endophytes have been isolated from all plants examined to date. The endophytes, including bacteria, fungi, actinomycetes, algae or protozoa, are important for their roles in improving fitness of plants. Bacteria living inside plant tissues as endophytes can be acquired horizontally (from the environment with each new generation), or vertically transmitted from generation to generation via seed.. Research has shown that endophytes cause phenotypic changes (in terms of growth, development, and production) of several crops (Rosenblueth and Martínez-Romero 2006; Gond et al. 2015b, White et al. 2015). Endophytic bacteria may colonise plant tissues, intracellularly or intercellularly (White et al. 2018a). Although endophytes may be isolated from all parts of plants, endophytes of seeds have developmental and evolutionary significance because seeds include the next generation of plant, and it is logical that seeds would be invested with all resources (including endophytes) needed to grow and compete. Some endophytes that tightly associate with seeds could have evolved to interact with ovaries and/or seedlings to modulate fruit and/or seeding development (Johnston-Monje and Raizada 2011; Gond et al. 2015b; Herrera et al. 2016). These seed endophytes are likely transmitted through successive generations and may be important endophytes in mature plants (Kaga et al 2009; Johnston-Monje and Raizada 2011; Hardoim et al. 2012).. Most reviews available on seed endophytes, focus on their diversity and distribution, while their developmental effects and functional roles have been largely unexamined (Rosenblueth and Martínez-Romero 2006; Ryan et al. 2008). In the present review, we will focus on seed-associated bacterial endophytes and their roles in seed germination, seedling development and establishment of the rhizospheric community with reference to nutrient acquisition and biocontrol of diseases.

Seed germination and endophytes

Seed germination comprises a series of events, including uptake of water by dry seeds (imbibition), rupture or softening of the seed coat, and emergence of the embryonic axes (root radical and/or coleoptiles) (Bewley and Black 1994; Bewley 1997).. Endophytic microbes have been isolated from surface-sterilized seeds of many plants, including rice, wheat, cotton, corn, tomato etc. (Gond et al. 2015a; White et al. 2015; Irizarry and White 2017; Verma et al. 2017, 2018; Verma and White 2018). This suggests that endophytic microbes live

protected within seeds with the dormant embryo or otherwise microbes are tightly associated with seeds and difficult to remove. Whatever the association, perhaps the microbes are important in facilitating development and establishment of seedlings.

Seeds are genetically and physiologically complete to develop into new plants under favorable conditions. A seed contains embryo and nutrient resources, often including a reserve of food in the form of endosperm. Some plant groups (e.g., orchids) do not have a reserve of food for the embryo. Previous findings have shown that endo-mycorrhizal fungi increase seed germination, and improve development in orchids; indeed this mutualistic association is necessary for successful germination of seeds (Masuhara and Katsuya 1989; Stewart and Zettler 2002; Rasmussen *et al.* 2015). Endo-mycorrhizal fungi supplement, carbohydrates, essential nutrients, and especially phosphate to germinating orchid seeds through the process of mycotrophy. Later, the fungus colonises roots and protocorms of orchids. *In vitro* studies have shown that symbiotic germination of orchid seeds requires a specific mycobiont association (Taylor and Bruns 1997; Batty *et al.* 2006; McCormick *et al.* 2006; Stewart and Kane 2006).

Systemic seed borne fungal endophytes may accelerate the seed germination of grasses (Clay 1987, Wali *et al.* 2009). In a study of fungal endophyte effects on seed biology, Clay (1987) demonstrated that seeds of perennial ryegrass and tall fescue infected with fungal *Epichloë* endophytes exhibited higher germination rates; also finding that infected seeds with endophytes produced significantly more biomass in both grass species. Wali *et al.* (2009), in a study of fine fescues, found that presence of endophytic fungus *Epichloë festucae* in seeds hastened seed germination; however this effect depended on habitat and germ line of fescue seeds. Gundel *et al.* (2006) in a study found that germination rate in annual ryegrass was not influenced by fungal *Epichloë* endophyte infection. Similarly, in an examination of several cool-season grasses Afkhami and Rudgers (2008) reported that *Epichloë* endophyte infection did not consistently enhance seed germination, although they did find many clear cases where endophytes presence increased germination. Shearin *et al.* (2017), in the study of *Phragmites australis* seeds and fungal endophytes, found that seed borne endophytes promoted seed germination. They have also found that colonisation of endophyte enhanced seedling growth in *Phragmites australis*. Hubbard *et al.* (2012) found that wheat seed germinated better under heat and drought stress when colonised by fungal endophytes (Hubbard *et al.* 2012); further seedlings infected with endophytes demonstrated fitness parameters similar to unstressed seedlings.

Although researchers have hypothesized that seed-borne endophytic bacteria move from seed to other plant parts during plant development, very little is actually known about how these bacteria colonise the seed tissues themselves during maturation of seeds (Truyens et al. 2015; Pitzschke 2016; Cope-Selby et al. 2016). In one study, it was found that endophytic colonisation of *Bacillus* in seeds induces faster germination, and further improves tolerance to stress in quinoa (*Chenopodium quinoa*) (Pitzschke 2016). Because it is often difficult to obtain endophyte-free seeds in many plants, it is difficult to evaluate the direct effects of endophytes on seed germination. Use of antibiotics to clean seeds may be successful to some extent, but it is still hard to be certain that seedlings are free of all endophytes. In a study of seeds of several plant species, including rice and soybean, Holland (2016) found that curing of seed of their symbiotic bacteria, PPFMs (Pink-Pigmented Facultatively Methylophilic bacteria), suppressed the percentage of germinating seeds, as well as the speed of germination. In experiments on effects of PPFMs on seedlings, Holland (2016) used old and new seeds of several plant species *Arabidopsis*, rice, soybean, and kidney bean, and found that bacterization of seeds improved ‘shelf life’ of seeds. Puente et al. (2009) tried several antibiotics including chloramphenicol, streptomycin sulfate, tetracycline, and penicillin with variable dosages to make bacteria-free cactus seeds. In other experiments we used streptomycin sulfate to clean rice and millet seeds (Verma et al. 2017, 2018) with some successful results. We found that removing microbes from seeds, slows the seed germination process in rice and millet (Verma and White 2018). Some bacteria in seeds may be unculturable, and it is unclear how they function in seeds because experiments are difficult to conduct without isolated microbes.

Emergence of the embryo in the process of germination is accompanied by cell expansion with loosening of cell walls and oxidative burst (Gimeno-Gilles et al. 2009; Pitzschke 2016). Generation of reactive oxygen species (ROS) is important for regulation of endophytic microbes during the germination process (Lamb and Dixon 1997; Huckelhoven and Kogel 2003). Pitzschke (2016) suggested that catalase and superoxide dismutase production by endophytes change the redox status of germinating seeds.

We hypothesize that presence of endophytic bacteria modulates the level of ROS at the time of germination. Some bacterial endophytes are also known to produce phyto-hormones. Auxin (IAA) is known for its role in cell wall loosening. IAA is also known to stimulate ROS production (Morre et al. 1995; Krishnamurthy and Rathinasabapathi 2013; Ivanchenko et al. 2013). IAA directly, or with ROS or other mediator molecules, may be involved in physiological interactions during seed germination (Gomes and Garcia 2013; Chen et al. 2014). Plant cell growth requires solubilization and mobilisation of stored food from endosperm to the embryo axes

(shoot and root). ROS roles have been recognised in weakening of endosperm and mobilisation of food reserve during seedgermination (El-Maarouf-Bouteau and Bailly 2008; Gomes and Garcia 2013). The exact roles of indigenous endophytes in nutrient mobilization from the seed endosperm during germination are unknown, but it is conceivable that endophytes could interact with seedlings to facilitate nutrient mobilization from endosperm to embryo. Although, many investigators are evaluating growth promotional effects of bacteria from diverse substrates (e.g., soils) on seeds of many agriculturally important crops, very little work is presently focused on elucidating the roles of indigenous bacterial endophytes of seeds.

Seedling growth and development of the rhizospheric community

In most plants, initial seedling growth and establishment is supported by metabolites hydrolysed from proteins, carbohydrates and oils stored in seeds. Endophytic microbes, native bacteria or fungi, from seeds have been reported to promote seedling growth (Nejad and Johnson 2000; Puente et al. 2009; Hardoim *et al.*, 2012; Verma and White 2018). Even bacteria from other sources or from wild relatives if compatible with a host may promote seedling growth (Gond et al. 2015a; Irizarry and White 2018). We found that seed endophytic bacteria from *Leersia*, a wild relative of rice, significantly promoted seedling growth in rice *Oryza sativa* (Verma et al. 2018). Indigenous endophytes of seeds have an advantage over most soil-borne microbes in colonization of seedling roots and shoots during seedling growth (Hardoim et al. 2012; Truyens et al. 2015). Vertical transmission of bacteria through successive generations via seed transmission has been observed by many investigators (Mukhopadhyay et al. 1996; Johnston-Monje and Raizada 2011; Gagne-Bourgue et al. 2013).

Seed-originated endophytes internally colonise seedlings, multiply on the surface of roots, and move into soil, developing in rhizospheric microbial communities (Truyens et al. 2015; López-López et al. 2010). This was demonstrated in one experiment using millet, where we found that three of four seed-endophytic bacteria, including *Curtobacterium* sp. (M1), *Microbacterium* sp.(M2) and *Bacillus amyloliquefaciens* (M4) colonized the rhizosphere and could be re-isolated from rhizospheric soil after 15 days; controls using axenic seeds grown in sterile potting mix did not result in rhizosphere colonization when sampled after 15 days (Verma and White 2018). Other investigations have shown the common presence of endophytic microbes in plants and their movement into soils (Compant et al. 2010; Santoyo et. al 2016). Growth promotion in seedlings may be attributed to microbe-produced plant hormones (Holland 2016; Verma et al. 2018) or to nutrient mobilization or to changing redox conditions of seedlings (White et al. 2018a). ROS are important signal molecules in plants, modulating plant growth and development (Foreman et al. 2003; Krishnamurthy and Thinasabapathi 2013;

Ivanchenko et al. 2013). Movements of seed inhabiting endophytic bacteria during germination and establishment of seedlings are divided into three types (figure 1). Seed endobacteria exit seedlings during germination and become a functional part of the rhizosphere community; bacteria attracted to root exudates around the root meristem tip and are internalized into root cells where they appear to be degraded in time to provide nutrition to roots. Paungfoo-Lonhienne et al. (2013) denominated this microbe internalization and degradation process which they denominated 'rhizophagy' to denote that roots appeared to be 'eating' microbes. The third movement of microbes is to exit root cells primarily from tips of elongating root hairs and return to the rhizosphere to acquire more nutrients. All these movements are primarily responsible for microbe mediated nutrient acquisition discussed in next section.

Microbe-mediated nutrient acquisition

Seedling-associated microbes (often bacteria) assist seedlings with acquisition of soil nutrients (White et al., 2015; Irizarry and White 2018). Nutrients supplied by microbes consist of two components as follows: 1) nutrients solubilized by microbes in the rhizosphere and absorbed into the root hairs; and 2) nutrients oxidatively-extracted from endophytic microbes in root cells in the rhizophagy cycle (Verma and White 2018; Paungfoo-Lonhienne et al., 2013; White et al. 2018a). Many endophytes have been found to promote plant growth through solubilization of rhizosphere nutrients, including nitrogen, phosphate, potassium, etc. (Verma et al., 2017, 2018). This is a process that is familiar to most biologists; where nutrient-solubilizing microbes mobilize nutrients in the rhizosphere, making them available for absorption by the plant roots (Paungfoo-Lonhienne et al. 2013; Soares et al. 2016; White et al. 2018; Verma et al. 2017).

In the rhizophagy process, microbes enter into root cells where they may be fully- or partially-degraded (Paungfoo-Lonhienne et al., 2010; Verma and White 2018; White et al. 2018d). In several studies, we have shown that seed-transmitted bacterial endophytes are not entirely degraded, instead these microbes alternate between a free-living soil phase and an intracellular/endophytic phase (White et al. 2018d; Verma and White, 2018; Verma et al., 2017). Plants cultivate microbes externally in root exudates (consisting of carbohydrates, vitamins, amino acids, organic acids) around the root tip meristem. Microbe internalization (perhaps by endocytosis) into root cells occurs primarily just beneath the root exudate zone around the root tip meristem (Beltran-Garcia et al., 2014; Paungfoo-Lonhienne et al., 2010; Prieto et al., 2017). Microbes are observed to retain cell walls and cell shapes (i.e., rods, tetrads, etc.) initially after internalization into root cells (White et al. 2018c). In the periplasmic space of root cells, microbes are subjected to reactive oxygen (primarily superoxide)

secreted onto them by root cell plasma-membrane bound NADH oxidase enzymes (White et al., 2018c,d).. It is likely that this exposure to reactive oxygen is the event that causes bacteria to lose cell walls and form spherical protoplast stages (called L-forms) (Verma and White 2018). Reactive oxygen, readily visualizable around intracellular bacterial protoplasts stained using reactive oxygen stain diaminobenzidine (White et al., 2014; Verma and White 2018; White et al. 2018b), functions in the extraction of nutrients from microbes (White et al. 2012, 2015, 2018d; Verma and White 2018). Reactive oxygen damages microbe plasma membranes and induces leakage of nutrients and electrolytes through microbe plasma membranes, resulting in liberation of nutrients that may be absorbed into root cells. Bacterial plasma membranes are vulnerable to plant-secreted reactive oxygen because bacterial membranes do not contain sterols and reactive oxygen readily penetrates through the bacterial membranes; plant cell plasma membranes are reinforced with sterols that reduce penetration of plant plasma membranes by the reactive oxygen, and protect the plant cell contents from oxidation (White et al. 2018c). Intracellular bacteria in the epidermal layer of roots, trigger root hair formation, and bacteria exit root cells through the tips of elongating root hairs, reforming bacterial cell walls as they emerge into the rhizosphere (Verma et al., 2017; White et al. 2018b, 2018c, 2018d).

Because degradation of microbes within plant root cells has been repeatedly observed (Paungfoo-Lonhienne et al., 2013; Verma et al., 2017; White et al. 2018b, 2018d), it is logical to conclude that the function of the rhizophagy process is nutritional. Work is still being done to determine the precise nutrients that plants extract from the oxidation of microbes within roots, although nitrogen transference has been previously demonstrated through nitrogen tracking experiments (Hill et al., 2011; Beltran-Garcia et al., 2014). The microbes that cycle between the soil and plant roots carry nutrients (macro- and micro-nutrients) from the soil into plant roots where nutrients are oxidatively extracted from them. Microbes involved in the rhizophagy process form a nutrient-mobilizing cycle (or ‘rhizophagy cycle’) that continuously functions to transport nutrients from soil to plant (Figure 1).

Endophyte role as bio-control agent during seedling development

During seed germination, seedlings are prone to be infected by soil borne pathogens. Seed-vectored endophytic bacteria act as competent bio-control agents during seedling formation (Bacilio-Jimeñez 2001; White et al. 2018a; Verma et al. 2017). Seed endophytes protect seedlings from biological enemies either directly through production of antimicrobial molecules or via stimulation of plant defense responses (Khalaf and Raizada 2018; Gond et al.2015a). Major soil-borne pathogens, including *Rhizoctonia solani*, *Fusarium* spp.,

Alternaria spp., *Phytophthora* spp., and *Pythium* spp., infect a wide range of economically important plants (Khalaf and Raizada 2018). In *Phragmites* seedling development, it was found that pseudomonad seed endophytes significantly reduced damping off disease caused by *Fusarium oxysporum* (White et al. 2018a). We also found that presence of seed-vectored endophytic bacteria, including *Enterobacter* sp., *Pseudomonas* sp., and *Pantoea* spp., reduced infection of rice seedlings by *Fusarium oxysporum* (Verma et al. 2017, 2018). Recently in the study of cucurbit seed endophytes, Khalaf and Raizada (2018) found that bacterial endophytes, including *Bacillus* spp., protected cucurbit plants from several soil borne pathogens as well as powdery mildew disease caused by *Podosphaera fuliginea*. Further, these authors showed that antifungal activity of bacterial seed endophytes was due in part to production of volatiles, which are known to induce plant defenses, including defense-related proteins (PR proteins). According to recent studies (Truyens et al., 2015; Khalaf and Raizada 2018), among the seed endophytes with biocontrol activities are *Bacillus* spp., *Pseudomonas* spp., *Pantoea* spp. and *Enterobacter* spp. Being endemic in seeds and seedlings, beneficial endophytic bacteria in seeds may also colonize developing plants and become part of the plant microbiome, increasing nutrient supplies and improving defense functions of host plants (Hardoim et al. 2012; Cope-Selby et al. 2016). Mechanisms of defense induction provided by endophytes to hosts depends upon endophyte attributes, e.g., induction of ROS in hosts, synthesis of antimicrobial metabolites, synthesis of lipopeptides, plant hormone production, induction of PR protein or host defense molecule synthesis (Gond et al. 2015a; Herrera et al. 2016; Khalaf and Raizada 2018) (Figure 2). Bacterial seed endophytes are relatively unexplored, but are gaining attention as a promising source of biofertilizers and biocontrol agents (Truyens et al., 2015; Khalaf and Raizada, 2018).

Conclusions

Though relatively unexploited, research on seed endophytes is gaining momentum because unlike other useful plant microbes, seed endophytes are more compatible with the host, and transmitted vertically through successive generations. Seed endophytes may be used to create seed inoculants that may function as biofertilizers and biopesticides. Although to date we have fragmentary reports, seed endophytes also function in the rhizosphere. Seed endophytes may function to continually mobilize nutrients from soil to the roots. Presence of these endophytes in seeds is important in modulation of plant development, and defense from pathogens. Some relevant questions that need to be answered with future research include: 1) Do all plant seeds vector endophytes that are important in modulation of seedling development? 2) What are the precise signals that pass

between endophytes and plants following germination? 3) How do seed endophytes improve tolerance of host plants to biotic and abiotic stresses?

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References

Afkhami ME, Rudgers JA (2008) Symbiosis lost: imperfect vertical transmission of fungal endophytes in grasses. *Am Nat* 172:405–416

Bacilio-Jimeñez M, Aguilar-Flores S, del Valle MV, Peñez A, Zepeda A, Zenteno E (2001) Endophytic bacteria in rice seeds inhibit early colonization of roots by *Azospirillum brasilense*. *Soil Biol Biochem* 33: 167-172

Batty AL, Brundrett MC, Dixon KW, Sivasithamparam K (2006) In situ symbiotic seed germination and propagation of terrestrial orchid seedlings for establishment at field sites. *Austr J Bot* 54: 375–381

Beltrán-García MJ, White JF, Prado FM, Prieto KR, Yamaguchi LF, Torres MS, Kato MJ, Medeiros MHG, Di Mascio P (2014) Nitrogen acquisition in *Agave tequilana* from degradation of endophytic bacteria. *Scientific reports* 4: 6938

Bewley JD, Black M (1994) *Seeds: Physiology of Development and Germination*. (New York: Plenum Press).

Bewley JD (1997) Seed Germination and Dormancy. *Plant Cell* 9: 1055-1066

Chen C, Twito S, Miller G (2014) New cross talk between ROS, ABA and auxin controlling seed maturation and germination unraveled in APX6 deficient Arabidopsis seeds. *Plant Signal Behav.* 2014; 9(12): e976489

Clay K (1987) Effects of fungal endophytes on the seed and seedling biology of *Lolium perenne* and *Festuca arundinacea*. *Oecologia* 73:358-362

- Compant S, Clement C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol Biochem* 42: 669–678
- Cope-Selby N, Cookson A, Squance M, Donnison I, Flavell R, Farrar K (2016) Endophytic bacteria in *Miscanthus* seed: implications for germination, vertical inheritance of endophytes, plant evolution and breeding. *GCB Bioenergy* 9: 57-77
- El-Maarouf-Bouteau H, Bailly C (2008) Oxidative signaling in seed germination and dormancy. *Plant Signal. Behav.* 3: 175–182.
- Foreman J, Demidchik V, Bothwell JH, Mylona P, Miedema H, Torres MA, Linstead P, Costa S, Brownlee C, Jones JD, Davies JM (2003) Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 422: 442–446
- Gagne-Bourgue F, Aliferis KA, Seguin P, Rani M, Samson R, Jabaji S (2013) Isolation and characterization of indigenous endophytic bacteria associated with leaves of switchgrass (*Panicum virgatum* L.) cultivars. *J Appl Microbiol* 114: 836–853
- Gimeno-Gilles C, Lelièvre E, Viau L, Malik-Ghulam M, Ricoult C, Niebel A, Leduc N, Limami AM (2009) ABA-mediated inhibition of germination is related to the inhibition of genes encoding cell-wall biosynthetic and architecture: modifying enzymes and structural proteins in *Medicago truncatula* embryo axis. *Mol Plant* 2: 108–119
- Gomes M P, Garcia Q S (2013) Reactive oxygen species and seed germination. *Biologia* 68(3): 351—357
- Gond SK, Bergen MS, Torres MS, White JF (2015a) Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. *Microbiol Res* 172: 79–87
Gond SK, Torres MS, Bergen MS, Helse Z, White JF Jr (2015b) Induction of salt tolerance and up-regulation of aquaporin genes in tropical corn by rhizobacterium *Pantoea agglomerans*. *Lett App Microbiol* 60: 392-399
- Gundel PE, Maseda PH, Ghersa CM, Benech-Arnold RL (2006) Effects of the Neotyphodium endophyte fungus on dormancy and germination rate of *Lolium multiflorum* seeds. *Austral Ecol* 31:767–775

Hardoim PR (2015) Heading to the origins – rice microbiome as functional extension of the host. *J Rice Res* 3: 133

Hardoim PR, Hardoim CCP, van Overbeek LS, van Elsas JD (2012) Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS ONE* 7: e30438

Herrera SD, Grossi C, Zawoznik M, Groppa MD (2016) Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. *Microbiol Res* 186: 37-43

Hill PW, Quilliam RS, DeLuca TH, Farrar J, Farrell M, et al. (2011) Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. *PLoS ONE* 6(4): e19220. doi:10.1371/journal.pone.0019220

Holland, M. A. (2016) Probiotics for Plants? What the PPFMs Told Us and Some Ideas about How to Use Them. Washington Academy of Sciences. *J Wash Acad Sci* 102: 31

Hubbard M, Germida J, Vujanovic V (2012) Fungal endophytes improve wheat seed germination under heat and drought stress. *Botany* 90: 137–149

Huckelhoven R, Kogel KH (2003) Reactive oxygen intermediates in plant-microbe interactions: Who is who in powdery mildew resistance? *Planta* 216: 891–902

Irizarry I, White JF (2017) Application of bacteria from non-cultivated plants to promote growth, alter root architecture and alleviate salt stress of cotton. *J Appl Microbiol* 122: 1110-1120

Irizarry I, White JF (2018) *Bacillus amyloliquefaciens* alters gene expression, ROS production, and lignin synthesis in cotton seedling roots. *J Applied Microbiology* 124: 1589-1603

Ivanchenko MG, De'sire'e den O, Monhausen GB, Dubrovsky JG, Bednarova A, Krishnan N (2013) Auxin increases the hydrogen peroxide (H₂O₂) concentration in tomato (*Solanum lycopersicum*) root tips while inhibiting root growth. *Ann Bot* 112: 1107-1116

Johnston-Monje D, Raizada MN (2011) Conservation and diversity of seed associated endophytes in *Zea* across boundaries of evolution, ethnography and ecology. *PLoS ONE* 6: e20396

Kaga H, Mano H, Tanaka F, Watanabe A, Kaneko S, Morisaki H (2009) Rice seeds as sources of endophytic bacteria. *Microbes Environ* 24: 154-62

- Khalaf EM, Raizada MN (2018) Bacterial Seed Endophytes of Domesticated Cucurbits Antagonize Fungal and Oomycete Pathogens Including Powdery Mildew. *Front Microbiol* 9: 42
- Krishnamurthy A, Rathinasabapathi B (2013) Oxidative stress tolerance in plants: Novel interplay between auxin and reactive oxygen species signaling. *Plant Signal Behav* 8: 10
- Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. *Annu Rev Plant Physiol Plant Mol Biol* 48: 251–275
- López-López A, Rogel MA, Ormeno-Orillo E, Martínez-Romero J, Martínez-Romero E (2010) *Phaseolus vulgaris* seed-borne endophytic community with novel bacterial species such as *Rhizobium endophyticum* sp nov. *Syst Appl Microbiol* 33: 322–327
- Masuhara G, Katsuya K (1989) Effects of mycorrhizal fungi on seed germination and early growth of three Japanese terrestrial orchids. *Sci Hortic* 37: 331-337
- McCormick MK, Whigham DF, Sloan D, O'Malley K, Hodkinson B (2006) Orchid–fungus fidelity: a marriage meant to last? *Ecol* 87: 903–911
- Morre DJ, Brightman AO, Hidalgo A, Navas P (1995) Selective inhibition of auxin-stimulated NADH oxidase activity and elongation growth of soybean hypocotyls by thiol reagents. *Plant Physiol* 107: 1285- 1291
- Mukhopadhyay K, Garrison NK, Hinton DM, Bacon CW, Khush GS, Peck HD, Data N (1996) Identification and characterization of bacterial endophytes of rice. *Mycopathologia* 134: 151–159
- Nejad P, Johnson PA (2000) Endophytic Bacteria Induce Growth Promotion and Wilt Disease Suppression in Oilseed Rape and Tomato. *Biological Control* 18: 208–215
- Paungfoo-Lonhienne C, Rentsch D, Robatzrk S, Webb RI, Sagulenko E, Nasholm T, Schmidt S, Lonhienne TGA (2010) Turning the table: plants consume microbes as a source of nutrients. *PLOS ONE* 5(7): e11915. doi: 10:1371/journal.pone.0011915
- Paungfoo-Lonhienne, C, Schmidt S, Webb R, Lonhienne T (2013) Rhizophagy- A new dimension of plant-microbe interactions, in de Briujn, F.J. (Ed.) *Mol Microbial Ecol Rhizosphere* 1: 1199-1207
- Pitzschke A (2016) Developmental Peculiarities and Seed-Borne Endophytes in *Quinoa*: *Omnipresent*, Robust *Bacilli* Contribute to Plant Fitness. *Front Microbiol* 7: 2

Prieto KR, Echaide-Aquino F, Huerta-Robles A, Valerio HP, Macedo-Raygoza G, Prado FM, Medeiros M, Brito HF, da Silva I, Felinto MCF, White JF, Di Masci, P, Beltran-Garcia M (2017) Endophytic bacteria and rare earth elements; Promising candidates for nutrient use efficiency in plants. Pp. 285-302, in Hossain M, Kamiya T, Burritt D, Tram L-SP, Fujiwara T. (Eds). Plant Macronutrient Use Efficiency. Academic Press, Cambridge, MA, USA.

Puente ME, Lib CY, Bashan Y (2009) Endophytic bacteria in cacti seeds can improve the development of cactus seedlings. *Environ Exp Bot* 66: 402-408

Rasmussen HN, Dixon KW, Jersáková J, Těšitelová T (2015) Germination and seedling establishment in orchids: a complex of requirements. *Ann Bot* 116: 391–402

Rosenblueth M, Martinez-Romero E (2006) Bacterial endophytes and their interactions with hosts. *Mol Plant–Microbe Interact* 19: 827–37

Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN (2008) Bacterial endophytes: recent developments and applications. *FEMS Microbiol Lett* 278: 1–9

Santoyo G, Moreno-Hagelsieb G, Carmen Orozco-Mosqueda, MC, Glick BR (2016) Plant growth-promoting bacterial endophytes. *Microbiological Research* 183: 92–99

Shearin ZRC, Filipek M, Desai R, Wesley A, Bickford, Kowalski KP, Clay K (2018) Fungal endophytes from seeds of invasive, non-native *Phragmites australis* and their potential role in germination and seedling growth. *Plant Soil* 422: 183

Soares, M.A., Li, H.Y., Kowalski, K.P., Bergen, M., Torres, M.S., and White, J.F. (2016) Functional roles of bacteria from invasive *Phragmites australis* in promotion of host growth. *Microb Ecol* 72(2): 407-17

Stewart SL, Kane ME (2006) Symbiotic seed germination of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. *Plant Cell Tissue Organ Cult* 86: 159–167

Stewart SL, Zettler LW (2002) Symbiotic germination of three semi-aquatic rein orchids (*Habenaria repens*, *H. quinquiseta*, *H. macroceratitis*) from Florida. *Aquat Bot* 72: 25–35

Taylor DL, Bruns TD (1997) Independent, specialized invasion of ectomycorrhizal mutualism by two nonphotosynthetic orchids. *Proc Natl Acad Sci USA* 94: 5410–5415

Truyens S, Weyens N, Cuypers A, Vangronsveld J (2015) Bacterial seed endophytes: genera, vertical transmission and interaction with plants. *Environ Microbiol Rep* 7: 40–50

Verma SK, White JF (2018) Indigenous endophytic seed bacteria promote seedling development and defend against fungal disease in browntop millet (*Urochloa ramosa* L.). *J Appl Microbiol* 124: 764-778

Verma SK, Kingsley K, Bergen M, English C, Elmore M, Kharwar RN, White JF (2018) Bacterial endophytes from rice cut grass (*Leersia oryzoides* L.) increase growth, promote root gravitropic response, stimulate root hair formation, and protect rice seedlings from disease. *Plant Soil* 422: 223-238

Verma SK, Kingsley K, Irizarry I, Bergen M, Kharwar RN, White JF (2017) Seed vectored endophytic bacteria modulate development of rice seedlings. *J Appl Microbiol* 22: 1680-1691

Wäli PR, Helander M, Saloniemi I, Ahlholm J, Saikkonen K (2009) Variable effects of endophytic fungus on seedling establishment of fine fescues. *Oecologia* 159: 49–57

White JF, Chen Q, Torres MS, Mattera R, Irizarry I, Tadych M, Bergen M (2015) Collaboration between grass seedlings and rhizobacteria to scavenge organic nitrogen in soils. *AoB PLANTS* 7.

White JF, Crawford H, Torres MS, Mattera R, Irizarry I, Bergen M (2012) A proposed mechanism for nitrogen acquisition by grass seedlings through oxidation of symbiotic bacteria. *Symbiosis* 57: 161–171. <http://doi.org/10.1007/s13199-012-0189-8>

White JF, Kingsley KI, Kowalski KP, Irizarry I, Micci A, Soares MA, Bergen MS (2018a) Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive seed grass (*Phragmites australis*). *Plant Soil* 422: 195-208
White JF, Kingsley K, Harper CJ, Verma SK, Brindisi L, Chen Q, Chang X, Micci A, Bergen M (2018b) Reactive oxygen defense against cellular endoparasites and the origin of eukaryotes, in: Krings M, Harper CJ, Cuneo NR, Rothwell GW (eds.). *Transformative Paleobotany: Papers to Commemorate the Life and Legacy of Thomas N. Taylor*. Elsevier, Amsterdam, Netherlands.

White J F, Kingsley K L, **Verma S K**, and Kowalski K P (2018d) Rhizophagy Cycle: An Oxidative Process in Plants for Nutrient Extraction from Symbiotic Microbes. *Microorganisms*, 6, 95; doi:10.3390/microorganisms6030095

White JF, Torres MS, Somu MP, Johnson H, Irizarry I, Chen Q, Zhang N, Walsh E, Tadych M, Bergen M. (2014) Hydrogen peroxide staining to visualize intracellular bacterial infections of seedling root cells. *Microscopy Research and Technique* 77: 566-573. DOI: 10.1002/jemt.22375

White JF, Torres MS, Verma SK, Elmore MT, Kowalski KP, Kingsley KL (2018c) Evidence for widespread microbivory of endophytic bacteria in roots of vascular plants through oxidative degradation in root cell periplasmic spaces. In: *PGPR Amelioration in Sustainable Agriculture: Food Security and Environmental Management* (Eds. Kumar A, Singh A, Singh V), Elsevier

Wilson D (1995) Endophyte—the evolution of term, a classification of its use and definition. *Oikos* 73: 274–276

Figures legends:

Fig1. Proposed model of movements of seed inhabiting endophytic bacteria during germination and establishment of seedlings: A. Seed endobacteria exit seedlings during germination and become a functional part of rhizosphere community; B. Bacteria are attracted to root exudates around the meristem tip and are internalized into root cells where they are subjected to reactive oxygen and nutrients are extracted from them (rhizophagy); C. Bacteria exit root cells primarily from tips of elongating root hairs and return to the rhizosphere to acquire more nutrients.

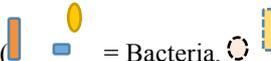
Where;  = Bacteria,  = Degraded bacteria).

Fig 2: Proposed role of bacterial endophytes in root cell during seedling establishment and defenses. Seed vectored bacterial endophytes exit during germination and colonise in rhizosphere and then in /on the root cells. They bring nutrients for plant and influence several physiological processes via growth regulators or by ROS or by both and induce plant growth related and defense related genes.

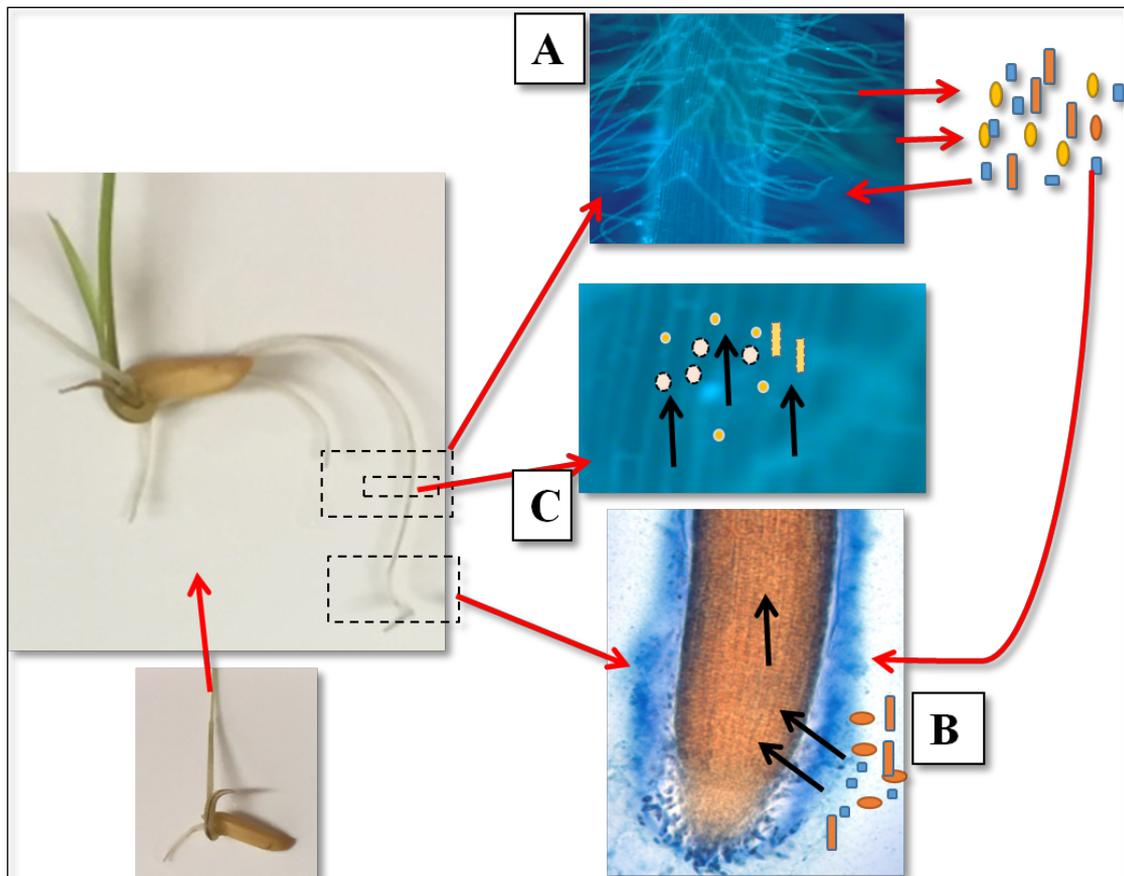


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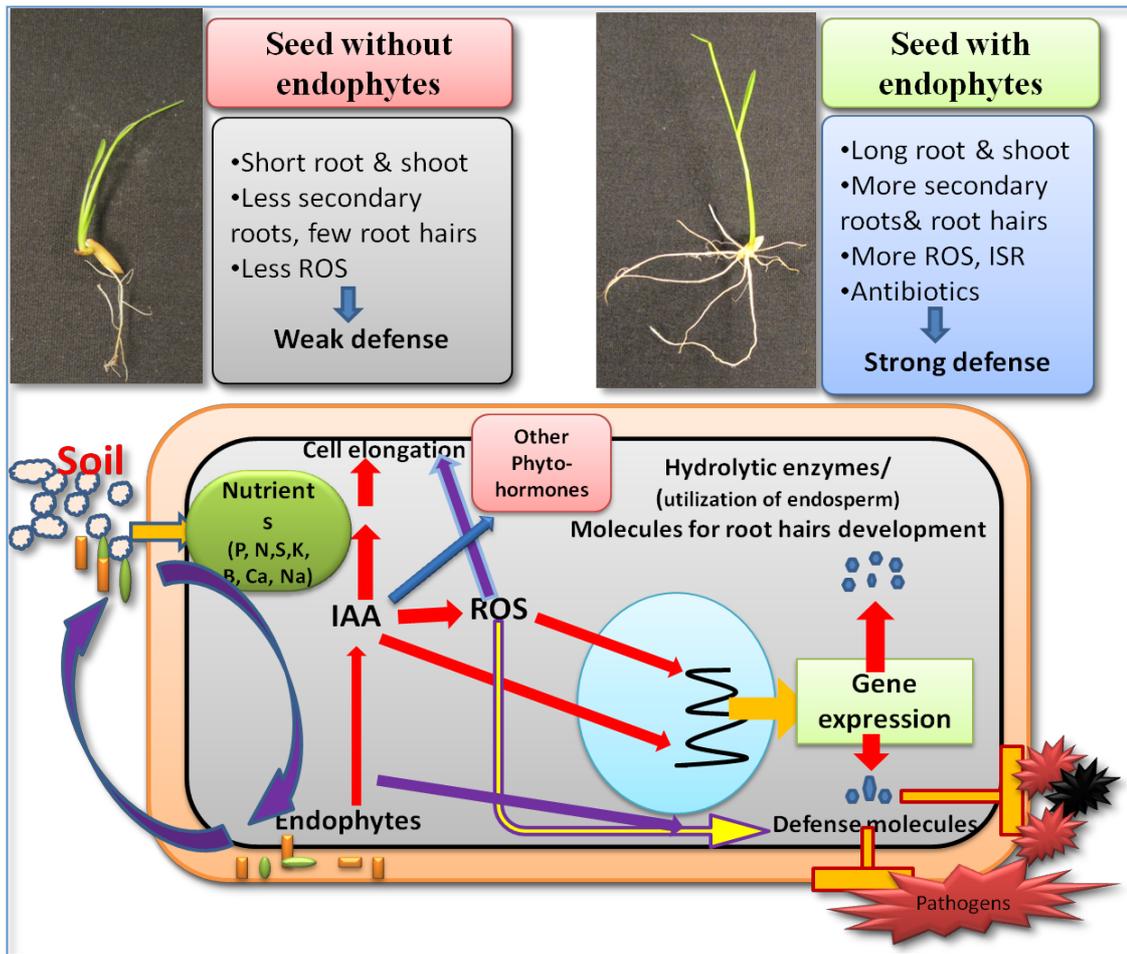


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