

## Seed Endophytic Microorganisms: The Seed We Eat is Not Sterile

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### SUMMARY

Seeds carry both beneficial and disease-causing microorganisms. Understanding their role in plants is important for better plant productivity. Managing the harmful seed-borne plant pathogens without killing beneficial microorganisms is a key challenge. This article highlighted beneficial seed endophytes and their role, further important seed-borne pathogens, and their detection, management, and quarantine associated with it.

### INTRODUCTION

The seed is a reservoir of both beneficial and harmful microorganisms consisting of bacteria, fungi, viruses, and nematodes. The beneficial microorganisms employ various direct and indirect mechanisms for plant growth promotion. Whereas, many disease-causing microorganisms hinder seedling growth and development, leading to a decrease in the crop yield. Pathogenic microorganisms involved in disease development are essential considerations for quarantine and seed certification of the seeds. In this article, we highlighted the role of endophytic microorganisms and pathogenic microorganisms present inside the seeds. Further, management of the seed-borne diseases have been discussed.

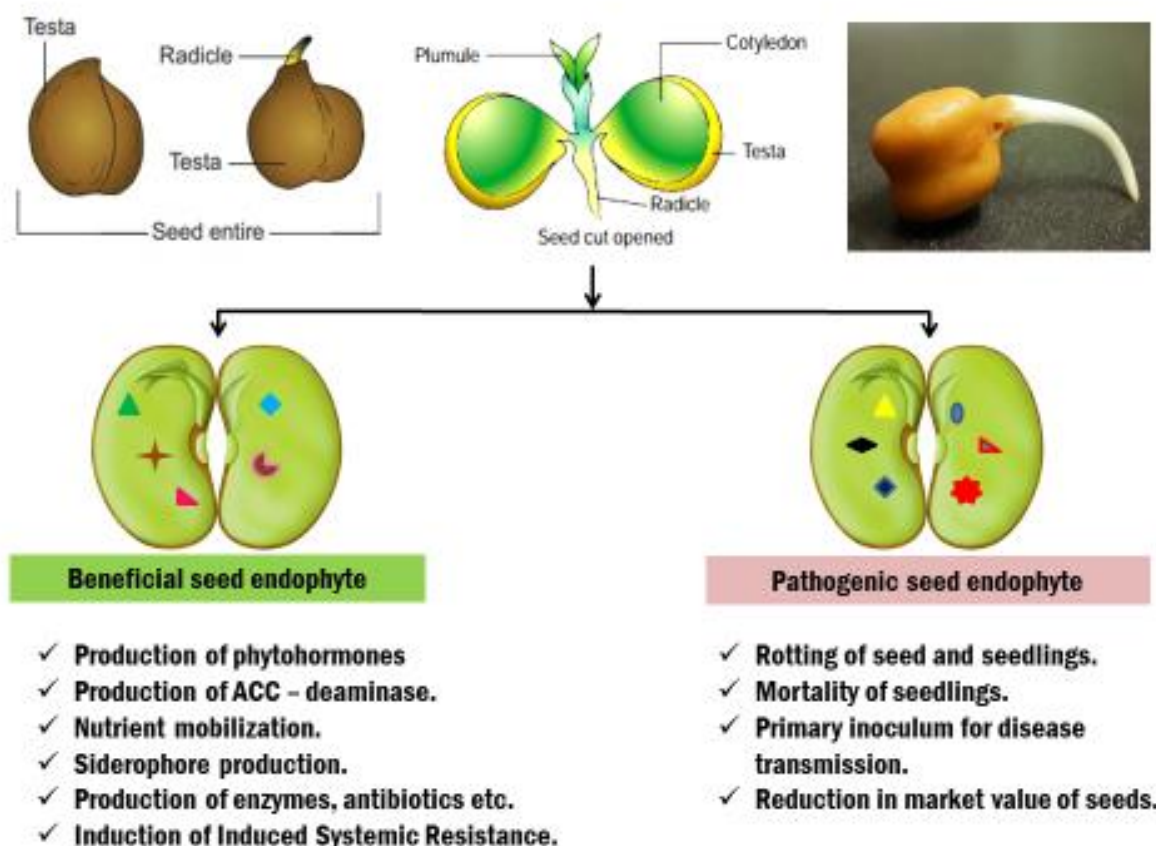


Fig. 1: Beneficial and pathogenic seed microorganisms and their role.

### Beneficial seed endophytes

Seed endophytes represent microorganisms present inside the seed. These microorganisms characteristically have cell motility, phytase enzyme activity, and endospore-forming ability, which enable them

to live inside the seeds. Fundamental mechanisms employed by seed endophytes for plant growth promotion are (i) releasing various phytohormones like IAA, GA3, and cytokinins, (ii) ACC-deaminase activity, which catalyzes ACC, a precursor of ethylene and thereby alleviates abiotic and biotic stresses, (iii) nutrient mobilization like phosphorus solubilization, (iv) siderophore production, (v) release of antifungal, antibiotics, enzymes, and secondary metabolite compounds, (vi) induction of induced systemic resistance (ISR). The predominant seed-borne bacterial genera are *Bacillus* spp., *Pantoea*, *Enterobacter*, *Paenibacillus*, *Burkholderia*, *Pseudomonas*, *Acinetobacter*, *Phyllobacterium*, *Rhizobium*, etc. Similarly, fungal endophytic genera are *Alternaria* sp., *Phoma* sp., *Cladosporium* sp., *Fusarium* sp., *Colletotrichum* sp., *Mucor*, *Penicillium*, *Chaetomium*, *Diaporthe* sp. etc. The source of these endophytic seed microorganisms is soil rhizosphere, phyllosphere, or the seeds of the previous generation. Many microorganisms are transferred from one generation to another generation, termed as vertical transmission. These seed endophytes play a role in seed germination and seed vigor. They mobilize the nutrients present in the seed by producing many extracellular enzymes like amylase, lipase, and protease for seed germination and seedling development.

### Pathogenic seed endophytes:

Seeds may carry pathogens externally or internally or as contaminants. These seed-borne pathogens cause rotting of seed and seedling, mortality in pre- and post-emergence of seedlings, and have small-sized and shriveled seed. It reduces the seed's market value, and further, it acts as a primary inoculum for the disease transmission to the next generation. The sources of seed-borne pathogens are from mother plants (ovary wall, vascular system, and floral parts), from environments (plant residue, soil, and storage), and human activities. Pathogen infection occurs during anthesis, seed development, and seed maturation. Therefore, disease management at these stages is very important. At anthesis, the developing embryo has greater chances of infection with smuts, bunts, ergots, bacteria, viruses, and nematodes. Since these pathogens survive in crop stubbles, weeds, and seeds, proper cultural management is required. Cultural practices like adjustment of sowing date, crop rotation, irrigation, and destruction of weed and previous crop stubbles could be done. Notable seed-borne diseases, which cause severe losses, are the late blight of potato, a brown spot of rice, loose smut, downy mildew of bajra, tobacco bud blight, and soybean mosaic virus.

## Seed-Borne Diseases

### Seed-Borne Bacterial Diseases

- Crucifers black rot - *Xanthomonas campestris* pv. *campestris*
- Cotton black arm - *X. citri* pv. *malvacearum*
- Tomato bacterial canker - *Clavibacter michiganensis* pv. *michiganensis*
- Soybean bacterial blight - *Pseudomonas syringae* pv. *glycinea*
- Wheat and barley bacterial black node - *P. syringae* pv. *syringae*
- Rice bacterial blight - *X. oryzae* pv. *oryzae*

### Seed-borne viral diseases

- Potato virus Y
- Pea seed mosaic virus
- Cowpea mosaic virus
- Barley stripe mosaic virus
- Soybean vein necrosis virus- Tomato spotted wilt virus (TSWV).
- Soybean bud blight- Tobacco ringspot virus (TRSV).

### Seed-borne fungal diseases

- Rice- blasts, false smut, root rot, seedling blight.
- Wheat – *Alternaria* leaf blight, loose smut, flag smut, karnal bunt, and *Fusarium* head blight.
- Maize- *Curvularia* leaf spot, *Aspergillus* ear, and kernel rot, and anthracnose leaf blight

- Soybean- Anthracnose and purple seed stain.
- Chickpea- *Ascochyta* blight, grey mold, and *Alternaria* blight.
- Sunflower- Downey mildew, charcoal rot, and anthracnose.
- Groundnut- Charcoal rot, crown rot, and *Aspergillus* mold.

#### Seed-borne nematode diseases

- Seed gall nematodes: *Anguina tritici*
- Rice white tip nematodes: *Aphelenchoides besseyi*
- Ground nut testa nematode- *Aphelenchoides arachidis*
- Rice ufra nematode- *Ditylenchus angustus*

#### Why seed-borne phytopathogen control is important

- Qualitative losses like seed abortion, shrunken and reduced seed, discoloration, and necrosis.
- Loss in seed germination and vigor.
- Toxin formation in the pathogen infected seeds.
- Disease development.
- Biochemical changes of protein, oil, and alteration of physical properties of seeds.
- Quantitative yield loss.
- The problem in exporting or importing the seeds in domestic and with other countries.
- Seed borne diseases could cause epidemics in new areas; a typical example of this situation is the Irish famine caused by late leaf blight of potato.

#### Detection of Seed-Borne Pathogens:

##### Bacterial detection:

- Visual examination of seeds.
- Bacterial ooze tests.
- Serial dilution in semi-specific media and identification.
- Immunofluorescence microscopy (IF), Enzyme-linked Immunosorbent assays (ELISA), and flow cytometry.
- PCR tests - BIO-PCR, Real-time- PCR, multiplex PCR, and Nested PCR
- Grow out tests

##### Viral detection

- Serological test- ELISA based tests.
- PCR based- RT- PCR, next-generation sequencing and DNA chips, and DNA microarrays.
- Biological tests- grow out test and indicator plant assay.
- Microscopy based- electron microscope and light microscope.
- Sensors- nanosensors

##### Fungal detection

- Seed samples incubation in selective media.
- Visual examination of dry seeds.
- Microscopic examination - NaOH seed soaking and whole embryo count method.
- Incubation methods- on agar and on blotter paper.
- Extraction and staining of seed embryo and tissues.
- In grow-out tests: observation of symptoms on seedlings.
- PCR tests - BIO-PCR, Real-time- PCR, multiplex PCR, and Nested PCR.

##### Nematodes detection

- Microscopic examinations.

- PCR based tests.

### Important points for controlling seed-borne phytopathogens:

- **Chemical treatment-** Seed treatments with fungicides before sowing the seeds. Ex seed treatment with carbendazim, thirum, mancozeb reduces the disease such as rice blast, anthracnose, *Alternaria* blight, and tikka leaf spot in the field. Seed treatment with metalaxyl reduces oomycetes fungus infection, i.e., *Phytophthora*, *Pythium*, downy mildew fungus. Similarly, seed treatment with carboxin reduces the smut of rice and sorghum, etc. Seed treatment with streptomycin able to reduce bacteria disease caused by genus *Xanthomonas* and *Pseudomonas* in crop plants.
- **Physical treatment:** Heat treatment, hot water treatment, or microwave heating before sowing the seeds. For Ex, Hot water treatment at 55 °C for 10 to 15 min reduces the white tip nematode of rice and loose smut of wheat, whereas 52 °C for half an hour reduces the diseases of sugarcane such as whip smut, grassy shoot, and red rot. Moist heat treatment of seed for 30 min reduces diseases in sugarcane and citrus. Dry air at 72 °C for 7-10 min eliminates barley leaf streak pathogen *X. campestris pv translucens*. Radiation such as UV, X-rays,  $\gamma$ ,  $\alpha$ , and  $\beta$  rays also helps in reducing seed-borne pathogens.
- **Biological treatments-** Biopriming with biocontrol agents or bioprotectants, e.g., *Pseudomonas* and *Bacillus*. Seed treatment with *P. fluorescence* @ 16 g per kg of maize seed reduces banded leaf and sheath blight of maize. Seed treatment with *Trichoderma* spp. reduces various soil-borne pathogens such as *Fusarium*, *Rhizoctonia*, *Macrophomina*, *Sclerotium*, and *Sclerotinia*.
- Epidemiological factors, method and duration of storage also determine the survival of pathogens in the seed. Ex., the fungus *Alternaria brassicicola* of rapeseed eliminated when storage temperature is above 35 °C. Similarly, *A. brassicicola* of cabbage does not grow below 15 °C.
- Seed certification for meeting the seed quality parameters.
- Phytosanitary certification requirement for exporting seeds to other countries.
- We should keep guidelines for the safe movement of germplasm for economically important seed-borne diseases as these diseases can spread from one territory to another.

### CONCLUSION

In the present situation for feeding the exponentially growing population, we need huge quantities of high-quality, disease-free healthy seeds to grow the crops for higher crop productivity. We should adopt low-cost, environmentally friendly, and sustainable solutions for controlling phytopathogens, i.e., the use of beneficial seed endophytic microorganisms.

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