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Review Article

Endophytic and Rhizosphere Fungi in Wheat: Ecology, Methodology and Prospects for Sustainable Crop Improvement"

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Abstract

Fungi play a central role in both natural and agricultural ecosystems because of their nutritional versatility, decomposer activity and close associations with plants. In cereal systems, especially wheat, endophytic and rhizosphere fungi contribute to plant growth, nutrient uptake and also to resilience against different abiotic and biotic stresses. However, modern wheat production is still highly dependent on agrochemicals, which can harm the environment and human health, and this push researchers to look more towards microbial-based strategies. This review paper tries to summarize the available knowledge about: (i) the classification and ecological roles of endophytic fungi, with special focus on wheat and related grasses; (ii) culture-based and culture-independent methods used to isolate and identify wheat-associated fungi; (iii) the structure and function of rhizospheremicrobiomes and their plant growth-promoting potential; (iv) the effect of agronomic practices like phosphorus fertilization and tillage on rhizosphere fungal communities; and (v) molecular and epigenetic mechanisms that underlie wheat fungus interactions. Overall, the literature supports the idea that endophytic and rhizosphere fungi, together with plant growthpromoting rhizobacteria, form a strong resource for developing microbial inoculants, biostimulants and biocontrol agents for more sustainable wheat cultivation.

Keywords:

Endophytic Fungi; Rhizosphere Fungi; Wheat (Triticumaestivum); Plant–Microbe Interactions; Plant Growth Promoting Fungi; Rhizospheremicrobiome; Fungal Biodiversity

1. Fungi in Plant–

Soil Systems and the Concept of Fungal Endophytes

Fungi play many interconnected roles in terrestrial ecosystems. They act as primary decomposers and nutrient recyclers, and they also can behave as symbionts or pathogens, sometimes both depending on the situation. Because of their wide enzymatic capacity and flexible lifestyles, fungi can colonize a wide variety of ecological niches, including the rhizosphere and the inner tissues of plants [Grigoriev 2013]. In association with plants, fungi may grow on the surface or inside the tissues of roots, stems, leaves and reproductive organs. These interactions range on a spectrum from mutualistic to parasitic. Fungal endophytes are usually known as fungi which live inside plant tissues for at least part of its life cycle without causing any visible disease. This group is now known to be almost ubiquitous. Endophytes have been found in nearly all plant species examined, from trees and grasses to lichens and herbaceous crops, in environments ranging from agricultural fields to natural ecosystems, and from tropical lowlands to high mountain regions [Hardoim et al., 2015].

A second, widely used framework separates clavicipitaceous (C-endophytes) from non-clavicipitaceous (NC-endophytes) fungi. C-endophytes (class 1) include Balansia spp., Epichloë spp. and Claviceps spp., which form systemic associations almost only with grasses, rushes and sedges. These fungi colonize shoot meristems, grow through intercellular spaces in developing shoots and are mainly transmitted vertically via seeds. Some Epichloë and Neotyphodium species can also be spread horizontally by infected leaf fragments falling on soil [Redman et al., 2002]. NC-endophytes, grouped into classes 2–4, have wider host ranges and tissue preferences. Class 2 fungi colonize both below- and above-ground tissues and can be horizontally and/or vertically transmitted. Class 3 Dikaryomycota are mostly restricted to aerial tissues of different hosts and are spread horizontally. Class 4 are dark septateendophytes, which colonize mainly roots and occupy inter- and intracellular spaces in the root cortex [O'dell et al., 1993].

2. Ecological Roles of Endophytic Fungi and Plant Fitness

Endophytic fungi can impact on its host plants in different ways and outcome is not always same. In some case they clearly support the plant, in others they seem almost no impact , and under certain conditions they may even add a little stress. How an endophytic fungus behaves in a plant depends on many things, such as which crop variety it is, what fungal species or strain is there, the local environment and which other microbes are living with the plant (Hardoim et al., 2015). Many of these fungi are thought to be useful because they can support plant growth, help the roots take up more nutrients and make the plant more tolerant to non-living stresses like drought, salty soils or very hot and cold weather. They may also lower disease by fighting with harmful microbes, by making substances that stop pathogens, or by turning on the plant's own defence system.Plants grown under sterile or microbe-poor conditions often show weaker growth and greater sensitivity to stress, which suggests that their natural microbial partners play an important role in plant health (Innerebner et al., 2011). In contrast, adding selected endophytic fungi has in some wheat studies led to higher seed germination and better viability of the next generation of seeds under heat and drought conditions (Hubbard et al., 2012). Because of such findings, there is growing interest in describing the full microbial communities of major crops like wheat and in picking out particular endophytic fungi that could later be developed as seed inoculants, plant growth stimulants or biological control agents.

3. Wheat as a Target for Microbiome Based Improvement

Wheat (Triticumaestivum L.) is the major cereal crop of world. It supplies a big part of the food energy for human beings and it also commonly used as feed for other animalstoo.In Europe, wheat production in 2018–2019 was more than 254 million tonnes, which is about one-third of the total world wheat output. Even though wheat is so important, wheat cultivation is now facing many problems. Due to climate change there are more frequent and severe droughts, late spring frosts and winter cold damage. When snow cover on the field is low or absent, the crop is less protected and frost injury can be more serious. These factors together make it hard to maintain high and stable wheat yields (Mäkinen et al., 2018). Over the last several decades, plant breeders have done a lot of work to improve wheat. They have raised the yield potential, improved grain quality and developed varieties that can better tolerate some types of stress (Araus et al., 2008).

But in most cases these improvements depend on heavy use of chemical fertilisers and different pesticides such as herbicides, insecticides and fungicides. This intensive use of chemicals can cause many problems: more greenhouse gases, pollution of rivers and groundwater, loss of useful plants and animals from fields and hedges, and possible harmful effects on human health. Because of these issues, more attention is now given to farming systems that use fewer chemicals, including organic and low-input systems.

In this new approach, helpful microorganisms around the wheat root, such as endophytic fungi, rhizosphere fungi and plant growth-promoting rhizobacteria (PGPR), are seen as important tools. These microbes can improve nutrient use, support growth and help the plant to face stress and disease, so that chemical inputs can be reduced. To use these microbes in a proper and planned way, we first need a good understanding of which fungi and bacteria live with wheat, how they behave in the field, and what roles they play in the growth and health of the crop.

4. Culture-Based Isolation of Endophytic Fungi from Wheat

Culture-based methods are still the basic approach for studying endophytic fungi in wheat, especially when we want to obtain pure isolates for functional tests or for making inoculants. In general, these methods include sampling of plant tissues (like roots, stems, leaves, spikes and grains), surface sterilization to remove epiphytic microbes, cutting tissues into small pieces and plating them onto different solid media [Comby et al., 2016]. The size of the tissue fragments is important. Gamboa et al. [2003] showed that smaller fragments led to higher apparent species diversity, probably because they reduce the dominance of fast-growing species and give a chance to more slow-growing or rare fungi.

In wheat studies, plant material is usually cleaned under running tap water to remove soil. Roots can be lightly brushed or scraped to get rid of soil particles [Cłapa et al., 2020]. Surface sterilization most often uses ethanol and sodium hypochlorite in sequence, followed by several rinses with sterile distilled water. For example, Larran et al. [2002] sterilized wheat leaves, stems, glumes and seeds by treating them with 96% ethanol, then 2% sodium hypochlorite, and again 96% ethanol, with sterile water rinses in between. Similar protocols are used for roots and other tissues, but exposure times or concentrations may be slightly adjusted depending on how sensitive the tissues are [Hubbard et al., 2014]. The success of sterilization is then checked by plating some of the final rinse water. If no colonies grow, the surface is considered clean.

5. Identification of Endophytic Fungi: Morphological and Molecular Approaches

Traditionally, fungal endophytes are identified based on their morphology. Macroscopic features like colony colour, texture, border shape, growth rate and pigmentation are recorded on culture plates. Microscopic structures, including hyphalseptation and branching, conidiophores and the size and shape of spores, are studied using light microscopy and sometimes scanning electron microscopy [Hyde &Soytong 2008]. Lactophenol cotton blue stain is often used for better visibility of hyphae and spores. While this classic approach is still useful, especially at genus or higher levels, it has several limitations. Isolates of the same species can show high phenotypic plasticity depending on culture conditions, and different species may have very similar morphologies (cryptic species). Convergent evolution can also lead unrelated taxa to look alike, and some fungi do not sporulate easily under laboratory conditions [Geiser 2004]. Moreover, traditional taxonomy does not always reflect evolutionary relationships when it ignores physiological, ecological and chemical differences [Raja et al., 2017].

6. RhizosphereMicrobiomes and Plant Growth-Promoting Microorganisms

The rhizosphere is the thin layer of soil around roots that is directly affected by root exudates and root-associated microbes. It is one of the most biologically active zones in soil. Microbial biomass in this region, including fungal mycelium, can immobilise significant amounts of mineral nutrients, for example up to about 20% of total soil nitrogen and phosphorus, acting as a temporary reservoir [Wu &Srivastava 2012]. In citrus orchards, Wu and Srivastava [2012] isolated several promising rhizosphere microbes, such as Bacillus polymyxa, Azotobacterchroococcum, Bacillus mycoides, Pseudomonas fluorescens and Trichodermaharzianum. They developed a microbial consortium from these strains, which worked well both in nurseries and in adult trees, improving plant performance and supporting rhizosphere health.

Conclusion :

The studies discussed in this review make it clear that endophytic and rhizosphere fungi form an important part of the wheat production system. They take part in nutrient cycling, support plant growth and help the crop to cope with different stresses. Their diversity and functions can be studied well when classical culture and morphology methods are combined with modern molecular tools, using proper sampling and surface-sterilisation procedures. Work on wheat and other crops also shows that these fungal communities, together with useful rhizobacteria, are influenced by farm practices such as fertiliser use, crop rotation and tillage, and that they can be managed to lower the need for chemical inputs. In the long run, a deeper knowledge of the ecology, taxonomy and mode of action of wheat-associated fungi will be needed to design reliable microbial inoculants and to fit them into breeding and agronomy programmes that aim at more sustainable crop production.

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