Field Crops Research 176 (2015) 11–21

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Bacillus cereus sensu lato strain B25 controls maize stalk and ear rot in Sinaloa, Mexico

Glenda Judith Lizárraga-Sánchez[a,b], Karla Yeriana Leyva-Madrigal[b], Pedro Sánchez-Peña[a,c], Francisco R. Quiroz-Figueroa[b], Ignacio Eduardo Maldonado-Mendoza[b,*]

[a] *Programa Regional del Noroeste de Doctorado en Biotecnología, Universidad Autónoma de Sinaloa, Ave. de Las Américas y Boulevard Universitarios s/n, Culiacán, Sinaloa, Mexico*
[b] *Departamento de Biotecnología Agrícola (BIOTECSIN), CIIDIR-SINALOA, Instituto Politécnico Nacional, C.P. 81101 Guasave, Sinaloa, Mexico*
[c] *Universidad Autónoma de Sinaloa, Facultad de Agronomía, Km. 17.5, Carretera Culiacán-El Dorado, C.P. 80000 Culiacán, Sinaloa, Mexico*

ARTICLE INFO

Article history:
Received 13 January 2015
Received in revised form 14 February 2015
Accepted 17 February 2015
Available online 9 March 2015

Keywords:
Maize
Fusarium verticillioides
Fumonisins
Biocontrol
Bacillus cereus sensu lato

ABSTRACT

Fungal pathogens causing maize stalk and ear rot are a potential threat to grain production in regions where monoculture extensions can reach over 500,000 ha per year. This particular problem is observed in northern Sinaloa, Mexico with the fungal pathogen *Fusarium verticillioides*. Three native *Bacillus* spp. strains isolated from the maize rhizosphere were tested for their potential as biocontrol agents (BCAs) against fusariosis in maize, during the 2011–2012 fall-winter growing season. Based on its performance, the *Bacillus cereus sensu lato* strain B25 was selected for further analysis. The effectiveness of maize seed inoculation with this strain was examined in two more consecutive growing seasons. The potential for B25 to control *Fusarium* stalk rot (FSR) and *Fusarium* ear rot (FER) of maize, as well as the accumulation of fumonisins in kernels, was thus assessed with white maize hybrids grown under different field conditions in northern Sinaloa, Mexico. FSR and FER incidence and severity were substantially reduced as compared to controls in all trials conducted. Fumonisin contamination in maize grains was also reduced (up to 93.9%) by B25 application, as compared to the control. Furthermore, B25 significantly increased grain yield in several trial sites or crop seasons, above the average of the untreated controls and consistently above the average of *F. verticillioides*-inoculated controls. Based on these findings, we propose that seed bacterization with strain B25, combined with adequate crop management practices, may become a useful tool for avoiding *Fusarium* stalk and ear rot of maize. This practice will also provide safe fumonisin grain levels for maize production in northern Sinaloa.

1. Introduction

Maize (*Zea mays* L.) is clearly the most important crop in Mexico on two levels: approximately half of the nation's area is dedicated to its cultivation; and it is an integral part of the culture and diet of the population. In 2013, Sinaloa state was the leading producer of maize in Mexico, harvesting 3,627,777.51 t (16% of the national Mexican production) and occupying a field surface of 426,856 ha (SIAP-SAGARPA, 2014). Due to its presence in all regions where maize is cultivated, *Fusarium* stalk, ear and root rot (SERR) of maize is a serious disease that inflicts important economic losses. *Fusarium verticillioides* (Saccardo) Nirenberg (teleomorph *Fusarium moniliforme* (Sawada) Wr), hereafter referred to as *Fv*, is an important fungus involved in the development of SERR on maize (Martínez et al., 2010). Plants infected with *Fv* can show SERR symptoms, as well as wilting, stalk thinning, and reduced aerial and root growth (Oren et al., 2003; Wu et al., 2011). This species produces carcinogenic mycotoxins known as fumonisins, in particular fumonisin B1 (FB1), B2 (FB2) and B3 (FB3), which are all accumulated in maize kernels (IARC, 2002). Fumonisins have been associated with human esophageal cancer, neural tube defects and leukoencephalomalacia in equines, and hepatotoxicity in different animals (Desjardins, 2006). These mycotoxins have been detected in a range of products for human and animal consumption derived from maize (Weidenbörner, 2007).

The incidence of maize SERR in northern Sinaloan fields is low (<10%). The presence of *Fusarium* in SERR symptomatic

* Corresponding author at: Boulevard Juan de Dios Bátiz Paredes #250, Col. San Joachin, 81101 Guasave, Sinaloa, Mexico. Tel.: +52 687 8729626x87652.
E-mail address: imaldona@ipn.mx (I.E. Maldonado-Mendoza).

http://dx.doi.org/10.1016/j.fcr.2015.02.015
0378-4290/

plants is associated with insect attack (Avantaggiato et al., 2003), and once it is present in the kernels, subsequent invasion of the ears by *Fusarium*, *Aspergillus* and yeast is commonly found (Quintero-Benítez and Apodaca-Sánchez, 2008). Rot caused by *Fv* on maize is difficult to control with chemicals, due to multiple factors such as the endophytic nature of the infection (Bacon et al., 2001). Another factor is that chemical control of this pathogen is applied to seeds before their planting, despite reports of the ineffectiveness of fungicides used in this manner and significant increases in fumonisin concentrations in plants resulting from fungicide-treated seeds (Pereira et al., 2007; Falcão et al., 2011). In spite of the significance of this disease to maize production, a more thorough study of the problem and effective control strategies are still necessary. Biological control is proven to be a promising alternative for the control of fungal plant pathogens (Heydari and Pessarakli, 2010). Several bacterial species have been reported to be highly antagonistic against *Fv*, the main causal agent of fusariosis (Bacon et al., 2001; Pereira et al., 2010, 2011). However, most studies have been developed mainly under controlled conditions that differ significantly from what happens in the field.

Bacteria with a potential for *Fv* biocontrol were previously selected both *in vitro* and *in planta* by screening a collection of 11,520 native maize rhizobacterial isolates from northern Sinaloa, Mexico (Figueroa-López et al., 2014; Cordero-Ramírez, 2013). In the present work we have evaluated the potential antagonistic activity of three such previously selected *Bacillus* spp. strains against *Fv* in the field. The goal of this work was to improve our understanding of their plant growth-promoting activities, by assessing their ability to reduce *Fusarium* stalk and ear rot (FSR and FER, respectively) and fumonisin content in maize kernels. *Bacillus cereus sensu lato* B25, the most potentially antagonistic strain during the first year field trials, was further tested for a total of three agronomic cycles. These findings demonstrate that B25 exhibits the best protective effect against SERR in the field.

2. Materials and methods

2.1. Microorganisms

Bacillus strains B5, B25 and B35 were used in the present study. Bacterial isolates were obtained from the maize rhizosphere of commercial maize fields in northern Sinaloa, Mexico (where the fungus displayed more negative effects on maize production). Strains were identified on the basis of their 16S rDNA gene sequence (GenBank accession numbers: *B. megaterium* (B5), JQ830832; *B. cereus sensu lato* (B25) JQ835946) (Cordero-Ramírez, 2013). B35 was only identified at the genus level as *Bacillus* sp. The strains were stored at −70 °C in Luria Bertani medium (LB, Sigma, No. Cat. L3022, USA) supplemented with glycerol (15%, v/v), and deposited in the CIIDIR-003 microorganism collection (CIIDIR – Sinaloa, Mexico).

Fungal isolate *Fv* P03 was used in experiments. It was isolated from infected maize roots and identified on the basis of partial sequences of the calmodulin (GenBank accession number KF641082) and elongation factor 1α (GenBank accession number KF640976) genes (Leyva-Madrigal et al., 2014). The pathogenicity of this isolate has been tested in multiple assays and in different maize hybrids (Figueroa-López, 2011; Leyva-Madrigal et al., 2014). A frozen stock (−70 °C) maintained in Potato Dextrose Agar (PDA; BD Bioxon, Edo. de México, México, Cat. No. 211900) and supplemented with 15% glycerol since 2009 was used as a starter inoculum for experiments conducted during this study.

2.2. Bacterial and fungal inocula

Bacterial isolates were reactivated on LB medium and grown at 25 °C for 24 h. A single colony was inoculated in 5 mL of LB broth and incubated for 18 h at 25 °C and 250 rpm. 1 mL of the latter culture was transferred to 50 mL of LB broth and incubated for 20 h at 25 °C and 250 rpm. Finally, 5 mL of the previous culture were transferred to an Erlenmeyer flask with 250 mL of LB broth and incubated for 24 h at 25 °C and 250 rpm. The concentration of the bacterial cultures (CFU/mL) was assessed by the serial dilution method (Supplementary Table 1). Maize seeds were treated with the corresponding inoculant and soaked in the bacterial suspension for 5 min prior to sowing. Bacteria remaining on maize seeds were assessed by the serial dilution method and reported as CFU g^{-1} of seed (Luna and Sánchez-Yáñez, 1991) (Supplementary Table 1). The inoculated seeds were sowed at a depth of 5–8 cm.

Supplementary Table S1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fcr.2015.02.015.

Fv isolate P03 was reactivated on PDA plates by incubation at 25 °C for 14 days. Twenty mycelial plugs (1 cm-diameter) were transferred to sterile plastic bags containing 500 g of sterile cracked maize, hydrated with 200 mL of sterile distilled water, and incubated at 25 °C for 14 days. Three kg of the maize–fungus mixture were manually inoculated to the furrows of each plot (four furrows of 10 m separated by a distance of 0.8 m between them). The control treatment received 3 kg of sterile non-inoculated cracked maize. Inoculum concentrations of *Fv* in the soil (CFU g^{-1}) were determined 6 days before sowing (Supplementary Table 1). To determine the natural population levels of *Fusarium* before inoculation with the fungus, soil samples were collected at three points from the usable area a depth of 0–30 cm. Subsamples were mixed, and one subsample was obtained for each treatment plot. Natural *Fusarium* populations were then quantified using the serial dilution method and cultivated on Nash–Snyder agar plates (Nash and Snyder, 1962), with incubation at 25 °C for 6 days. To determine the population levels of *Fusarium* after soil inoculation, three plants per treatment were randomly selected 20 days after emergence of maize plants. Rhizosphere samples of these plants were collected and *Fusarium* populations were quantified by serial dilutions, as described by Nash and Snyder (1962) (Supplementary Table 1).

2.3. Field trials

Three field trials were conducted during the three consecutive fall-winter growing seasons of 2011–2012, 2012–2013 and 2013–2014. Trials were conducted at four locations in northern Sinaloa, Mexico: El Realito (site A), La Noria (site B), Santa Rosa (site C) and El Burrión (site D). Geographic information for each experimental field is reported in Table 1.

Experimental plots in each trial consisted of four furrows of 10 m separated by 0.8 m. The usable area consisted of the two central rows, so as to avoid "border effects" for each experimental plot. Seeds were mechanically sown (1053P 1010 MaxPlanter Seeder, John Deere) during the recommended dates for this region (November 1–December 31), with the following exceptions: sowing was manually performed for field trial I; and experiments commenced after the recommended dates in site A, for two growing seasons. Maize sowing and harvest dates for each growing season and field are provided in Table 2. A density of 90,000 plants ha^{-1} was used in all field trails (INIFAP, 2002). During the tested crop cycles four maize hybrids: Garañón, Cebú, and Gorila (Asgrow), and DK2038 (DeKalb) were evaluated. These maize hybrids are the most commonly used in the region due to their agronomic characteristics:. According to the FAO classification, they all belong to maturity class 900 (ultra-late with a vegetative cycle ≥150 days).

Nitrogen was supplied in two applications using ammonia as the source: 220–250 kg N ha^{-1} before sowing and 60–80 kg N ha^{-1}, 60 days after sowing (according to the soil analysis results; data not shown).