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## Seed priming with endophytes: A novel approach for future prospects

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**Abstract**

In the current scenario of climate change, numerous strategies have been employed in the area of sustainable agriculture or plant science to generate plants which can withstand various types of biotic and abiotic stresses. Seed priming has been developed as a crucial method to germinate the seed or increase plants resistance against various biotic and abiotic stresses. Seed priming with microbial inoculum, termed as 'bio-priming', involves the application of beneficial microbes, such as bacteria, fungi and actinomycetes to seed that enhance the germination, vigour, protection from diseases and pests leading to increase in yield. Seed priming with the use of endophytic microbial strains appears as more beneficial or stable than rhizospheric microbial strains due to better colonization adaptability and suitability under biotic and abiotic stress conditions. Microbial endophytes are symbionts dwelling within plant tissues without appearance of disease symptoms on host plant and have been recently investigated for their plant growth-promoting properties and their beneficial functions associated with plant responses under salinity, drought, temperature, heavy metal stress, and nutrient stress through different mechanisms and also provide protection from diseases and pests.

**Keywords:** Endophytes, seed priming, abiotic stress, biotic stress, management

**Introduction**

The improved quality of seed to fulfill the higher demand of agriculture has been recognized as a major challenge globally. In this aspect, improving the germination, vigour and health of seed through 'seed priming' is a sustainable approach to enhance yields and performance of plants (McDonald 2000) [27]. It has been broadly mentioned that priming of seeds mitigates the adverse impact of various biotic such (phytopathogens, plant diseases; Van Hulst *et al.*, 2006) [45], and abiotic (drought, salinity, flooding) stress factors, that affect the physiology and metabolism of plants via different mechanisms (Kausar and Ashraf 2003; Basra *et al.*, 2005; Kumar *et al.*, 2016) [21, 2, 23].

Seed bio-priming involves the integration of beneficial microbes including bacteria, fungi and actinomycetes for improved plant growth and development. Nature harbors a large diversity of microbial communities, among them endophytes have received increasing attention worldwide because of their promising hidden potential against various biotic and abiotic stress factors, and also their potential applications in growth promotion of plants via modulating growth hormones, nutrient availability, siderophore production, etc. Priming has been supposed to induce cellular metabolic processes, hence exposure to any detrimental environmental factors would allow them to respond rapidly and nullify the stresses in an effective manner as compared to non-primed seeds.

**What are endophytes?**

The term "endophyte" is derived from the Greek word "endon" means "within" and "phyte" means "plant" so the term includes all organisms that, during a variable period of their life, symptomlessly colonise the living internal tissues of their hosts (Stone *et al.*, 2000) [39]. Different parts of plants were used for isolation of microbial endophytes such as meristem, scale primordia, resin ducts (Pirttilä *et al.*, 2003) [30], leaf segments with midrib and roots (Hata *et al.*, 2002) [20], leaf blade, stem, petiole, bark, and buds (Pirttilä *et al.*, 2008) [30].

**Colonization of endophytes**

Endophytic fungi insert through the hyphae and enter the kernels in the seeds of plant cells that come below vertical transmission. A variant was detected in horizontal and vertical transmission of the endophyte species invading the host plant cells (Tintjer *et al.*, 2008) [42]. The endophytic fungal species transmits horizontally by sexual spores or asexually between different plants in community or a population (Tadych *et al.*, 2014) [41].

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The microbial community such as bacteria, algae, fungi, and actinomycetes colonizes the host plant roots (Saharan and Nehra 2011; Prashar *et al.*, 2014) <sup>[34, 31]</sup>. Among microbial population found in the rhizosphere, actinobacteria are considered the second most abundant microorganisms, and they comprise more than 30% of the total microorganisms in the soil (Glick 2014) <sup>[15]</sup>. Endophytes are transmitted between the soil rhizosphere across the seeds. They spread quickly between endo-rhizosphere through the lateral root junction instigated through microbial phyto-pathogens or nematode (Chi *et al.*, 2005) <sup>[7]</sup>. Also, bacterial endophytes can enter their host plant roots through spaces between root hairs and epidermal cells (Hardoim *et al.*, 2008) <sup>[18]</sup>.

### Endophytic fungal strains

The most common endophytic fungi isolated and identified from numerous plants are *Alternaria infectoria*, *Aspergillus* sp., *Penicillium* sp., *Colletotrichum musae*, *Colletotrichum gloeosporioides*, *Nigrospora oryzae*, *Phomopsis* sp., *Nigrospora sphaerica*, *Guignardia* sp., *Cordana musae*, *Rhizoctonia* sp., species of *Phialocephala sphaeroides*, *Xylaria* (Wilson *et al.*, 2004) <sup>[47]</sup>, *P. chrysogenum* Pc\_25, *A. alternata* Aa\_27, and Sterile hyphae Sh\_26 (Fouda *et al.*, 2015) <sup>[11]</sup>. On the other hand, endophytic *P. chrysogenum* Pc\_25 was mediated biosynthesis of ZnO nanoparticles (Fouda *et al.*, 2019) <sup>[12]</sup>.

### Endophytic bacterial strains

In addition, various endophytic bacterial strains were isolated from economically important plant species. Several of the novel endophytic bacterial species belong to the *Arthrobacter* spp., *Actinobacter* spp., *Aeromonas* spp., *Enterobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Bacillus* spp., *Flavobacterium* spp., *Azospirillum* spp., *Azotobacter* spp., *Pseudomonas* spp., *Burkholderia* spp., *Beijerinckia* spp., *Enterobacter* spp., *Flavobacterium* spp., *Erwinia* spp., *Rhizobium* spp., and *Serratia* spp. were characterized and identified (Gray and Smith 2005) <sup>[16]</sup>. In the last periods, other endophytic actinobacteria such as *Streptomyces*, *Amycolatopsis*, *Nocardia*, *Microbispora*, *Micromonospora*, and *Streptomyces capillispinalis* Ca-1 have been positively isolated from different plant species (Shi *et al.*, 2009; Ruanpanun *et al.*, 2010; Hassan *et al.*, 2018) <sup>[36, 33, 29]</sup>.

Bacterial endophytes are consistently reported present in the root, stem, leaf, fruit and tuber tissues of a wide range of agricultural, horticultural and forest species are given below.

Endophytes	Plant species
<i>Azorhizobium caulinodans</i>	Rice
<i>Bradyrhizobium japonicum</i>	Rice
<i>Gluconacetobacter</i> sp.	Sorghum
<i>Diazotrophicus</i> sp.	Sugarcane
<i>Enterobacter sakazakii</i>	Soybean
<i>Microbacterium testaceum</i>	Maize
<i>Rhizobium radiobacter</i>	Carrot
<i>Citrobacter</i> sp.	Banana
<i>Methylobacterium mesophilium</i>	Citrus
<i>Enterobacter asburiae</i>	Sweet potato

### Biocontrol of phytopathogens by endophytic bacteria

#### Endophytes as biocontrol agents

Bacteria from the phylloplane have provided some biological disease control. Unfortunately, most of these biocontrol agents have not fulfilled their initial promise, their failure usually being attributed to poor rhizosphere competence and

the difficulties associated with the instability of bacterial biocontrol agents in long term culture (Schroth *et al.*, 1984; Weller, 1988) <sup>[48]</sup>. However, intimate relationship between endophytic bacteria and their hosts make them natural candidates for selection as biocontrol agents (Chen *et al.*, 1995) <sup>[6]</sup>.

#### Mechanism of biocontrol

The mechanism by which endophytes can act as biocontrol agents include the production of antifungal or antibacterial agents (Lambert *et al.*, 1987) <sup>[24]</sup>, siderophore production (Kloepper *et al.*, 1980) <sup>[22]</sup>, nutrient competition (Lockwood, 1990; Kloepper *et al.*, 1980) <sup>[22]</sup>, niche exclusion and indirectly through the induction of systemic acquired host resistance or immunity (Chen *et al.*, 1995; Tuzun and Kloepper, 1994) <sup>[6, 44]</sup>. Thus bacteria that enhance emergence and promote growth in the face of pathogen attack may show no apparent benefit in the absence of that disease pressure.

#### Endophytes act as inducers of systemically acquired resistance

Plant possesses a variety of latent defense mechanisms conferring quantitative protection against a broad range of microorganisms. These mechanisms become systemically activated following exposure to stress or infection by phytopathogens or other microorganisms (Sticher *et al.*, 1997) <sup>[38]</sup>. Endophytic bacteria have been implicated in such induced protection responses, and some plant defense strategies can involve endophyte mediated *de novo* synthesis of structural compounds and fungitoxic metabolites at sites of attempted fungal penetration. Sturz *et al.*, (1999) <sup>[40]</sup> demonstrated that in certain communities of endophytic bacteria defence against pathogens may be related to bacterial adaptation to location within a host and may be tissue type and tissue site specific. Smith and Metraux (1991) <sup>[37]</sup> showed that pre-inoculation with *Pseudomonas syringae* pv *syringae* of the first true leaf in the rice plants induced a systemic resistance to *Pyricularia oryzae* suggesting that the response is elicited by the initial inoculation that cross protects the plant against subsequent infections. Cameron *et al.*, (1994) <sup>[4]</sup> induced systemic acquired resistance against virulent pathogens in *Arabidopsis thaliana* by pressure infiltrating *Pseudomonas syringae* pv. tomato into one leaf.

Application of root endophytic fungi *Piriformospora indica* belonging to class basidiomycetes in order to enhance the disease resistance, tolerance to salinity stress and increase in grain productivity of barley (*Hordeum vulgare* L) is documented (Waller *et al.*, 2005) <sup>[46]</sup>. Endophyte mediated induction of disease resistance was observed to be systemic in nature. The improved defense responses were demonstrated to result from the enhanced antioxidative behavior conferred by ascorbate-glutathione cycle, leading to increase in grain productivity. The fresh shoot weight of four-week-old endophyte infested barley was recorded to be 1.65 fold higher compared to the control group. The grain yield increase for two different barley cultivars i.e. 'Annabell' and 'Ingrid' was found to be 11 and 5.5%, respectively and was attributed mainly to the rise in number of ears per plant. Thus, the increase in shoot fresh weight was directly correlated with the increase in grain yield. Interestingly, the endophyte was also capable of enhancing grain yield in soil systems receiving high nitrogen input. The easy *in vitro* cultivation of the *Piriformospora indica* without the requirement of host cells suggests the effective utilization of fungus for improving the resistance against plant diseases and enhanced grain yield.

Induction of seedling growth in the wheat by inoculation with plant growth promoting endophytic bacterium *Bacillus subtilis* strain 11BM was documented (Egorshina *et al.*, 2012) [10]. The inoculation with endophyte was found effective in increasing root and shoot weight as compared to control sets. Wheat seeds treated with endophyte spores culminated into the transient rise of hormonal status of IAA and IBA in the seedlings of root as well as shoot. The considerable alteration in wheat plant hormones was considered as a prime mechanism responsible for induced seedling growth.

### Endophytes to combat abiotic stress environments

The introduction of beneficial bacteria can improve the plant performance under stress environments enhancing yields (Bensalim *et al.*, 1998) [3]. In plant bacterial coculture, plant growth promotion reported increases in plant height (Chanway *et al.*, 1994) [5], root and shoot biomass (Pillay and Nowak, 1997) [29], tuber production, lignification of xylem vessels (Frommel *et al.*, 1991) [13] and root nodule formation. Bacterized plantlets grown *in vitro* were found to be greener and had elevated levels of cytokinins (Lazarovits and Nowak, 1997) [25]. Levels of phenylalanine ammonia lyase (PAL) and free phenolics were also higher in bacterized plantlets and both are linked to induced plant resistance responses to adverse abiotic stresses and phytopathogen attack (Richards, 1997) [32]. The overall effect of a more vigorous plant is increased drought resistance and reduced transplanting shock (Lazarovits and Nowak, 1997) [25]. The bacterium *Pseudomonas* strain PsJN improves stomata function in bacterized *in vitro* plantlets similar to those of green house-hardened transplants. Improvement of water relations under osmotic stress have also been recorded in wheat seedlings cocultured with *Azospirillum brasiliense* strain SP245 (Creus *et al.*, 1998) [8]. It has been theorized that the responses of plants to abiotic and biotic stresses are dependent on chemoperception systems - signal recognition and transduction modulated by microbial substances produced by endophytes (Harmon, 1997) [17]. Thus, the development of new methods of microbial culture may eventually help to establish more stable and mutually beneficial associations between plants and endophytic bacteria.

Endophytic bacterial species equipped with plant growth promoting traits may induce tolerance to salt stress by modulating the morphological, physiological and biochemical characteristics of plants (Mahmood *et al.*, 2016) [26], suggesting their utilization in crop enhancement under stress conditions. Priming of seeds from two barley genotypes (Haider-93 and Frontier-87) with endophytic bacterial strain *Enterobacter* spp. FD17 was performed to elucidate the role of biopriming in alleviation of salt stress (Tabassum *et al.*, 2018) [43]. Seed priming was helpful in improving grain yield, photosynthetic pigments, soluble protein accumulation, membrane stability and osmolyte concentration. The increased osmolyte concentration under salinity stress may be the outcome of endophyte induced enhanced expression of genes governing the synthesis of osmolytes (Miotto-Vilanova *et al.*, 2016) [28]. Moreover, the bacterially synthesized osmolytes may act in synergistic fashion with plant produced osmolytes to enhance the tolerance against the salinity stress (Dimkpa *et al.*, 2009) [9]. In conclusion, endophytic bacterial association was positively associated with seedlings biochemical and physiological attributes under salinity stress. Effect of inoculation of two different endophytic bacterial strains namely, *Burkholderia phytofirmans* (PsJN) and *Enterobacter* sp. (FD17) in combination with biochar has

been performed to alleviate the negative consequences of salinity stress in maize (Akhtar *et al.*, 2015) [1] by reducing the absorption of sodium ions in xylem or by maintaining the nutrient balance of plant system. The better activity (in terms of reducing sodium ion uptake) in biochar added soil was recorded for *Enterobacter* sp. (FD17) as compared to *Burkholderia phytofirmans* (PsJN). Such improvements in plant physiological processes under saline environment could be employed for enhancing the crop productivity in a sustainable manner.

The endophytic fungus *Piriformospora indica* induced alternation in plant metabolites under drought stress was recently elucidated (Ghaffari *et al.*, 2019) [14]. The barley seedlings primed with endophyte cell suspension under mild drought stress was presented to have changes in abundance of total 145 plant proteins in contrast to 104 in untreated seedlings. On the other hand, under severe drought stress environment, plant proteins showing considerable changes in their abundance were recorded as 144 and 462, respectively for endophyte treated and untreated ones. The plant proteins showing changes were related to primary plant metabolic activities and documented to be involved in alleviating the negative effects imposed by oxidative stress under drought. Root colonization by endophytic fungi was associated with enhanced biological functions of photosynthetic machinery and electron transport pathway, in addition to induced build up of proteins with protective role in different biological processes including energy production, primary metabolic pathways and autophagy. Resource redistribution in host cells along with maintenance of water channels (aquaporins) in endophyte inoculated plants as the effective mechanisms to cope up with the detrimental consequences of drought stress could be considered for managing agricultural productivity under changing environmental conditions.

### Future perspective

The application of beneficial endophytes to enhance plant performance under natural environmental conditions is of immense importance in the area of agricultural sciences. Since, impacts imparted by endophytic microbes used for seed priming is greatly influenced by host plant as well as prevailing environmental conditions, critical investigations under field conditions should be performed to harness the potential of seed primed with endophytes in crop productivity enhancement.

The interactive effects of other priming techniques with endophytic microbe based bio-priming could provide better outcomes for the management of crop productivity. More studies under field conditions for widely distributed endophytic arbuscular mycorrhizal and other endophytic fungi could provide novel and beneficial approaches to combat the problem of reduced crop productivity imposed by abiotic and biotic stresses.

The search for newer endophytic bacterial and fungal species would provide many opportunities for different plant species. Moreover, the application of nitrogen fixing endophytic bacteria for seed priming have potential to enhance the seed characteristics in terms of seed vigor, germination rate and overall crop productivity under changing environmental conditions.

The identification and transfer of endophytic fungal and bacterial species genes responsible for crop improvement such as disease resistance and stress tolerance genes would be helpful in simultaneous improvement of seed quality and enhancement in agricultural productivity. In this context, the

findings that the resistance genes harbored by uncultivable endophytes give a potential avenue for application in plant disease management. Further, the detailed investigations of improved plant disease resistance under the influence of microbial endophytes would help to unveil the precise molecular mechanisms of host protection.

### Conclusion

Application of endophytes for seed priming has several promising potentials in the field of seed technology and agricultural productivity. Available evidence has shown the positive influence of priming on seed quality, seedling growth, crop productivity even under stress conditions. Priming with endophytes has also shown increases in disease resistance, and tolerance to numbers of biotic and abiotic stresses. Few studies regarding the combined application of endophyte based priming with other priming methods like osmo-priming and hydro-priming may have more conspicuous impacts on plant performance under natural environmental conditions as compared to those manifested by bio-priming alone.

Endophytes can be used as alternative strategies to plants that adapted to many stresses like drought, salinity, temperature, nutrient stress, and heavy metals. Further studies of endophytes will provide a better understanding of their relationship with host plant and maximize its utilization as promoters of plant growth as well as its ability to protect the plant from many harmful factors.

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