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Endophytes in plant system: Roles in growth promotion, mechanism and their potentiality in achieving agriculture sustainability

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Abstract
Intensive agriculture practices which depends heavily on unsustainable level of agrochemical inputs are environmentally harmful and the expansion of these practices to meet future needs are not economical and ecologically Sustainable. Endophytes are the group of microorganisms that inhabit plant, live inside the host microenvironment, receive protection from environmental stresses, and have greater access to nutrients with lesser competition from other microbes. Bacterial and fungal endophytes are widespread inhabitants inside plant tissues and have been shown to assist plant growth and health. They exert beneficial effects upon plants and enhance plant growth and yield either directly or indirectly via several mechanisms. Present review paper critically focused on the role of these endophytes in plant growth promotion, there different mechanism of action and their practical utility in agriculture system. This comprehensive review is based on critical study of different research works and investigations all around the globe. Paper also depicts evaluation of endophytes role in enhancing crop productivity and maintaining soil health aiming towards sustainability of agriculture in long run.

Keywords: endophytes, plant growth, mechanism, soil health, sustainability

1. Introduction

The long-term approaches of organic and inorganic fertilizers beside to pesticides are urgently required for the enhancement of influence on plant growth and health which effectively ameliorates agricultural traits and improve soil quality and nutrient cycling (Khan AL, 2013) [11]. Soil fertility in modern agricultural systems is maintained by the application of fertilizers, and pathogens and pests are controlled by various agrochemicals. Groups of microbes, such as mycorrhizal fungi and nitrogen-fixing bacteria, have long been known to benefit plant growth (Berendsen *et al*., 2012) [2]. In addition, some endophytic microbes residing within plant tissues have been shown to promote plant growth and endow protection against biotic and abiotic stresses under laboratory conditions (Baltruschat *et al*., 2008) [1]

Endophytes are the group of microorganisms that live inside host microenvironment, receive protection from environmental stresses, face lesser competition from other microbes, restore heavy metal contaminated soil by various agriculture practices and have greater access to nutrients. Endophytes promote plant growth and yield and can act as biocontrol agents. Plants benefit extensively by harbouring these endophytic microbes; they promote plant growth (Compant *et al*., 2005) [4] and confer enhanced resistance to various pathogens (Clay and Schardl, 2002) [3] by producing antibiotics. The main role of endophytic organism in plant growth promotion include that they can inhabit plant tissues and the close linkage of endophytes inside plant tissues facilitates nutrients exchange and enzymes activity (Khan A, *et al*., 2015) [11]. The distribution of growth-promoting hormones produced by endophytic microorganisms towards plant tissues positively promotes plant growth. Endophytes possess vital ability to mobilize insoluble phosphate and provide nitrogen to their host plants (Matsuoka H, 2013) [15]. Endophytes also produce unusual secondary metabolites of plant importance (Taechowisan *et al*., 2005) [23] they also help in production of phytohormones, biocontrol of phytopathogens in the root zone by production of antibacterial and antifungal compounds and enhancing availability of nutrients. Bacterial endophytes play a key role in adaptation of the host plant to a changing environment (Hallmann *et al*., 1997) [10]

Endophytes can also be beneficial to their host by producing a range of natural products that could be harnessed for potential use in medicine, agriculture or industry. In addition, it has been shown that they have the potential to remove soil contaminants by enhancing phytoremediation and may also play a role in soil fertility through phosphate solubilization and nitrogen fixation. There is increasing interest in developing the potential biotechnological applications of endophytes for improving phytoremediation and the sustainable production of nonfood crops for biomass and biofuel production. Endophytes belonging to genera Acinetobactor, Enterobactor, Paneoea, Pseudomonas and Ralstonia showed plant promotion charactesistics in vitro and include phosphate solubilization activity, indole acetic (IAA) and production of siderophore. However there is some metal resistance endophytes fungi (*Microsphaeropsis, Mucor, Phoma, Peyronellaea, Aspergillus, collatotrichum, Neotyphodium, Epichloe, Piriformospora, Serendipita*) decrease plant metals uptake and accumulation. Endophytic fungi also show potential to enhance phytoremediation (Lodewyckx, 2001) [14].

There are several mechanisms of plant growth enhancement by endophytes have been documented. Direct growth mechanisms include: (1) solubilization of immobilized mineral nutrients such as phosphorus and zinc or mineralization of organic phosphorus compounds; (2) associative nitrogen fixation; (3) production of different types of phytohormones like auxins, cytokinins, and gibberellins; (4) sequestration of iron by siderophores; (5) oxidation of sulfur; (6) production of aminocyclopropane- 1-carboxylic acid (ACC) deaminase (Grayston *et al.,* 1990) [8] and (7) production of volatile growth stimulants such as acetoin and 2,3-butanediol (Ryu *et al*., 2003) [17]. The indirect plant growth mechanisms include: (1) antibiosis; (2) siderophore production; (3) induced systemic resistance (ISR); (4) competition for limited resources; (5) hydrogen cyanide (HCN) production; and (6) production of a wide range of cell wall degrading enzymes (Zahir *et al*., 2004) [25]. Thus plant growth promoting endophytes has potential role in increase the nutrient availability to plant and indirectly increase the plant growth and yield of crop.

1.1 Endophytes

Microorganisms play a very crucial role in the environment. Endophytes are defined as the “microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effects”. The term endophyte was originally defined by De Bary in 1866 as “Any organism occurring within plant tissues”. The word endophyte means -in the plant (derived from the Greek-endon = within, phyton=plant) this term can be used for a wide spectrum of potential hosts inhabitants, e.g. bacteria, fungi, etc. (Stone *et al*., 2000) [21]. Various forms of microorganisms that have been recovered as endophytes, include bacteria, fungi, actinomycetes and mycoplasma.

1.2 Endophytic bacteria

Bacterial endophytes are defined as those bacteria that can be isolated from surface-disinfected plant tissues or extracted from within the plant and that do not visibly harm the plant (Hallmann *et al*., 1997).

1.3 Endophytic fungi

Fungal endophytes primarily *ascomycetous* fungi, are found in abundance, whereas *basidiomycetes, deuteromycetes* and *oomycetes* are rarely found (Saikonnen *et al*., 1998) [18]. Although they do not show host specificity, certain fungal lineages appear with greater frequency in plants representing particular families and thus denote host preference. There is tremendous diversity of endophytic fungi and their ecological roles along with the amazing chemical variety of their secondary metabolites.

2. Methodology for isolation of endophytes

2.1 Collection of plant sample for isolation

The bacterial and fungal endophytes were isolated from roots, stems and leaves of the healthy flowering plants. The isolation was done from the plant immediately after collection. The plant samples were washed running tap water for 10-15 mins. to remove adhering soil particles, air-dried and roots, stems and leaves were separated out. The separated plant roots, stems and leaves were weighed up to one gram on a weighing balance. The weighed samples were soaked in distilled water and drained. The samples were then surface-sterilized by dipping in 70% ethanol for 1 minute, stems and leaves with 4% sodium hypochlorite for 5 minutes and roots with 2% sodium hypochlorite for 10 minutes and then treated with 70% ethanol for 30 secs. followed by rinsing five times in sterilized distilled water. The surface sterilized samples were blot-dried using sterile filter paper.

2.2 Isolation of Bacterial Endophytes

These surface sterilized samples were then macerated in one ml of distilled water in pestle and mortar. For each macerated sample that is root, stem and leaves serial dilutions were made upto 10-5 dilutions. One hundred micro liters from each dilution of the respective sample was then poured in their respective petri plates so labeled from 10-1 to 10-5 containing Nutrient Agar Medium and then spread with spreader for the isolation of the bacterial endophytes. The plating was done in triplicate for each dilution. The plates were incubated at 370 C for 72 - 96 hours. Sterility check was performed by imprinting the surface sterilized plant samples of roots, stems and leaves in the media.

2.3 Isolation of Fungal Endophytes

The surface sterilized samples were macerated in one ml of distilled water in pestle and mortar. For each macerated sample that is roots, stems and leaves serial dilutions were made upto 10-5 dilutions. One hundred micro liters from each dilution of the respective sample was then poured in their respective petri plates so labeled from 10 -1 to 10 -5 containing Potato Dextrose Agar Medium and then spread with spreader for the isolation of fungal endophytes. The plating was done in triplicate for each dilution. The plates were incubated at 280 C for two weeks. Sterility check was performed by imprinting the surface sterilized plant samples of roots, stems and leaves in the media.

3. Role of endophytes in plant growth promotion.

Endophytes may enhance plant growth and yield either directly or indirectly via several mechanisms.

3.1 Direct growth promotion mechanism

3.1.1 Biological nitrogen fixation

Biological Nitrogen Fixation (BNF) is the second most important biological process after the photosynthesis. The biological nitrogen fixation is restricted only to prokaryotic organisms. The degree of association between plant host and nitrogen fixing bacteria could be either through symbiotic, endophytic, or free living associations (asymbiotic). Endophytic bacteria, which form intimate associations with plants, are capable of fixing nitrogen in various crops without forming nodule-like structures. Bacterial and fungal endophytes residing inside the plant interior are protected from competition with other microbes, and are supplied with nutrients directly from the host plants. In return, the plant interior, which is rich in carbon and low in oxygen, provide favorable conditions for fixation of nitrogen which can then be transferred by the bacteria to their hosts (Ladha and Reddy, 2003) [13].

3.1.2 Phosphate solubilization

Phosphorus (P) is one of the main macronutrients required for plant growth in relatively higher amounts. It is not found in a form that is readily available for plant uptake. To remedy phosphorus deficiencies involves either the application of chemical phosphate fertilizers or of biofertilizers. Several endophytic bacterial and fungal genera have the capacity to solubilize insoluble inorganic phosphorus compounds and make them available for plant uptake. Such microorganisms are referred to as phosphate solubilizing microorganism (PSM) (Rodriguez and Fraga, 1999) [16].

3.1.3 Siderophores synthesis

Siderophores are low-molecular-weight iron binding compounds that are produced by many soil microorganisms under iron deficiency conditions (Crowley, 2006) [5]. Siderophores are synthesized by microbes which chelate Fe^{3+}, and transport it back to their cells where it becomes available for microbial growth. Endophytic bacteria have been reported to produce siderophores, a mechanism which is highly important for their growth (Sessitsch *et al*., 2004) [20]. Siderophore producing bacteria can enhance growth of their host plant either as biofertilizers (i.e., increase iron availability in the immediate surrounding area of their host plant roots) or by their biocontrol activities.

3.1.4 Phytohormone (IAA) production

Diverse bacterial and fungal species possess the ability to produce several types of plant growth regulators or phytohormones such as auxins, cytokinins, gibberellins, ethylene, and absicisic acids (Zahir *et al*., 2004) [25]. Production of indole acetic acid is common among many genera of bacteria and fungi. Endophytic bacteria and fungi are also able to synthesize indole acetic acid (Sessitsch *et al*., 2004) [20]. Indole 3-acetic acid involved in cell division and differentiation ultimately increases root length and root hair abundance providing more sites for infection and nodulation. This modification of root patterns enhance root surface area and thus increase plant ability to absorb more nutrients, which in turn stimulate plant growth (Gravel *et al*., 2007) [9].

3.2. Indirect growth promotion mechanisms

The indirect growth enhancement of the host plants by their endophytes occurs through suppression of phytopathogenic microorganisms in a process termed as biological control, in which the endophytes produce biocontrol traits lethal to the pathogenic microorganisms or compete with them for nutrients supply and root colonization sites.

3.2.1 Biological control of plant pathogens

Soil-borne plant pathogens continue to be a major threat to agricultural development and productivity worldwide. Several plant disease control methods have been implemented to protect crops against a wide range of phytopathogens but most of the approaches tend to have one or the other side effect associated with them. An attractive method to control plant disease is the use of plant rhizosphere associated beneficial microorganisms, which are called Biological Control Agents (BCAs) (Whipps and Gerhardson, 2007) [24]. Many biological control agents are known to reduce the incidence and severity of plant disease. *Pseudomonas* and *Bacillus* spp. are the predominant bacterial biological control agents, whereas *Trichoderma* spp. is the most important fungal biological control agent (Gerhardson, 2002) [7]. Bacterial endophytes also exhibit antagonistic activities against a broad spectrum of fungal pathogens.

4. Conclusion

A vast diversity of endophytic bacteria and fungi isolated from a large number of agricultural plants suggests that the bacteria play an integral role in balancing plant physiology, contribute to plant growth, help in restoration of available nutrient and phytoremediation. Endophytes may also benefit host plant by preventing pathogenic, predatory organism by colonizing them. There are various studies demonstrate potential role of endophyte bacteria and fungi in plant growth promotion and adaptability to biotic or abiotic stresses. It is studied that endophytic bacteria can also play important role in phytoremediation. Exploiting the usefulness of endophytic microorganisms for improvement of soil quality, plant growth promotion, phytoremediation, reclamation of problem soils etc. are the key to replace heavy agrochemical input in agricultural systems. Therefore, understanding of composition and functioning of plant associated microbial communities has a large potential of enhancing plant growth and restoration of soil quality.

5. Future Prospects

The recent research on endophyte fungi and bacteria on plant growth promotion and remediation of metal contaminated soil show a brilliant baseline for the successive studies. However, many key issue in this field need to be further studied as the investigation had been mostly focused on endophytic bacteria while fungi have been ignored. More integral approaches to study plant associated microbes and their positive interactions should be a matter of concern. Conversely study on plant pathogen interaction have been seldom reported. By considering all this situation, the study on different endophyte fungi and bacteria show brilliant prospect for successive studies for sustainability of soils and agriculture ultimately.

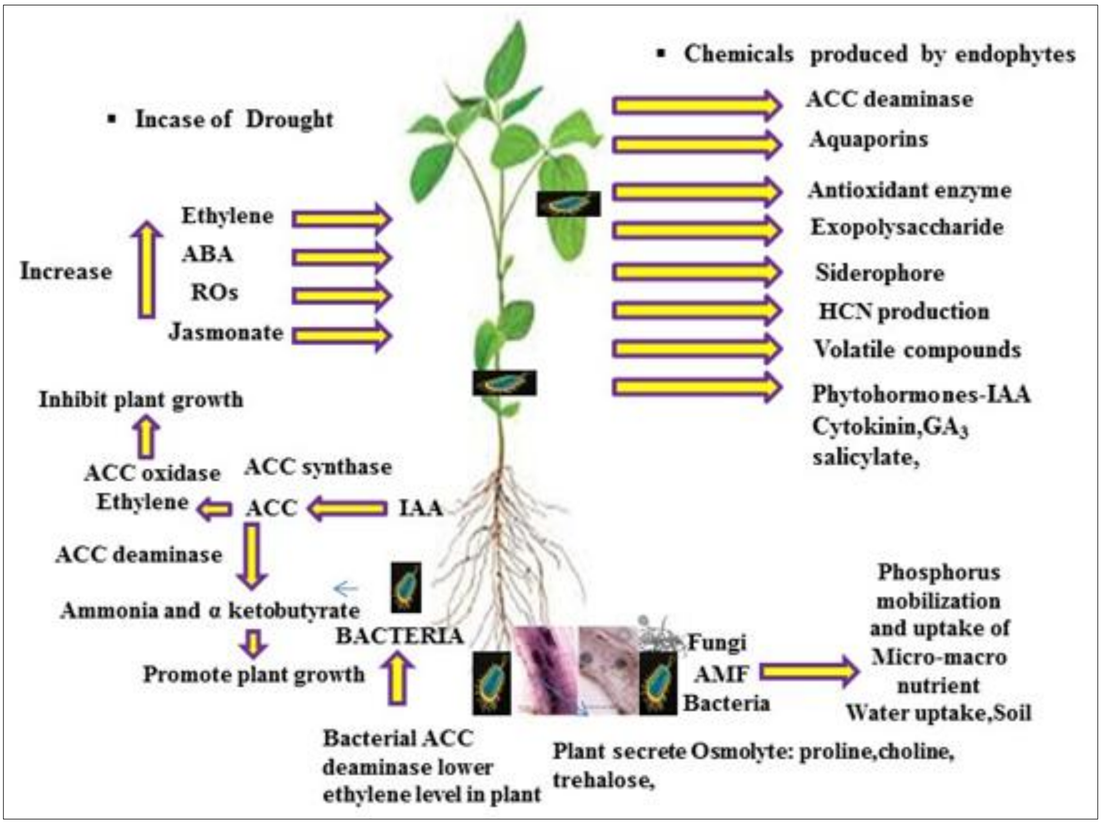

Fig 1: Mechanism and enzymes, osmolytes and phytohormones involved in draught mitigation, plant nutrition etc. by Endophytes

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