

**POLICY
PAPER**

71

Role of Root Endophytes in Agricultural Productivity



**NATIONAL ACADEMY OF AGRICULTURAL SCIENCES, NEW DELHI
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Role of Root Endophytes in Agricultural Productivity



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Preface

One major challenge for the twenty first century will be the production of sufficient food for the global human population which is estimated by United Nations Population Fund to reach 10 billion by 2050. If global food production is to keep pace with an increasingly growing population, the great challenge for agricultural scientists is to boost plant productivity in an environmentally sustainable manner. Sustainability refers to “Successful management of resources for agriculture to satisfy changing human needs while maintaining or enhancing the quality of the environment and conserving natural resources”. The solution for the above lies in developing new technologies with reduced use of fertilizers and pesticides besides maintaining a sustainable level of productivity. What has been largely ignored is the important role of microbial communities that interact with plants to influence plant health, productivity and biodiversity.

Endophytic microorganisms which live within plants have been shown to aid N and P nutrition and protect plants from diseases, pests and abiotic stresses. They also produce secondary metabolites which are industrially important and also in curing human diseases. Out of the 300,000 plant species existing on earth, endophytes have been isolated and studied only from a few plant species. Thus an understanding of the endophytic organisms associated with different crops assumes importance in agriculture, human health and microbial ecology taking into account the potential significance of such organisms in plant growth promotion, protection against biotic and abiotic stresses, sources of novel biomolecules and agents in bioremediation and determinants of soil and environmental health. However there are some constraints in using endophytes in low input sustainable agriculture.

The National Academy of Agricultural Sciences (NAAS) organized a Brainstorming Session on “Role of Root Endophytes in Agricultural Productivity” on July 5, 2013 to identify strategies in research, policy and development programmes with a goal to promote use of endophytes in sustainable agriculture.

On behalf of the Academy I would like to compliment the Convener Dr. D.J. Bagyaraj, NASI Senior Scientist & Chairman, CNBRCD, Bangalore and Dr. Ajit Varma, Co-Convener, Director General, Amity University, Noida for their contributions. In addition to the valuable presentations by experts in their relevant areas, there

was active participation from twenty one participants. My thanks are due to all distinguished participants of the Brainstorming Session and the Editors of the Policy Paper.



S Ayyappan
President

Role of Root Endophytes in Agricultural Productivity

1. PREAMBLE

Endophytes are microorganisms that colonize the plants internally without adversely affecting them. Fossil records show this association to be more than > 400 million years old. It has played a pivotal role in the adaptation and movement of plants from water onto land. Usually these endophytes grow intercellularly but sometimes also intracellularly and have been isolated from different plant parts, more frequently from roots. They get nutrition and protection from host plants and in return, they confer several benefits to plants. They accelerate seedling emergence, promote plant growth and increase yield, suppress pathogens, help to remove contaminants, and improve plant nutrition. Several of them produce phytohormones and also increase the tolerance against abiotic stresses such as drought, salinity, heat and heavy metals (Arnold, 2007). Several of them exhibit antagonistic effects against soil-borne plant pathogens (Schulz and Boyle, 2005). They are also a potential source for the production of secondary metabolites as antimicrobial compounds, enzymes etc., that have been exploited in medical and industrial areas (Kusari and Spiteller, 2011).

Most plants are host to one or more endophytes. Few of these plants have been studied in relation to their endophytic biology but there is opportunity to find new endophytic microorganisms among myriads of plants in different settings and ecosystems.. The feasibility of seed/seedling inoculation with endophytes is an added advantage as it is a simple inexpensive method for adapting sustainable agriculture with reduced fertilizer/ pesticide application that would improve soil health and reduce environmental pollution. Thus, an understanding of the association of endophytes and different crops assumes importance in agriculture and human health. Positive interactions between endophytes and their host plants may result in a range of beneficial effects.

2. THE ISSUES

The study of endophytes is a method-dependent process and technique used severely influence their isolation. Different hosts and plant parts require different methodologies for investigations which have to be standardised. Schultz *et al.* (1998) introduced a testing method which efficiently eliminate epiphytes. The most

frequently isolated endophytes are the fungi, though several endophytic bacteria are also known. It has been speculated that about 1.5 million fungal species may be existing but about 1,00,000 have been described (Hawksworth, 2001). The rest of them perhaps exists as endophytes, a rich and reliable source of genetic bio-diversity.

The role of mutualistic N-fixing rhizobia has been well documented for decades, but not much information on exchange of nutrients during the symbiosis of plants and bacteria is available. The plant attracts nitrogen-fixing bacteria to invade the cells in the root and provide them with carbohydrates as a food source while the bacteria reduce atmospheric nitrogen that is then used by the plants. Recent investigations have shown that not only rhizobia form nodules in roots of legumes but, several other α -proteobacteria like *Phyllobacterium*, *Methylobacterium*, *Ochrobactrum* and β -proteobacteria like *Burkholderia*, *Cupriavidus*, *Devosia* also form nitrogen fixing nodules in legumes (Moreira, 2008). The information available on these newer legume nodulating bacteria is meagre. Certain bacteria form stem nodules in legumes like *Sesbania rostrata* and *Aeschynomene indica*. The capacity of stem nodules of *S. rostrata* to fix nitrogen is speculated to be exceeding 150 kg N per hectare. The bacterium involved has been named as *Azorhizobium caulinodans*. This system offers a unique potential as a green manure in rice cultivation.

A variety of endophytic associative nitrogen fixing bacteria colonizing roots of several plants of economic importance have been described from different parts of the world. The important genera are *Azospirillum*, *Gluconacetobacter*, *Herbaspirillum*, *Azoarcus*, etc. *A. brasilense* and *G. diazotrophicus* members of α -proteobacteria have shown to be colonizing and fixing nitrogen inside tissues of rice and sugarcane. In addition, inoculation with *Azospirillum* increases the level of the phytohormones (IAA, GA3 and ABA) which promotes plant growth. *H. seropedicae* is an active plant colonizer with a low survival rate in plant-free soil and has been shown to promote plant growth and increase grain production in maize, wheat and sorghum. Though considerable work has been done on *Azospirillum* and *Gluconacetobacter* in India, only scanty information on *Herbaspirillum* and *Azoarcus* is available, requiring further investigations.

Phosphorus a major plant nutrient is of much importance as it is not only found deficient but applied P fertilizers get fixed in soil in plant unavailable form. Mycorrhizal fungi forming symbiotic association with the roots of most plants help in the uptake of available phosphorus from soil. There are different types of mycorrhizal associations. The most common association occurring in crops important in agriculture, horticulture and forestry is the arbuscular mycorrhiza

(AM), which belong to the phylum Glomeromycota and include 26 genera and 228 species. The commonly occurring, genera of AM fungi are *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora* and *Entrophospora*. These fungi being obligate symbionts have not been cultured so far on media. Though not host specific, these fungi exhibit host preference thereby enabling the researcher to screen and select the best AM fungi for inoculating a particular crop. Many workers have investigated the mechanism of improved plant growth induced by mycorrhizal inoculation. Greater soil exploration by mycorrhizal roots as a means of increasing phosphate uptake is well established. Phosphate ions are fairly immobile and a phosphate depletion zone often develops around roots in phosphate deficient soils. The hypha is able to reach beyond this zone and directly translocate nutrients from the soil to the root cortex where transfer to the plant occurs. Experiments with ³²P labeled phosphate indicate that AM hyphae obtain their extra phosphate from the labile pool rather than by dissolving insoluble phosphate. These fungi in addition to enhancing uptake of diffusion limited nutrients like phosphorus, zinc, copper etc., also improve plant growth through increased water uptake, production of plant growth promoting substances like IAA, cytokinins and protecting plants against soil-borne plant pathogens. AM fungi also have synergistic interaction with beneficial soil organisms like nitrogen fixers, phosphate solubilizers and other plant growth promoting rhizo-microorganisms (PGPRs). Field studies in our country have shown that 50% of phosphatic fertilizer can be saved through inoculation with efficient AM fungi selected for a particular crop (Bagyaraj, 2011).

A morphologically defined group, the dark septate endophytes (DSE), which have been isolated from numerous plants, including crops, is represented by different species between Ascomycota and Basidiomycota. They colonize almost all the plant tissues like roots, stem, bark, leaves, floral parts and seeds and some of them may show tissue specificity. Yuan *et al.* (2010) have demonstrated the ubiquitousness of DSE and sclerotium-like structures in root tissues of wild rice (*Oryza granulata*). Basidiomycota were the most dominant endophytes. But many of the isolates belonging to unknown taxa *Exophila*, *Cladophialophora*, *Harpophora*, *Periconia macrospinosa* and *Ceratobasidium/ Rhizoctonia* complex were the important DSE groups. Inoculation with these dark septate endophytes leading to improved plant growth has been reported by some workers through production of growth promoting substances or enhancement of nutrient uptake (Jumpponen, 2001).

There are some examples of different types of endophytes having antagonistic effects against soil-borne fungal, bacterial and nematode pathogens, and also inducing systemic resistance in plants against pathogens. Diversity of endophytic bacterial population and their interaction with the plant pathogens has been reported. Endophytic

bacteria like *Achromobacter*, *Acinetobacter*, *Pseudomonas* etc., and actinomycetes like *Streptomyces*, *Microbispora* etc., protecting plants against root pathogens has been reported as potential biocontrol agents (Coombs and Franco, 2003). Similarly endophytic fungi like *Phomopsis*, *Cloridium* and AM fungi showing antimicrobial activity against plant pathogenic fungi *Botrytis cinerea*, *Fusarium oxysporum*, and plant pathogenic bacterium *Ralstonia solanacearum* have been reported. Several of them alleviate severity of disease caused by plant parasitic nematodes *Meloidogyne incognita*, *Radopholus similis*, *Pratylenchus goodeyi*, etc. (Huang *et al.*, 2008). Endophytes are known to have anti-insect properties. Endophytic fungus *Phomopsis oblonga* protects elm trees against the beetle *Physocnemum brevilineum* and perennial ryegrass *Lolium perenne* against the sod webworm. Some endophytic isolates act against multiple pests simultaneously. More investigations are required at the field level.

Mutualistic fungal endophytes can confer tolerance to abiotic stresses like drought, salinity, heat, cold, oxidative stress, heavy metal toxicity, etc. Some examples of fungal endophytes that confer drought/water stress tolerance are *Colletotrichum magna* in tomato and pepper, *Fusarium culmorum* in rice and tomato, and *Trichoderma hamatum* in cacao (Singh *et al.*, 2011). *Curvularia protuberante* offers heat tolerance in rice, tomato, wheat and watermelon. The drought tolerance of endophyte colonized plants is attributed to the induction of drought responsive enzymes like peroxidase, catalase, superoxide dismutase as well the expression of transcripts of drought-related genes.

An endophytic bacterium isolated from cortical cells of endangered *Ginkgo biloba* roots has been evaluated for its plant growth promotion and biocontrol properties (Pandey *et al.*, 2009). The psychrotolerant bacterium was identified as *Pseudomonas* sp. and possesses potential for propagation of *G. biloba* under temperate climatic conditions. *G. biloba* has received attention for its propagation and conservation mainly due to its medicinal value and existence of only about 30 established plants all over the country, mainly in hilly tracts (Purohit *et al.*, 2007). The endophyte may play a role in the propagation of this endangered, threatened and rare plant.

The covert and integral association of endophytes with the tissue culture systems in cultivable and non-cultivable forms suggest that some of the activities of tissue cultured plants could be linked or attributable to the associated endophytes. Some of the endophytes show considerable potential for improved rhizogenesis or organogenesis *in vitro*. It is possible to exploit such organisms for improved rooting *in vitro* and in enhancing acclimatization and in improving plant vigour *ex vitro* (Thomas, 2012).

3. ACTION PLAN FOR DEVELOPMENT AND UTILIZATION OF ENDOPHYTES

3.1 Gaps in Basic Research

- a. It is high time to isolate endophytes from the different climatic zones and unique habitats of India such as Western Ghats, Thar Desert of Rajasthan, North-Eastern region, Mangroves of the Sunderbans and the Himalayas, rainforests, medicinal and bioenergy plants and the stressed niches where they are supposed to be used. These collections have to be housed in an appropriate repository. The following points are to be taken care of during/ after collection;

Identification of the endophyte through morphology and molecular methods and their metabolites that may have distinct applications.

Documentation, preservation and deposition of isolates and sequences in recognized repository centers.

Barcoding of endophytes.

Bioprospecting of these isolates for N fixation, P and K mobilization, production of phytohormones, biocontrol activity against plant pathogens and insect pests, and production of secondary metabolites which may confer tolerance to abiotic stresses.

Basic research to elucidate the host-endophyte associations and interactions.

- b. Both fungi and bacteria are the most common microbes existing as endophytes. Recent studies have revealed a diverse group of endophytic actinobacterial species from various plant species with different functions. Most of the reports on endophytic actinobacterial diversity are from China or other Asian countries. Though, India is home to rich plant genetic diversity, no report so far recorded a new species or genera of endophytic actinobacteria. Many other microbial forms exist in plants as endophytes such as mycoplasmas, rickettsia and archaeobacteria but they are not culturable employing common laboratory techniques, making their presence and role in plants even more intriguing. Future research using metagenomic approaches can help monitor such microbes.
- c. It is well known that endophytes are microbes that colonize living internal tissues of plants without causing any immediate negative effects. This leads to some very basic questions on which research is needed.

Does a plant preferentially select endophytes for its own benefits?

How do endophytes circumvent host defense response?

What are the signaling mechanisms and mutual recognition systems of communications in establishing symbiotic associations between plants and endosymbionts?

- d. In symbiotic N fixing legume nodulating bacteria, plant breeders and microbiologists should work together and select high 'nod' variants in segregating populations during varietal development and select for improved symbiosis through high nitrogen harvest index. It is also suggested to strengthen research on symbiotic N fixing actinobacteria in non-legumes like *Casuarina*, *Alnus*, *Myrica*, etc.
- e. In associative N fixing endophytes, we need to initiate research to answer the questions:

How much nitrogen is fixed inside the tissue contributing to the N economy of the plant?

Are cyanobacteria true endophytes in higher plants?

- f. Recent studies have shown that co-inoculation of AM fungi with other beneficial soil microorganisms has synergistic effect, thus there is need for development of suitable microbial consortia. Further experiments to standardize tracking down the introduced AM fungi to study their competitive ability with indigenous AM fungi, using molecular techniques are needed. We have to research on the laboratory media and techniques for the cultivation of AM fungi, which will aid easy and cost-effective mass multiplication.
- g. Regarding the biocontrol potential of endophytes, there is need to identify and evaluate endophytes with broad spectrum of biocontrol activity and their metabolites with multiple actions against pathogens and insect pests so that one biological treatment / application of BCAs controls more than one problem.
- h. Our understanding on endophytic interactions contributing to abiotic stress tolerance, especially drought, salinity and heavy metal toxicity, is still in the stage of infancy. Hence, future research should focus on the following areas:

Culturing and evaluation of endophytes under appropriate abiotic stress condition(s).

Identification of the endophytes promoting abiotic stress tolerance.

Elucidation of mechanisms underlying signaling, adhesion, endophytic competence and overcoming plant defense response for a compatible plant-endophytic interactions under abiotic stress conditions.

3.2 Best Proven Cases for Pilot Studies / Scaling-up / Business Model

- a. Some of the nitrogen fixing endophytes like *Rhizobium*, *Azospirillum* etc., have given significant economic advantage in increasing crop productivity and also in saving the application of nitrogenous fertilizer, thus reducing the cost of cultivation. These endophytes and few others like *Gluconacetobacter*, *Piriformospora indica* and AM fungi are commercially produced and available in the market.
- b. The role of AM fungi in improving plant growth is now well documented. The beneficial effects include improvement in the uptake of diffusion-limited nutrients, synergistic interaction with beneficial soil microorganisms, production of plant growth promoting substances, greater ability to withstand water stress and root pathogens. Most of the studies have been carried out under greenhouse conditions. Hence, field evaluation to validate the results of pot culture trials should be carried out. The possibility of growing AM fungi on laboratory media like any other fungi should be given high priority which will enable easy mass multiplication of the fungus and to provide quality inoculum to the farming community.
- c. An advantage of *Piriformospora indica* over the AM fungi is that it can be cultured axenically. This property gave the opportunity to mass multiply the fungus under *in vitro* conditions. The use of this fungus has to be tested extensively, especially for legumes, under the field conditions before its use is recommended.
- d. Potential biocontrol agents have to pass through several tests in order to be commercially viable. After thorough, large-scale field testing at multiple locations, under different soil and climatic conditions, these agents can be recommended for registration with the government agencies. The technology must be transferred to some firms which can take up the mass production of the product.

4. PUBLIC AWARENESS/ POLICY ISSUES

- a. Some of the endophytes like *Rhizobium*, *Azospirillum*, etc. have been discovered and researched for the last few decades, but widespread use of these products is still to take place. Availability of good quality biofertilizers and biopesticides to the farmers is still an issue along with lack of awareness about the products and their benefits. There are several constraints in the production and commercialization of these endophytes. These may be physical, chemical, biological, technological, infrastructural, financial, market related or concerned

with human resource development. These constraints have been detailed in a review (Bagyaraj, 2003).

- b. Substandard quality of inoculants is one of the most important factors resulting in failure at the field and lack of farmers' confidence in the product. The issue of quality control should be dealt with stringently to ensure quality products to the end users. Very often, locally formulated products are available in the market in plenty, but quality of those cannot be ascertained. Adequate number of laboratories should be established to test the quality control norms. There should be honesty in the production units to produce quality product, especially as it is going to marginal and poor farmers.
- c. Most of the farmers in the country do not have sufficient and clear knowledge on the use of endophytic microbes as biofertilizers and biopesticides. The farmers need to be made aware of this frontline technology through demonstration trials on the cultivator's field, as seeing is believing. Publicity programmes can also be done through media like TV, radio, seminars, exhibitions, write ups in local papers, etc. There is also a need to sensitize stake holders particularly the departments of agriculture, horticulture and forestry on the importance of promoting endophytes as biofertilizers and biopesticides.

5. RECOMMENDATIONS

1. A network project with emphasis on endophytic bacteria including actinomycetes, dark septate fungi and AM species should be developed. This will result in the exploration of newer microorganisms which will help in understanding our national heritage of endophytic microorganisms, which have not been understood and conserved so far. This can be achieved through:

Isolation, identification, characterization and documentation.

Preparing a distribution map of endophytes in terms of density and diversity for different crops/genotypes and agro-climatic zones.

Conservation and maintenance of endophytes.

Assay of endophytic microorganisms for their potential to fix N, mobilize P, to produce plant growth promoting substances, control insect pests and plant pathogens, protect crop plants against abiotic stresses through laboratory studies, glasshouse investigations and field trials.

Studies on the factors affecting efficiency of endophytes.

Developing mass multiplication protocols for the potent endophytes and their metabolites.

Human resource development by providing training to researchers in the field of endophytic microorganisms, especially on molecular characterization.

2. Suitable techniques should be followed to confirm the endophytic status of microorganisms. This is a must because the surface sterilization of the plant tissue if not done properly, non-endophytes will appear and can be mistaken as endophytes.
3. All the cultures of endophytic microorganisms available with different researchers/ institutes/SAUs/ private collections must be deposited in national repositories for proper conservation and utilization.
4. Strengthening basic research to understand the host-endophyte associations and interactions and the role played by the organisms as given earlier.
5. Exploitation of beneficial endophytes for improved organogenesis or rhizogenesis *in vitro* and at acclimatization during micropropagation of plants.
6. Application of endophytes conferring abiotic stress tolerance to crop plants which can enhance crop productivity under dry-land agriculture.
7. Recent studies have shown that co-inoculation of AM fungi with other beneficial soil microorganisms is more useful in improving plant growth, thus suggesting the need for development of suitable microbial consortia for inoculating different crop plants.
8. Different endophytes help in increased growth of plants / trees in addition to fixing atmospheric carbon in soil thus enhancing the carbon sequestration. This will help reduce global warming and climate change.
9. Possible rhizosphere engineering for increasing the density and diversity of endophytes and relating it to crop productivity.
10. Training workshops on “Isolation, identification, cultivation and use of endophytes in plant health” should be conducted at periodic intervals for the young researchers.
11. A crop based atlas of agriculturally important endophytes may be developed for use by researchers.

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Note: The designations and affiliations of the participants as on date of BSS.

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