

Use of the Plant Growth Promotion Rhizobacteria as Biocontrol Agents: Perspectives for the Use of *Azospirillum brasilense* to Control Barley Yellow Dwarf Virus

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Abstract

Plant growth promoting rhizobacteria (PGPR) are soil-dwelling microorganisms that live in association with plant roots. PGPR can play an important role in agricultural by enhancing crop production as well as increasing plant resistance against damaging pests and diseases. In the case of the destructive viral disease of cereal crops barley yellow dwarf virus (BYDV), there is currently no treatment available after a plant becomes infected. Control of this aphid-transmitted viral disease is limited to resistant plant cultivars and/or chemical control of vector populations. With increasing environmental pressures to move towards sustainable agriculture, PGPR-based disease control provides a viable alternative to chemical pesticide applications. Here, we aimed to review the potential use of PGPR *Azospirillum brasilense* to protect wheat plants against barley yellow dwarf virus, and the underlying mechanisms for PGPR-mediated changes on plant-virus-vector interactions.

Keywords: Crop protection, Induced defense, Plant defense, Plant growth-promoting rhizobacteria, *Rhopalosiphum padi*, Symbiosis.

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INTRODUCTION

Since plants are often attacked by multiple herbivores and pathogens, they have developed a complex system of defence against these organisms. However, positive associations commonly occur between plants and other organisms, such as rhizobacteria, contributing in a beneficial manner to plant survival.

Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria that colonize the rhizosphere and interact symbiotically with the plant, creating a positive effect [1] by the nitrogen fixation and inducing systemic resistance against herbivores [2-5] and plant diseases [6-11]. Since PGPR stimulate plant growth and greatly improve crop yields, their use has been incorporated successfully into agricultural practices in many countries [12, 13].

PGPR can benefit plant growth by modulation of plant hormone levels, such as 3-indole-acetic acid, cytokinins, auxins and gibberellins [14], as well as improving their nutritional status, via associative nitrogen fixation, phosphate solubilization and absorption, and phytosiderophore production [15].

These rhizobacteria can also stimulate an increased plant resistance against pathogenic microorganisms that inhibit the plant growth [10, 16] regulating from siderophores, antibiotics synthesis, enzymes or fungicidal compounds [17]. Moreover, PGPR trigger induced systemic resistance (ISR), characterized as a state of alert in the plant given by the expression of associated genes. As a result, plant defensive responses are induced faster and more intensively after being infected by pathogens or attacked by insects [18, 19].

Despite the significant advances in the field and the promising use of PGPR as a biocontrol agent to enhance plant immune system against vector-borne viruses, so far, there are few studies that investigated the effect of PGPR colonization in plant-insect vector interactions. It is of the utmost important to know if PGPR colonization changes the interaction between the host plant and the insect-vector in order to assess if PGPR actually enhance plant protection against insect-borne viruses. Also, as the benefits provided by PGPR depend on the strain/plant cultivar combination, it is necessary to carry out specific studies for each crop and plant disease of interest. Finally, a more comprehensive understanding on the mechanisms involved in PGPR-

induced resistance should provide useful baseline figures for developing a potential integrated pest management strategy to greatly reduce yield losses due to pathogen infections.

Barley yellow dwarf (BYDV) is the most widely distributed and economically important aphid-transmitted viral disease of cereal crops in the world [20, 21]. As there is no treatment after plants get infected, the main control tactics are the use of resistant plant cultivars [21], or control vector populations using chemicals [50]. Nevertheless, considering the benefits of PGPR as biotic agents to control or suppress plant diseases, the use of these microorganisms can be an interesting strategy for disease management.

Induced systemic resistance by PGPR inoculation

The mechanisms involved in the induction of plant growth by association with PGPR can vary according to the target species. Volatile organic compounds (VOCs) emitted by two PGPR species, *Bacillus subtilis* GB03 and *B. amyloliquefaciens* IN937, appear to be responsible for promoting growth in *Arabidopsis thaliana* [22]. VOCs emitted by rhizobacteria may also trigger induced systemic resistance (ISR) in *Arabidopsis* [23].

Initially, ISR triggered by PGPR colonization showed similarities to the systemic acquired resistance (SAR), induced by infection of pathogenic microorganisms. SAR is mediated by salicylic acid (SA) and characterized by the activation of pathogenesis-related genes, which express antimicrobial proteins [24]. Indeed, ISR activated by root PGPR colonization can be SA-dependent [25], but, in some cases, can also be activated by pathways dependent on jasmonic acid and ethylene [26].

It is also expected that the interaction between signaling pathways activated by PGPR and those mediated by induced defense responses will modify plant-insect interactions [27]. However, the effect of PGPR colonization seems to vary according to the herbivore feeding strategy with the host plant. For example, the ISR induced by *Pseudomonas fluorescens* in *Arabidopsis* had a positive effect on the biology of the general aphid *Myzus persicae*, whereas a neutral effect was observed for the specialist aphid *Brevicoryne brassicae* [28]. Besides, for chewing insects, the activation of ISR and SAR in the same plant reduced the growth and development of *Spodoptera exigua*, but did not affect any biological parameter of the specialist *Pieris rapae* [29].

Additionally, ISR induction by PGPR colonization has been reported to reduce the severity of some diseases. In beans, *Bacillus pumilus* and *B. subtilis* conferred greater protection against the insect-borne pathogen bean common mosaic virus (BCMV) [30]. Tomato plants inoculated with *B.*

amyloliquefaciens, *B. subtilis* and *B. pumilus* presented reduced disease severity caused by infection with tomato mottle virus (ToMoV) [6]. Likewise, cucumber and tomato plants upon colonization by *Pseudomonas fluorescens* and *Serratia marcescens* also become more resistant to cucumber mosaic virus (CMV), an aphid-transmitted plant virus [8].

Barley Yellow Dwarf Disease

Barley yellow dwarf virus (BYDV) is one of the most dangerous viral diseases in winter cereals [20]. Such as most viruses belonging to *Luteovirus* genus (Luteoviridae), the BYDV has isometric viral particles of approximately 25nm in diameter. The viral genome is composed of single-stranded and positive-sense (ssRNA +) strand with ca. 5,600 nucleotides [31].

BYDV is restricted to plant phloem and is transmitted only by aphids in a persistent, circulatory and non-propagating manner [32, 33]. The acquisition of viral particles occurs during aphid feeding on phloem cells [34]. Inside the insect body, the virus penetrates the lining of the intestine and reaches the hemolymph. Through the circulatory system, the viral particles are transported to the insect salivary glands where the viral proteins are recognized and connect the glycoprotein of the basal lamina of the accessory salivary glands. The transmission to uninfected plants occurs when the insects feed and the virus is excreted through the salivary channel [35].

The viruses associated with yellow dwarf disease (YDD) were initially classified according to the specificity of transmission by the vector species: RPV (*Rhopalosiphum padi*); RMV (*R. maidis*); MAV (*Sitobion avenae*, old *Macrosiphum avenae*); SGV (*Schizaphis graminum*); PAV (*R. padi*, *S. avenae*) [36].

The infection occurs mainly between leaf emergence and tillering of the plants [49]. The virus symptoms observed on the plants are more frequent on the flag leaf four weeks after virus inoculation. Moreover, the virus induces stunting, chlorosis, reddening and yellowing of older leaf tips depending on susceptibility of the cultivar [20].

Azospirillum genus and the perspectives for its use as a biocontrol agent against BYDV

The genus *Azospirillum* (Rhodospirillaceae) comprises gram-negative, microaerophilic, non-fermentative and chemoorganotrophic bacteria, belonging to the alpha-subclass of proteobacteria [48]. These bacteria are facultative endophytes [37], with a versatile carbon and nitrogen metabolism, making them adapted to establish in the competitive environment of the rhizosphere [38].

Since its discovery, more than 40 years ago [39], *Azospirillum* has been isolated from the rhizosphere of different species of grasses and cereals

worldwide [47], being one of the most studied plant-associated bacteria [40]. Also, it is an extensively commercialized PGPR in countries, such as Argentina, France, India, Italy and Mexico [41]. In Brazil, *A. brasilense* has been widely used as a commercial inoculant in corn and wheat [42]. Like most of the PGPR, *Azospirillum* is also known to enhance the growth of plants whose roots are colonized, and their state of defense against the attack of pathogens and herbivorous insects.

Aphids are one of the most important insects responsible for the transmission of plant viruses [43] and in Brazil, *Rhopalosiphum padi* (Aphididae) is among the main pests of wheat crop for direct damage and by the transmission of the BYDV. This plant virus is the most widely distributed and economically important aphid-transmitted viral disease of cereal crops in the world [20, 21]. Since there is no efficient treatment for plants infected by BYDV, the main control tactics are the use of resistant plant cultivars [21, 44], or chemical control of insect vectors [45]. Nevertheless, considering the benefits of PGPR as biotic agents to control or suppress plant diseases, the use of these microorganisms can be an interesting strategy for disease management.

It is already known that a single inoculation of *A. irakense* in wheat was not effective to deter the severity of BYDV infection in the field, when the inoculant was applied before and after the infection, and the symptoms were reduced. Despite that, the mechanism involved in this interaction was not investigated [46].

CONCLUSIONS

The PGPR is widely used around the world, with benefits mainly related to the growth and plant development. On the other hand, this mini-review addresses new perspectives about the effect of these microorganisms on plant defense against insect herbivores and pathogens. *Azospirillum* genus is one of the most studied and commercial PGPR in several countries. Although it is mainly used in order to improve plant growth and yield in many agricultural systems, we still have little information about the effect of these bacteria on plant resistance. The use of *Azospirillum* against the worldwide distributed BYDV was reported to be effective to reduce disease severity. Finally, a more comprehensive understanding of the mechanisms involved in PGPR-induced resistance should provide useful baseline figures for developing a potential integrated pest management strategy to reduce yield losses due to BYDV infections.

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