ESTABLISHMENT OF AN ENTOMOPATHOGENIC FUNGUS AS AN ENDOPHYTE IN THE COMMON BEAN PLANT

Jannet Edith Salinas Hernández, [1] Socorro Guajardo González, [1] Laiju Kuzhuppillymyal Prabhakarankutty[2]*

[1]Centro de Investigación y Desarrollo de Educación Bilingüe, Unidad Mederos, Universidad Autónoma de Nuevo León, Avenida Lázaro Cárdenas, Monterrey, Nuevo León, México.

[2]Facultad de Agronomía, Universidad Autónoma de Nuevo León, Francisco Villa S/N, Ex Hacienda El Canadá, C.P. 66050, General Escobedo, Nuevo León, México.

*Correspondence: laijuprabha@gmail.com

Abstract

The endophytic capacity of some entomopathogenic fungi has gained the attention of many scientists worldwide. Beauveria bassiana is one of the potential candidates because of its ability to resist biotic and abiotic challenges in agricultural plants and its role as a growth promoter. In this research, we would like to share our preliminary results about the successful colonization of the fungus Beauveria bassiana in bean plants by seed treatments. Initial results indicate successful colonization, which could contribute to enhanced drought tolerance and pest resistance. These findings align with prior studies on maize, suggesting broad applicability across crop species. Future research should focus on long-term impacts and optimal application methods.

Keywords: Beauveria bassiana, Phaseolus vulgaris, Seed treatments, Biotic factors, Abiotic factors.

COLONIZACIÓN ENDOFÍTICA DE UN HONGO ENTOMOPATÓGENO EN LAS PLANTAS DE FRIJOL

Resumen

La capacidad endofítica de algunos hongos entomopatógenos ha captado la atención de muchos científicos en todo el mundo. Beauveria bassiana es uno de los candidatos potenciales debido a su capacidad para resistir desafíos bióticos y abióticos en las plantas agrícolas y su papel como promotor del crecimiento. En esta investigación, nos gustaría compartir nuestros resultados preliminares sobre la colonización exitosa del hongo Beauveria bassiana en las plantas de frijol mediante tratamientos de semillas. Resultados preliminares indican una colonización exitosa, lo que podría contribuir a una mayor tolerancia a la sequía y resistencia a las plagas. Estos hallazgos están alineados con estudios previos en maíz, sugiriendo una amplia aplicabilidad en diversas especies de cultivos. La investigación futura debería centrarse en los impactos a largo plazo y en métodos óptimos de aplicación.

Palabras clave: Beauveria bassiana, Phaseolus vulgaris, tratamiento de semillas, factores bióticos, factores abióticos.

Feeding a global population of 10 billion by 2050 demands innovative and sustainable solutions to overcome biotic stress like insects, herbivores, parasites etc. and abiotic stress like temperature, ultraviolet radiation, salinity, drought etc. Leveraging scientific advancements and promoting integrated agricultural practices can enhance food security and ensure a stable food supply for future generations (Jaiswal D.K. et al. 2022). Addressing pesticide resistance and its associated impacts requires a shift towards sustainable agricultural practices that minimize reliance on chemical inputs, thereby protecting human health, preserving environmental integrity, and reducing economic burdens (Brian P.B. et al., 2020). Microbiologists, plant pathologists, entomologists, and other researchers across the globe have concluded that biological control can play a significant role in sustainable agriculture. The increasing consumer demand for sustainable agricultural practices, driven by the awareness created by researchers, academicians, and non-governmental organizations, is accelerating the shift toward biological control, which addresses the environmental and health issues associated with today's agricultural practices but also enhances global food security by promoting sustainable and resilient agricultural systems (Jaiswal D.K. et al. 2022). Biological control is a cost-effective, eco-friendly, and long-term solution for plant protection against biotic and abiotic stresses.

Biological control, distinguished from natural control by its human intervention, encompasses a range of techniques aimed at managing pest populations through natural enemies. The main biological control techniques are classical (or inoculative), augmentative, and conservation control. Each method has its unique application and effectiveness depending on the crop and the stress (Balel J.S, et al., 2018). Classical biological control involves introducing small numbers of natural enemies to control exotic pests over long periods, typically in perennial crops. Augmentative biological control includes periodic releases of natural enemies, providing immediate, but often temporary, pest control and seasonal inoculative control allowing for population build-up within a growing season. Conservation control focuses on enhancing the effectiveness of indigenous natural enemies through various ecological manipulations (Balel J.S, et al., 2018). These methods highlight the versatility and potential of biological control as a sustainable alternative to chemical pesticides. Integrating these practices, agricultural systems can reduce reliance on synthetic inputs, mitigate environmental damage, and improve crop health and productivity.

Globally, the inoculative method has recently gained attraction because an endophyte, to effectively combat stress, needs to be present in the plant tissue when the stress occurs. Successful colonization of an endophyte in a plant tissue depends on abiotic factors such as temperature, rain, humidity, and UV rays, as well as biotic factors including plant age, susceptibility, physiology, competition with other endophytes, and the plant's immune system (Kuzhuppillymyal-Prabhakarankutty. L, et al., 2021). Plants' symbiosis with beneficial microorganisms, evolved escape strategies, including avoidance, tolerance, and activities to reduce the effects of abiotic stresses like drought (Vigani. G, et al., 2018) and biotic stress like Spodoptera frugiperda in maize plants. (Kuzhuppillymyal-Prabhakarankutty. L. et al., 2020). Beauveria bassiana, traditionally known as an entomopathogenic fungus, has garnered interest for its endophytic capabilities. Studies have demonstrated that B. bassiana can colonize various plants such as corn, potato, cotton, tomato, sorghum, palm, banana, cocoa, poppy, coffee, pine, sugarcane, etc. (Donga. T.K, et al., 2018, Bamisile. B. S, et al., 2018) offering benefits beyond pest control, including improved drought tolerance and enhanced growth.

In 2002, Wagner and Lewis for the first time reported that B. bassiana can be inoculated artificially into maize plants (Wagner BL, Lewis LC 2000). Recently more studies have reported on the artificial inoculation of endophytes in various plant species. Various inoculation methods, such as soil drenching, leaf spraying, stem injection, irrigation, and seed treatment, have been used to introduce the fungus into plants (Bamisile. B.S, et al. 2018).

B. bassiana is vulnerable to direct sunlight and ultraviolet radiation, which can significantly reduce its efficacy (Kaiser. D, et al. 2019). Recent advancements have shown that the use of formulations containing natural substances can enhance conidial survival and viability under UV exposure. Kaiser, D. et al (2018) reported that natural UV-protective additives such as humic acid, extracts from Reseda luteola and Hippophae rhammoides, as well as oils like colza and sesame, and teas have proven effective in increasing B. bassiana resistance to UV radiation. These formulations represent a promising strategy to enhance the practical application of B. bassiana in various agricultural settings, ensuring its effectiveness as a biocontrol agent while mitigating the adverse effects of environmental stress. According to our previous studies, we have decided to use cornstarch as the addictive medium for inoculating B. bassiana blastospores to the seeds of the common bean plant.

Fungal Culture Activation and Preparation

Beauveria bassiana (GHA) strains (Kindly donated by Dr. Ek Ramos, Facultad de Ciencias Biologicas, UANL) were reactivated by plating stock cultures onto potato dextrose agar (PDA) and incubating in darkness at 25 ± 2 °C for one week. A single colony was inoculated into a 500 mL Erlenmeyer flask with 200 mL potato dextrose broth (PDB) and incubated at 25 °C on a rotary shaker at 120 rpm for five days until blastopore production. Blastospores were counted and adjusted to a final concentration of 1 × 10^6 spores/mL. (Fig. 1).

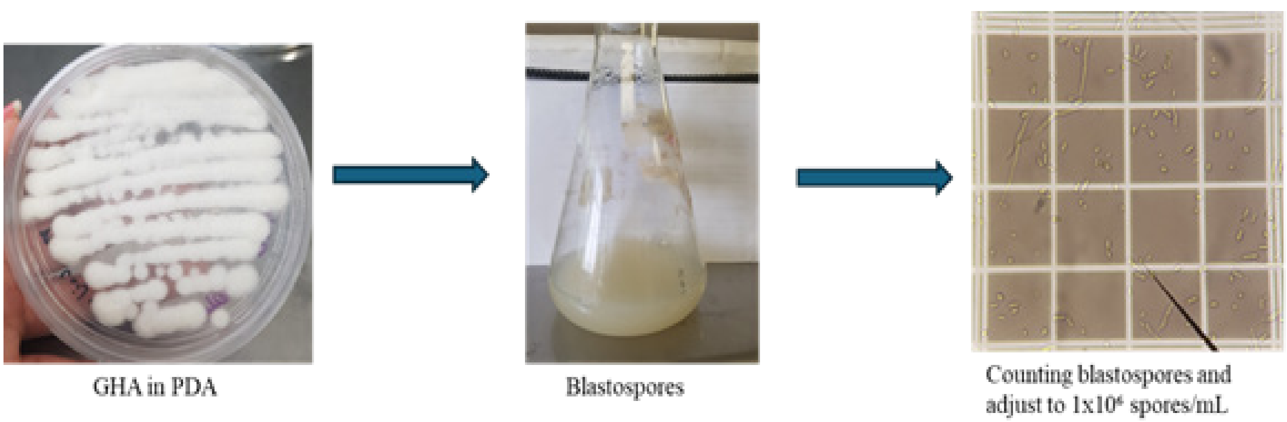
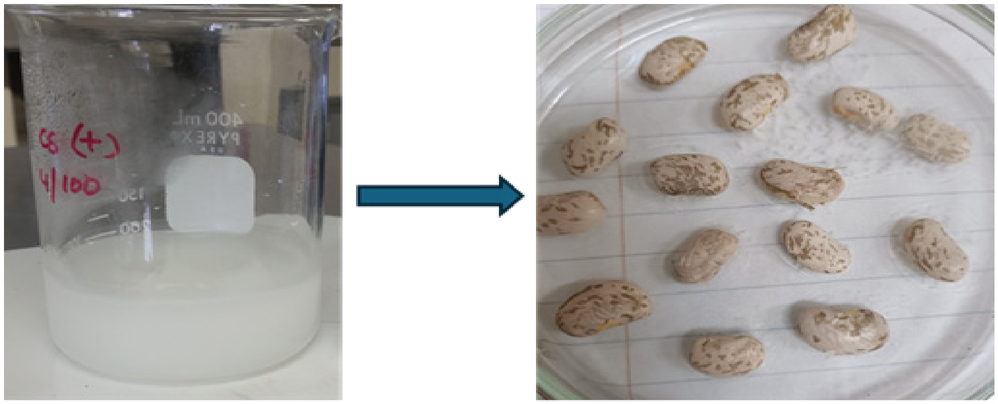


Figure 1- Fungal culture activation and preparation.

Seed Treatment

Blastospores were mixed with cornstarch (CS) (4% final concentration) for adequate seed attachment. CS was pre-gelatinized in boiling distilled water. Blastospores were added to cool CS adherent material to form a homogeneous suspension. Seeds (15 seeds) were coated with the spore-adherent mixture and dried at 25 °C for 24 hours. Included untreated seeds without the fungus (Fig. 2).



Blastospores mixed in corn starch

Inoculated bean seeds

Figure 2- Seed treatment.

Plant Growth and Maintenance

Treated seeds were sown in autoclaved commercial soil and placed in seedling trays. Trays were kept at room temperature (23-28ºC). Seedlings were watered daily with 5 mL of distilled water (Fig. 3).

Inoculated seeds sown in autoclaved soil

14 days old bean plants

Figure 3- Plant growth and maintenance.

Endophytic Colonization Assessment

Fourteen-day-old plants were surface sterilized by sequential rinsing in tap water, ethanol (2%) and sterile distilled water, followed by plating of the final rinse water to verify sterilization efficiency. Plant fragments (1–2 cm) from leaves, shoots, and roots were plated on PDA to assess colonization. Typical B. bassiana colonies were identified by hyphal and conidial structures observed under a microscope. (Figure 4)

Sterilized plants cut into 1-2 cm pieces

Plant tissues plated on PDA plates

Endophytic establishment of *B. bassiana* on bean plant root tissue

Figure 4- Endophytic colonization assessment

Conclusion

This study demonstrates the successful colonization of B. bassiana in P. vulgaris through seed treatment. It provides next-level assessment like the effect of endophytic B. bassiana as a growth promoter, tolerance to drought, and resistance to insect pests, both laboratory and field levels. Continued research, education, and implementation are crucial for advancing biological control and ensuring its role in sustainable agriculture worldwide.

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Conflict of Interest

The authors declare no conflict of interest.

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