The endophytic capacity of the entomopathogenic fungus *Beauveria bassiana* caused inherent physiological response in two barley (*Hordeum vulgare*) varieties

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Abstract

In this study, the endophytic capacity of *B. bassiana* was determined for two barley (*Hordeum vulgare*) varieties, Josefa and Esmeralda, inoculated with a seedling immersion at three different concentrations (1×10^6 , 1×10^7 and 1×10^8 conidia/mL). Seedling length and chlorophyll content were found to be not affected when inoculated with the entomopathogenic fungus, in both barley varieties. However, the colonisation percentage was found to be significantly lower with the inoculum concentration 1×10^6 conidia/mL for both barley varieties ($P < 0.05$) when compared to the other concentrations. Furthermore, a principal component analysis indicated that 96.23% of the variability in the data could be explained with two components. This analysis showed that the seedling length and chlorophyll content were positively correlated in both barley varieties for the 1×10^7 conidia/mL concentration. Likewise, a positive correlation was observed for colonisation percentage and treatment with 1×10^8 conidia/mL in the Josefa variety only. This is the first study in which the endophytic capacity of *B. bassiana* was evaluated in two different barley varieties, with the Josefa variety found to be the most susceptible.

Keywords *Beauveria bassiana* · Endophytic capacity · Barley · Colonisation · Principal component analysis

Introduction

Endophytic organisms are able to colonise vegetal tissues and structures without causing any damage to the host (Wilson 1995). Nevertheless, diseases have been seen to appear after inoculation by endophytic organisms (Petrini 1991). Some fungal species with an endophytic capacity have been shown to improve host tolerance to environmental stress, damages caused by insects, and diseases caused by pathogens (Hyakumachi and Kubota 2004).

Beauveria bassiana is an entomopathogenic fungus (EF) widely used to control harmful insect populations (Zimmermann 2007). Additionally, this EF is able to colonise some plant species (Bamisile et al. 2018; Gurulingappa et al. 2010; Jaber and Owley 2018). These include rice, bean, tomato, potato, cotton, wheat, onion, maize, coffee, cacao, pumpkin, grape, cassava, palm tree, and pine (Akutse et al. 2014; McKinnon et al. 2017; Ownley et al. 2008, 2010; Parsa et al. 2013; Renuka et al. 2016; Vega 2008, 2018). Furthermore, it has been reported that *B. bassiana* can control and even eliminate pathogens by antibiosis, substrate competence or microparasitism when inoculated into different crops (Ownley et al. 2008, 2010).

Barley (*Hordeum vulgare*) is a crop used in many agricultural industrial processes as well as animal feeding. According to Servicio de Información Agroalimentaria y Pesquera (SIAP) (2017), specifically in Mexico approximately 324,000 ha was used for barley crops, with an estimated production greater than 590,000 tons, representing 0.4% of the worldwide production. Josefa and Esmeralda barley are two malting barley varieties produced in the Mexican plateau. They are rust resistant and have high production

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yield, making barley an important agricultural product of Mexico. Due to the high importance of these barley varieties, agrochemical products are almost exclusively used to protect it (Secretaría de Agricultura Ganadería, Desarrollo Rural, Pesca y Alimentación, SAGARPA). The aim of this study was to evaluate the endophytic capacity of *B. bassiana* in Josefa and Esmeralda barley crops cultivated in Mexico.

Materials and methods

Entomopathogenic fungus

The strain of *B. bassiana* Bb 882.5 was used, belonging to the fungal collection of Universidad Autónoma Metropolitana-Iztapalapa. Propagation was carried out in 250-mL Erlenmeyer flasks containing 50 mL of modified Sabouraud Maltose agar (2%) (agar 15 g/L, maltose, 20 g/L, casein peptone 2.5 g/L, yeast extract 0.5 g/L and oat flour 15 g/L) previously sterilised in an autoclave (120 °C for 15 min). The flasks were incubated at 28 °C ± 1 °C for 8 days with a specific photoperiod of 12:12 h (light–dark) (Pérez-Guzmán et al. 2016).

Barley growth conditions

Seeds of two varieties of barley (*H. vulgare*) was used, Josefa and Esmeralda. To eliminate pathogens and promote seed germination, a sterilisation process was carried out by immersing the seeds in sodium hypochlorite (0.5%) for 2 min and ethanol (70%) for 2 min. Rinsing was performed in triplicates. The substrate was prepared using Peat Moss (Premier®, Canada) and leaf litter (1:1), which was sterilised in an autoclave (120 °C for 30 min). Once the substrate was cooled, four 7-in. plant pots were filled for each conidial concentration, described below, for each barley variety, and for the control (Parsa et al. 2013). Then, 10 g of each seed variety was planted at depth of 2 cm. The pots were placed at room temperature in the laboratory without light regime. One week after planting, a solution of triple 16 fertiliser (16% N, P₂O₅, K₂O) (10% w/v) (Ultrasol®, Mexico) was added. The pots were watered each 3 days with sterile water (120 °C for 15 min).

Inoculation of barley seedlings

Three conidial suspensions of *B. bassiana* were prepared (1×10^6 , 1×10^7 and 1×10^8 conidia/mL) and 20 mL of Tween 80 (0.05%) was added to the Erlenmeyer flasks used in the propagation, and stirred during 10 min. Then, the suspension was filtered through a sterile gauze. The conidial count was determined using a Neubauer chamber (Marienfeld-Superior®, Germany) and diluted to obtain each specific

concentration. Additionally, germination and viability were assessed, as described by Garza-López et al. (2012), obtaining values higher than 90% in both variables.

Four weeks after planting, the barley seedlings of both varieties were inoculated with each conidial suspension by immersing the aerial part for 15 s (Parsa et al. 2013; Tefera and Vidal 2009).

Determination of growth parameters

Chlorophyll content was measured using a SPAD-502 Plus meter (Konica-Minolta®, Japan). Determinations were made from ten seedlings per plot and the data reported as SPAD units (Gianquinto et al. 2003). Simultaneously, the length of the seedlings was determined using a Vernier caliper. The determinations were carried out each 7 days.

Determination of colonisation percentage

To corroborate that *B. bassiana* colonised the seedlings, 3 weeks after inoculation, ten 1-cm² pieces of leaves were cut and rinsed in sodium hypochlorite (1%) for 2 min, ethanol (70%) for 2 min, and distilled water for 2 min. Subsequently, the pieces were placed in Petri dishes containing 25 mL of Sabouraud Dextrose agar, previously sterilised (120 °C for 15 min). The Petri dishes were incubated at 28 °C ± 1 °C for 7 days with a specific photoperiod of 12:12 h (light–dark) (Arnold et al. 2000; Tefera and Vidal 2009). To evaluate the efficacy of the surface sterilisation method, 20 µL of the water used to rinse the pieces of leaves, after surface sterilisation, were plated on Petri dishes containing 25 mL of agar–agar and spread with a sterile glass rod, then incubated at 28 °C ± 1 °C for 7 days to count colony forming units (Tefera and Vidal 2009). However, no growth of microorganisms was observed after this time.

Statistical analysis

The sample size was determined according to Cochran's formula ($n = 168$). The experimental design was completely random. All experiments were performed in triplicates and repeated six times to obtain 180 plants tested. Data obtained were analysed using one-way analysis of variance (ANOVA) and the Tukey–Kramer multiple comparison test, with statistical significance set at $\alpha = 0.05$ (SPSS 21, IBM®, USA). Additionally, a principal component analysis (PCA) was used to determine patterns in the standardised data (Ramette 2007) (XLSTAT Version 2014.3.04, Addinsoft®, USA).

Results

Principal component analysis (PCA)

The PCA allowed to identify possible patterns between variables and treatments assessed. The first component (PC1) represented the largest portion of variability in the data and successively the other components (PC2, PC3, etc.) represent the remaining variability. The analysis indicated that 96.23% of the variability can be explained by two components, PC1 (73.89%) and PC2 (22.34%). The three variables

Fig. 1 Principal component analysis (PC1 vs PC2; 96.23% of accumulated variance explained) of the determined variables. Triangles represent the different barley varieties and the inoculum concentrations of *Beauveria bassiana*

determined were positively correlated among them, as the inoculum concentration increased (Fig. 1). The variables of seedling length and chlorophyll content were found to be positively correlated with the treatment of 1×10^7 conidia/mL in both barley varieties. Additionally, colonisation percentage was positively correlated with the Josefa variety inoculated with 1×10^8 conidia/mL. However, all variables were negatively correlated with the treatment of 1×10^6 conidia/mL in both barley varieties.

Seedling length

The seedling length of the Josefa variety can be seen in Fig. 2a. It was found that the seedlings inoculated with the concentration 1×10^7 conidia/mL showed the most growth 2 weeks after inoculation. However, it is important to mention that there was no significant difference between the different treatments ($P > 0.05$). Similarly, the seedlings of the Esmeralda variety showed longer length when inoculated with 1×10^7 conidia/mL (Fig. 2b), which was significantly different in comparison with the other treatments ($P < 0.05$). Moreover, the uninoculated seedlings showed the shortest length, which was significantly different ($P < 0.05$) to the rest of treatments. This suggests that *B. bassiana* did not affect the growth of the Josefa variety.

Chlorophyll content

The chlorophyll content was determined to evaluate the seedlings' health indirectly. The chlorophyll content of the Josefa variety can be seen in Fig. 3a. It was observed that the chlorophyll content showed a similar response to that

Fig. 2 Seedling length of two barley varieties inoculated with different conidia concentrations of *Beauveria bassiana*. **a** Josefa variety, **b** Esmeralda variety. Error bars represent standard deviation.

Open square: control; cross: 1×10^6 conidia/mL; filled circle: 1×10^7 conidia/mL; filled triangle: 1×10^8 conidia/mL

Fig. 3 Seedling chlorophyll content of two barley varieties inoculated with different conidia concentrations of *Beauveria bassiana*. **a** Josefa variety, **b** Esmeralda variety. Error bars represent standard deviation.

of the seedling length, with all treatments, including the controls, showing between 20 and 35 SPAD units, and no significant difference ($P > 0.05$). Similarly, in the Esmeralda variety when inoculated with 1×10^6 conidia/mL, the lowest chlorophyll content was observed, while the greatest chlorophyll content was obtained with the 1×10^7 conidia/mL inoculum (Fig. 3b). It is important to mention that after 3 weeks from inoculation, the seedlings inoculated with 1×10^7 conidia/mL were significantly higher than the other treatments ($P < 0.05$). In fact, the differences in the growth parameters evaluated could be due to inherent variability of the plants.

Colonisation percentage

Regarding the colonisation percentage, it was observed that treatment with 1×10^6 conidia/mL caused significantly lower colonisation than the rest of the treatments in both barley varieties ($P < 0.05$) (Fig. 4). In the case of the Josefa variety, the highest colonisation percentage was obtained with the treatment of 1×10^8 conidia/mL (68%) and the lowest percentage was observed with 1×10^6 conidia/mL (17%). Similarly, in the Esmeralda variety, the same conidia concentrations (1×10^6 and 1×10^8 conidia/mL) were presented with the lowest and the highest colonisation percentages, respectively (8% and 57%). However, it is important to mention that the colonisation percentage obtained with the inoculum concentrations of 1×10^7 and 1×10^8 conidia/mL did not cause significant difference ($P > 0.05$). Therefore, when comparing the two varieties, only with 1×10^6 conidia/mL was observed significant differences ($P < 0.05$).

tion. Open square: control; cross: 1×10^6 conidia/mL; filled circle: 1×10^7 conidia/mL; filled triangle: 1×10^8 conidia/mL

Fig. 4 Colonisation percentage of barley seedlings at different conidia concentrations of *Beauveria bassiana* (black filled bars: Josefa variety; open bars: Esmeralda variety). Error bars represent standard deviation. Capital letters distinguish significant differences between barley varieties (Tukey: $P < 0.05$). Lowercase letters distinguish significant differences between treatments (Tukey: $P < 0.05$)

Discussion

In this study, *B. bassiana* was able to colonise barley (*H. vulgare*) seedlings, previously not reported. However, the colonisation level of the barley depended on the variety. Previously, it was observed that *B. bassiana* has endophytic capacity in coffee, banana, wheat, bean, and grapevine (Parsa et al. 2018; Rondot and Reineke 2018; Sánchez-Rodríguez et al. 2018; Vega et al. 2010). Consistent with this study, the growth of different plants such as

cotton and tomato were not affected by endophytic capacity of *B. bassiana* (Ownley et al. 2008, 2010).

The use of SPAD units in the determination of the chlorophyll content has been widely used, mainly to correlate nitrogen requirements in different plants, such as maize, wheat, tomato, potato and sweet pepper (Gianquinto et al. 2003; Madeira et al. 2003). Although, such correlations are specific to each crop. Additionally, it has been reported that differences between SPAD units was dependent on different substrates and the nitrogen doses used. However, there are few studies in which SPAD units were used in the measurement of the chlorophyll content in inoculated plants with entomopathogenic organisms. The authors measured SPAD units to determine differences among *B. bassiana* and *Metarhizium anisopliae* treatments (Greenfield et al. 2016).

On the other hand, the colonisation percentage of *B. bassiana* has been previously shown to be dependent on the crop and inoculation method used. Three different inoculation methods (seed immersion, leave spraying, and soil inoculation) have been previously evaluated and it was reported that the best method was leave spraying with a maximum colonisation percentage of 90% (Tefera and Vidal 2009). However, when the same methods were used again, a maximum colonisation percentage of 40% was observed with the method of leave spraying (Parsa et al. 2013). Similarly, a colonisation percentage higher than 80% was attained in wheat with leave spraying (Sánchez-Rodríguez et al. 2018).

Furthermore, there have been numerous studies that recommend alternative inoculation methods. Posada et al. (2007) obtained better colonisation in coffee using stem injection. Additionally, the previous studies used 1×10^8 conidia/mL as the inoculum concentration and different concentrations have not been compared. In this study, the same colonisation percentage was attained with a tenfold smaller inoculum concentration. Furthermore, few studies have used different varieties of plants to explore the colonisation percentage.

In maize, high nutrient availability increased plant growth when inoculated with *B. bassiana* compared to low nutrient conditions, suggesting that the addition of fertiliser affects the plant–fungus interaction (Tall and Meyling 2018). In this study, the concentration and type of fertiliser used is recommended by barley growers.

In conclusion, *B. bassiana* was able to colonise both Josefa and Esmeralda barley varieties; notably Josefa variety was more susceptible. Furthermore, the growth of both varieties was not affected by inoculation with this entomopathogenic fungus. This is the first study that evaluated the endophytic capacity of *B. bassiana* in barley crops.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest in the publication.

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