

Prospect of Endophytic Fungal Entomopathogens for Pest Management

Xin Zhao, Shuaishuai Huang, Zhibing Luo, Yongjun Zhang*

Biotechnology Research Center, Academy of Agricultural Sciences, Southwest University, Chongqing, China

ABSTRACT

Endophytic fungal entomopathogens could offer a number of benefits to their host plants, which include protection against the primary pests, resistance against microbial pathogens, stimulation of growth, or increase of heavy metal or drought tolerance. However, all reports, so far, indicate that colonization of some plants by entomopathogenic fungi seemly confer only certain benefits to their hosts or else only one parameter was examined. Our study underscores the practicability of introduction of specific insect fungal pathogen strains into host plants as endophytes that could simultaneously promote host plant growth and offer plant protection against microbial pathogens, and/or insect pests, which highlights the prospect of endophytic fungal entomopathogens for pest management. Here, we further commented on the study-involved issues and the factors that possibly affect colonization by fungal entomopathogens and the benefits to the host plant offered by the endophytes. Moreover, we put forward the possible link of some plant morphological changes and/or stimulation of some plant defense pathways with the resistance of the host plants against insect/ microbial pathogens, although the underlying mechanism remains somewhat of a mystery that should be uncovered in the future.

Keywords: Endophytic fungal entomopathogens; Pest management; Resistance to plant pathogen; Plant growth promotion

INTRODUCTION

Fungal entomopathogens occupy a specific niche as insect pathogens as well as plant endophytes. Most research on insect fungal pathogens has focused on development of biocontrol agents for direct control of the target insect pests [1]. Advance demonstrated that several insect fungal pathogens, such as the genera *Beauveria* and *Metarhizium*, naturally have a plant colonization life cycle that form intimate associations with various plants, suggesting a larger role of these fungi in the ecosystem beside insecticidal action only [2]. Most importantly, these insect fungal pathogens could be artificially colonized into many plants as endophytes that provide beneficial effects to their host plants, i.e., stimulation of growth, increase of heavy metal or drought tolerance, or increase of resistance to microbial pathogens, aside mere protection against the primary pests, suggesting an alternative strategy to using fungal agents as plant colonizers.

LITERATURE REVIEW

Several insect fungal pathogens have been artificially colonized different plants as endophytes, however, plant colonization and effects on host plant growth, resistance against pest insects or microbial pathogens have been reported to vary significantly between different fungal species, the method of inoculation, as well as the physiological status of the plant [3]. For example, both *B. bassiana* and *M. anisopliae* were shown to endophytically colonize cassava roots following soil drench inoculation, however neither was found in the leaves or stems of the treated cassava plants. Several strains of *B. bassiana* and *M. anisopliae* were also able to colonize the common bean (*Phaseolus vulgaris*) via inoculation of bean seeds in conidial suspensions by a simple soaking protocol. Whereas, colonization was generally higher in roots than on stem and leaves [4]. Difference in colonization success has also been reported among different insect fungal pathogen treatments [5]. The entomopathogens *B. bassiana*, *Lecanicillium lecanii* and *Aspergillus parasiticus* could individually

Correspondence to: Dr. Yongjun Zhang, Biotechnology Research Center, Academy of Agricultural Sciences, Southwest University, Chongqing, China, E-mail: yjzhang@swu.edu.cn

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colonized the seedling of all six crop plants, cotton, wheat, bean, corn, tomato and pumpkin by spraying conidia on leaves or applying conidia in soil. However, colonization, persistence and plant tissue localization of the fungi, as well as their effects on the plant growth, were highly variable in plant species, inoculation approaches and fungal species [3,6]. In this study, Qin, et al. found that tobacco (*Nicotiana benthamiana*) seedlings were endophytically colonized by *B.bassiana* strains via a simple co-culturing of conidial suspensions with seeds prior to planting [7]. Different colonization rates by several *B.bassiana* strains were also examined. However, all of the colonized *B.bassiana* cells could be detected and recovered from the plant leaves and stems 50 days post-inoculation, although more fungal cells have been detected in stems as compared to leaves and roots, demonstrating that inoculated *B.bassiana* strains have systematically colonized the seedlings, including leaves, stems and roots. Moreover, this study provides a new insight into the inoculation protocol of insect pathogenic fungi as endophytes, that is the seed-inoculation prior to planting which would greatly avoid or decrease labor and cost intensively as compared to those inoculation methods, i.e., via soil drench and foliar spray for seedling.

In addition to colonization of plant to form intimate (endophytic) associations, it is important to examine and confirm potential benefits including promotion of plant growth, increased resistance to plant microbial pathogens, and increased protection against insect pests. Similar to other fungal endophytes, it is expected that introduction of insect fungal pathogens into plant as endophytes offers a number of benefits for host plants. However, all reports, so far, indicate that colonization of some plants by entomopathogenic fungi seemly confer only certain benefits to their hosts or else only one parameter, i.e. resistance against pest insects or microbial pathogens, stimulation of growth, or increase of heavy metal or drought tolerance [8,9]. These results might be dependent on the aim of artificially colonization of plants by these fungi to some extent. Of course, endophytic fungal strain/species variation might be the most important factor on their offered benefit parameters for the host plants [10]. Moreover, inoculated methods that affect those benefits for plants have also been reported [11]. Despite those factors that might affect colonization and the benefits for the plants, this study by Qin, et al. underscores the practicability of introduction of specific insect fungal pathogen strains into host plants as endophytes that could promote host plant growth and offer plant protection against microbial pathogens, and/or insect pests [7].

DISCUSSION

The main purpose of artificial introduction of insect fungal pathogens into plants is for (indirect) control of pest insects. The target pests managed by endophytic insect fungal pathogens cover many insects of *Diptera*, *Homoptera*, *Orthoptera*, *Lepidoptera*, *Hemiptera*, *Coleoptera* and *Hymenoptera*, which are able to feed on many plants [3]. Similarly, this study by Qin, et al. demonstrated that colonization of tobacco (*Nicotiana benthamiana*) seedlings by several *B. bassiana* strains offered resistance against aphids (*Myzus persicae*), confirming potential of

insect fungal pathogens as endophytes for control of insect pests [7]. However, distinct effects on aphid control were also seen in the plants colonized by several *B. bassiana* strains. In the six tested *B. bassiana* strains, four stains (Bb07, Bb08, Bb0062 and Bb029)-colonized seedlings displayed significantly increased resistance against the aphids, with little effects for Bb025 (another strain)-colonized plants. However, no obvious difference in aphid control was examined between the Bb02-colonized seedlings and control seedlings (no-*B.bassiana* treatment).

Protection against microbial pathogens as a result of endophytic association of insect fungal pathogens with plants has also been reported. Most research has focused on control of plant fungal pathogens, such as *Rhizoctonia solani* and *Pythium myriotylum* (tomato and cotton pathogens), *Fusarium oxysporum* (causing basal rot of onion), and *Plasmopara viticola* (causing downy mildew on grapevine leaf) [12-14]. Moreover, it was also reported that colonization by some *B. bassiana* strains offered plant protection against viruses (i.e., Zucchini yellow mosaic virus), and phytoparasitic nematodes (i.e, *Heterodera filipjevi*) [15,16]. This study showed that colonization of tobacco seedlings by specific *B. bassiana* strains significantly increased plant tolerance/resistance against the bacterial pathogen *Ralstonia solanacearum* Yabuhi besides against fungal pathogens, such as *Alternaria alterana* and *Botrytis cinerea*, which expands the insight into biocontrol potential of insect fungal pathogens as endophytes for management of plant microbial pathogens. Similarly, the fungal strain variation was seen in control of plant pathogens in this study. In all of the six tested *B.bassiana* strains, only Bb0062-colonized seedlings displayed significantly increased resistance against bacterial pathogen, *R. solanacearum*, with smaller effects seen for Bb02 as compared to untreated control plants. However, no such effect was observed in the plants colonized by other four *B. bassiana* strains. With respect of control of plant fungal pathogens, all the tested strain except for Bb025 could enhanced plant resistance against *A. alterana*, while only Bb0062, Bb08 and Bb02-colonized seedlings displayed increased resistance to *B. cinerea* [7].

Promotion of plant growth is another benefit offered by the endophytic colonized insect fungal pathogens, i.e., improvement of plant height, weight, rood development and/or weight of seed [3]. Similar to reports previously [10], distinct effects of endophytically colonized insect fungal pathogens on plant growth were also seen in this study by Qin, et al. [7]. Four of the six tested *B. bassiana* strains, Bb02, Bb08, Bb0062 and Bb07, distinctly improved growth of the tobacco plant, with significantly increased leaf number, plant height, leaf area (of the first, second and third leaves), root development, i.e., the primary root length and number of lateral roots, as well as overall plant biomass (both aboveground and belowground parts). Little growth promotion was examined in the plants colonized by another strain, Bb029, although development of the primary root, i.e. root length, was stimulated by the fungal strain. However, colonization by the remaining strain, Bb025, showed less pronounced effects as compared to other strains. In addition, colonization by five strains, Bb02, Bb08, Bb07, Bb0062 and Bb29, resulted in a significant increase in the 1000-grain weight of newly produced tobacco seeds. Moreover,

colonization by strains Bb0062, Bb07, Bb08 and Bb02, significantly increased net photosynthesis rates of tobacco plants with smaller effects seen for Bb025 and Bb029 as compared to untreated control plants, which seemed consistent with their effects on plant growth promotion despite of varied chlorophyll (a and b) content in their colonized leaves of the plants.

Altogether, the study by Qin, et al. demonstrated that although *B. bassiana* strain variation was detected in plant growth effects, pest control and/or resistance/tolerance against microbial pathogens, there was one strain, such as Bb0062, that simultaneously boosted plant growth, increased resistance to aphids and the plant bacterial and fungal pathogens [7]. These data suggest that it is practical to simultaneously boost host plant growth and increase resistances against insect pests and microbial pathogens by introduction of appropriate species/strains of insect fungal pathogens as endophytes, which highlight the potential of endophytic fungal entomopathogens for pest management.

Moreover, this study by Qin, et al. found that tobacco seedlings colonized by *B. bassiana* displayed morphological changes, including increased stomatal and trichome density, which might offer the resistance against fungal pathogens and aphids via physiologically hindering them from infection on leaves to certain extent [7]. In addition, stimulation of plant Salicylic Acid (SA) and/or Jasmonate Acid (JA)-associated defense pathways was detected in some *B. bassiana* strain-colonized plants, potentially indicating induction of increased systemic resistance pathways in host plants, which might contribute to the observed increased resistances to the microbial pathogens and/or insect pests. However, some puzzles on those issues need to be unveiled in the future. (1) How the endophytic insect fungal pathogens affect plant morphology and stimulate plant defense pathways; (2) How the endophytic insect fungal pathogens promote plant growth, protect host plants against microbial pathogens and/or insect pests; (3) What the underlying mechanism of the fungal strain variation in plant growth effects, pest control and/or resistance/tolerance against microbial pathogens is.

CONCLUSION

Uncovering those mechanisms would be helpful in understanding intimate associations of insect fungal pathogen with host plants and beneficial effects on host plant offered by those associations, meanwhile would provide theoretical guidance for screen of entophytic fungal entomopathogens of a given plants for pest management.

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