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Endophytic population in wild banana cultivars and its defence role against pathogen

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Abstract
Endophytes are microbes (mostly bacteria and fungi) present asymptomatically in plants. Endophytic microbes are often functional there in they may carry nutrients from the soil into plants, modulate plant development, increase stress tolerance of plants, suppress virulence in pathogens, increase disease resistance in plants, and suppress development of competitor plant species. Due to the effective functions of endophytic microbes, we propose that endophytic microbes may significantly reduce use of agrochemicals (fertilizers, fungicides, insecticides, and herbicides) within the cultivation of banana plants. The loss of endophytic microbes from banana plants during domestication and long-term cultivation might be remedied by transfer of endophytes from wild relatives of crops to crop species.

Keywords: Endophytic, population, banana, cultivars, pathogen

Introduction
Banana is one of the most common herbaceous plant of the genus Musa and is found to be the oldest cultivated plant. The banana is a general term embracing the number of species or hybrids between *Musa accuminata* and *M. balbisiana* in the genus *Musa* and family *Musaceae*. Banana plants are perennial cotyledons commonly grown in the tropics situated at latitude 20 degree above and below the equator. Banana are found to be useful not only as fruits but also as drug, utensil, and for various tradition and cultures. Therefore it is referred to as "Kalpatharu" (plant virtues) due to its multifaceted uses (Praveena *et al*., 2018) [24]. This popularly demanded fruit tree faces so much severe biotic challenges due to bacteria, fungi, viruses and nematodes etc. (Blomme *et al*., 2017) [2]. This factor not only discourages the growth and development of plant but also affect the fruit quality thereby reducing the commercial demand in the market. Moreover, as the pathogens are evolving, chemical or physical treatment are no longer in use against pathogen growth. From the very early stages of plant, many microorganisms are associated with the growth and development, out of which some are beneficial and others are pathogenic. It is observed that the beneficial microorganisms have the potentiality to control the growth of pathogens by following different mechanisms. This beneficial microorganism survive either in phyllosphere and rhizosphere like *Rhizobium leguminosarum, Burkholderia cenocepacia, Pseudomonasfluorescence* etc or some adapt themselves to survive in the environment present inside the plant without causing damage to the plant health. This type of microorganisms is known as endophytic microorganisms (Santoyo, 2021). Anton De Bary coined the term Endophytes in 1866 to describe microorganisms that colonize internal tissues of stems and leaves. There is a huge potential of these endophytes in production of secondary metabolites, especially in life saving drug discovery.
Endophytic population are found in close association with the plant, and are gaining commercial and scientific interes for its plant growth promotion activities through IAA production, Nitrogen fixation, siderophore production and mineral solubilisation. The growth of endophyte communities are generally influenced by a combination of host species identity, host genotype, season, and environment. Ikeda *et al*., (2014) [24] also demonstrated that endobacterial diversity varies among the cultivars of the same plant species. Therefore different cultivars of the same plant show different growth rate and their responses to biotic and abiotic stresses are different. It is generally observed that the growth of wild banana plant is highly vigorous and they are found to express higher level of resistance to wide range of pathogens. This wild cultivars harbours a wide range of endophytes, and they perform versatile role giving an awesome health to the plant.

This wild banana plant are less or not treated by chemical pesticides and very less interrupted by human beings, so colonization of endophytic population is very less affected. Thus the wild banana plants favours the survival of endophytes (Hallmann *et al*., 1997; Kuklinsky-Sobrel *et al.*, 2005) [10, 15]

Colonization of bacterial endophytes

The colonization process always includes interaction between two partners. The process usually starts from the roots and the endophytes recognise the specific compounds present in the root exudates of plant. (Bulgarelli *et al*., 2012; Kawasaki *et al.*, 2016) [5, 14]. The endophytic bacteria colonise inside the plant environment in a sequence of events similar to those of rhizospheric bacteria (Hallmann *et al*., 1997) [10]. Once they enter inside the plant, they can systematically infect the adjacent plant tissues. During colonization the sub-population ranges from 10^5cfu /g fresh weight (Hallmann, 2001) [9]. This involves bacterial contact with the cell surface structures, which is mediated by their polysaccharide pili and bacterial adhesions (Hori and Matsumoto 2010) [12]. The bacteria then reach the root entry sites, like lateral root emergence and wounds, using type IV pili mediated twitching motility. Penetration can be either active or passive in nature. Passive penetration can occur through cracks in root tips, root emergence created by many other factors. Active penetration occur through dedicated machinery of attachment and proliferation, which involves lipopolysaccharides, flagella, pili, twitching motility and quorum sensing which can affect colonization and movement inside the plant. (Berne *et al.*, 2015; Zheng *et al.*, 2015) [1, 31] entry into the roots,the bacteria can spread systematically to the above ground parts. Bacterial migration is supported by either through bacterial flagella or plant transpiration stream (Compant *et al*., 2015) [6] Migration along intercellular spaces occur through degradation of cell wall by degrading enzymes like cellulose and pectinases, inxylem it occur through perforated plates that allow movement of bacteria though pores. And the final sink is the leaf tissues.

Colonization of fungal endophytes

Colonization pattern seems to be different according to the host plant. At the first fungus comes in contact with the plant host by producing running hyphae that try to penetrate through the intercellular spaces of roots. It starts with the single cells colonization parallel to the main axis of the roots, between epidermal cells and cortical cells and intercellular colonization occurs as formation of microsclerotia (O`Dell *et al* 1993) [21]. In case of *Trichoderma*, hyphae grow around roots to form appressorium-like structures, to penetrate through cortical cells and epidermal layers. Deposition of cell wall material and production of phenolic compounds by the surrounding plant cell (Bora *et al.*, 2021) [3] takes place for the growth of intercellular hyphae which showed contriction at sites where the fungus traverse from epidermal to cortical cells. Complete colonization without infection of vascular cells is observed 2-4 weeks after inoculation.

Action mechanism of Endophytes

Endophytic microbes play its role in boosting plant health through different pathways that involves both direct and indirect mechanisms.

a) Direct mechanism

It is the endophyte–pathogen interaction. In this mechanism endophytes directly produce antibiotics, which exhibits antibacterial and antifungal properties thus help in inhibiting the growth of phytopathogens (Gunatilaka, 2006) [8]. Many metabolites with antimicrobial properties have been discovered recently like flavonoids, steroids, terpenoids, alkaloids, polyketides (Mousa and Raizada, 2013) [19]. The association of pathogen with the plant triggers the secretion of secondary metabolites by endophytes. Example – Altersetin, a novel alkaloid which was isolated from endophytes *Alternaria* spp., exhibited a strong antibacterial effect against many gram-positive bacteria that are pathogenic (Hellwig *et al.*, 2002) [11].

Endophytes that colonise the surface of plants produce numerous enzymes like cellulose, hemicellulose, chitinase etc. that help in degrading the plant cell wall. Enzymes may not directly act as antagonist, but it enhances the antagonistic activities when combined with other mechanisms.

Competition with pathogen is a strong mechanism in plant protection used by the endophytes. They carry out this mechanism though colonization, either locally or systematically (Latz *et al*., 2018) [16] and then taking nutrients and space that otherwise is occupied by pathogen for their activities. This mechanism in combination with other activities is found to be effective against pathogenic microbes.

b) Indirect mechanisms

Endophytes improves the plant defence mechanism through the production of secondary metabolites and enhances its resistance. There are some rapid changes seen during interaction with the pathogens like biochemical and morphological changes, cellular necrosis and phytoalexin production (Fadiji *et al*., 2020) [7].

Endophytes induced the production of ISR (Induced Systemic Resistance) and is moderated by ethylene or jasmonic acid which is not linked with production of pathogenesis –related (PR) protein (Yu *et al*., 2022) [29]. PR protein have lytic enzymes which cause lysis of pathogen invading cells and also strengthening the cell wall boundaries and cell death. ISR induced by endophytes helps in activation of genes that are expressed in pathogenesis.

Secondary metabolites help the plant in adaptation to different environment. Endophytic colonization induced the production of hydrolase in plant cells that help in reducing the growth of fungi. Thus endophytes act as elicitors by using hydroxylation production. Examples of elicitors like glycoprotein, polysaccharides, lipopolysaccharides that triggers the defence mechanism and induce secretion of plant secondary metabolites (Fadiji *et al*., 2020) [7].

Increase growth of the plant means increase vigour and improved resistivity to biotic and abiotic stresses. This is one of the effective strategies developed by plant against pathogens. Example – Endophytes *Collectotrichum spp.*, isolated from *Artemesiaannua* produces IAA which helps in regulating plant physiology (Xin *et al.,* 2009).

Endophytes also attacks the pathogens by using hyperparasitisms. Endophytes capture the pathogen by twisting and penetrating the hyphae or by production of lyase which destroy the cell wall of pathogen. In microbial predation, endophytes exhibit their predatory characteristics during the time of nutrient deficient condition. Example –

enzymes attacking the cell wall of fungal pathogens are found to be directly produced by *Trichoderma spp* (Bora *et al*., 2021 [3]

Endophytes associated with wild banana (*Musa accuminata*)

Fusarium solani, F. oxysporum, F. semitectum, are some promising endophytes found to have effective biocontrol properties against *Fusarium oxysporum* f.sp. *cubense* and other pathogens (Zakaria *et al*., 2011) [30]. Fast competitive growth of *Cordana* sp. and antibiotic producing endophyte *Nodulisporium* sp., showed a high percentage of inhibition against anthracnose disease of banana both *in vitro* and *in vivo* screening proving them as potential biological control agents (Nuangmek *et al*., 2008) [20]. *Aspergillus, Peniophora, Meyerozyma, Saccharicola, Hypocreales, Nigrospora, Byssochlamys, Periconia, Myrothecium, Peroneutypa, Acrocalymma* are the endophytic fungal genus strains isolated from banana leaves (CAS Junior *et al*., 2018) [27]. The *Enterobacteriaceae* strains *Enterobacter* sp., *Kosakonia* sp., and *Klebsiella* sp. Isolated from wild banana spp. engineered by 1-aminocyclopropane-1-carboxylate (ACC) deaminase on the bacterial cell walls showed resistance to the *Fusarium* wilt disease (Liu *et al*., 2019) [17]. *Serratia, Pantoea, Streptococcus, Neisseria, Bacillus, Arsenicicoccus, Sphingobacterium, Herbaspirillum, Lactococcus, Variovorax, Pseudorhodoferax, Brevibacterium.* are some endophytic bacterial strains isolated from banana leaves, that helps in promoting plant growth along with protection against a wide range of phytopathogen (CAS Junior *et al.* 2018) [27].

Conclusions

Microbial endophytes and soil microbes might be employed to improve plant health and enhance productivity directly in commercial crop plants. Increasing crop productivity without harming the health of agricultural soils and compromising food quality with agrochemicals are often a challenge using present agricultural practices. Endophytes could help cultivate crops with less fertilizers, fungicides, insecticides, or herbicides. Supplementing microbial diversity through microbe amendments to soils and plants that function to bring nutrients to plants, while simultaneously suppressing virulence in pathogens, deterring insect feeding, and reducing growth of competitor weeds, may result in less environmental contamination and agricultural practices that are more parsimonious with natural processes.

Conflict of interest

The authors declare that they have no conflict of interest.

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