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POTENCIAL DE CONTROL DE BIOLÓGICO DE BACTERIAS ASOCIADAS A LA SEMILLA DE MAÍZ CONTRA SUS PATÓGENOS

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EL PRESENTE TRABAJO SE REALIZÓ EN EL **LABORATORIO DE AGROECOLOGÍA** DEL INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD DE LA UNIVERSIDAD AUTÓNOMA DE MÉXICO, BAJO LA ASESORÍA DEL **DR. JOHN LARSEN** Y LA **DRA. SYLVIA P. FERNANDEZ PAVÍA**, Y EN EL **INSTITUTO DE CIENCIAS AGRÍCOLAS** DE LA UNIVERSIDAD DE AARHUS, DINAMARCA, BAJO LA ASESORÍA DE LA **DRA. SABINE RAVNSKOV**, CON EL APOYO DE LA UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO Y EL CONSEJO NACIONAL DE CIENCIA Y TECNOLOGÍA (CONACYT).

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I. ABSTRACT

A major part of plant development is attributed to microorganism associations (Larsen, Jaramillo-López, Nájera-Rincón & González-Esquivel, 2015). Bacteria is a core group of plant microbiota (Philippot, Raaijmakers, Lemanceau, & Van Der Putten, 2013). Interest in endophytic bacteria and their importance on plants health has been point out (Rijavec, Lapanje, Dermastia & Rupnik, 2007). Presumably, endophytes are better adapted to plants life, colonize plant niches and respond to plant stimuli more effectively than non-endophytic microorganisms (Nelson, 2004; Cankar, Kraigher, Ravnikar, & Rupnik, 2005). Interestingly endophytes community structure varies in relation to plants genotype, this may suppose different response to biotic and abiotic pressures accordingly plant genotype (Johnston-Monje, Mousa, Lazarovits & Raizada, 2014; Johnston-Monje, Lundberg, Lazarovits, Reis & Raizada, 2016). The main objective of the present study, was to assess the role of maize seed endophytic bacteria in maize plants root health. For this purpose, a pot experiment under greenhouse conditions was first conducted, ten maize genotypes infected in different treatments with four common maize root pathogens (*Fusarium graminearum*, *F. verticillioides*, *Pythium arrhenomanes* and *Pythium* sp.) were executed, the objective was to select tolerant/resistant and susceptible maize genotypes to root pathogens in order to isolate their associated seed endophytic bacterial communities for subsequently assessment for their capability to provoke resistance or susceptibility in susceptible or resistant maize genotypes respectively. In this study, *Rhizophagus irregularis* was also investigated for its capability to reach higher root pathogen biocontrol in association with maize seed endophytic bacteria, both are common inhabitants of maize roots, their interaction is of particular interest since plant response to root pathogens may be altered (Budi, Van Tuinen, Martinotti & Gianinazzi, 1999).

For this purpose, a second pot experiment under glass house controlled conditions were set up wherein maize tolerant and resistant genotypes were inoculated with *R. irregularis* and maize seed endophytic bacteria communities in order to assess biocontrol potential against *P. arrhenomanes*. The results of the first experiment showed that only maize plants infected with *P. arrhenomanes* presented symptoms of infection, maize root architecture, shoot and root biomass were negatively affected. The effects caused in maize plants by *P. arrhenomanes* were dependent of maize genotype. Statistically, E. Occidentales, NB9 and PUMA were not affected. On the contrary, H318 and R. espada genotypes were highly affected. The endophytic bacteria communities selected for the second experiment were those of E. occidentales, NB9 and H318, the first two were considered as those capable to confer resistance to H318 maize genotype against *P. arrhenomanes*; meanwhile the endophytic bacteria community of H318 were considered as the one conferring susceptibility to NB9 maize genotype against *P. arrhenomanes.* The results obtained showed that maize endophytic bacteria communities from E. occidentales and NB9 were not capable to ameliorate *P. arrhenomanes* negative effects on H318 maize plants and bacteria communities from H318 did not increase negative effects in maize plants of NB9 infected with *P. arrhenomanes*, rejecting our main hypothesis that maize root health depends on associated seed endophytic bacteria. In the same way, *R. irregularis* neither alone nor in interaction with maize endophytic bacteria of NB9 and E. occidentales showed any health improvement in plants infected with *P. arrhenomanes*. However, *R. irregularis* and bacterial communities affected plant biomass and root architecture, either alone or in interaction. These effects were different among maize genotypes and microbial interaction.

II. RESUMEN

Una gran parte del desarrollo de las plantas se atribuye a los microorganismos con los que establecen asociaciones, siendo las bacterias un grupo clave (Larsen, Jaramillo-López, Nájera-Rincón & González-Esquivel, 2015; Philippot, Raaijmakers, Lemanceau, & Van Der Putten, 2013). El papel que juegan las bacterias endófitas en la salud de las plantas ha sido señalado como importante (Rijavec, Lapanje, Dermastia & Rupnik, 2007). Aparentemente, los endófitos están mejor adaptados a la vida de las plantas, son capaces de colonizar los nichos que ofrecen estas y responden a sus estímulos con mayor efectividad que los microorganismos no-endófitos (Nelson, 2004; Cankar, Kraigher, Ravnikar, & Rupnik, 2005). Interesantemente la comunidad de bacterias endófitas varía según el genotipo de la planta, esto podría suponer una respuesta diferente a las presiones bióticas y abióticas según el genotipo de esta (Johnston-Monje, Mousa, Lazarovits & Raizada, 2014). El objetivo principal de este trabajo fue evaluar el papel de las bacterias endófitas de semillas de maíz en la salud de las raíces de las plantas de maíz. Para lo cual primero se estableció un experimento en macetas bajo condiciones de invernadero donde se evaluaron diez genotipos de maíz infectados en diferentes tratamientos con cuatro patógenos comunes de raíces (*Fusarium graminearum*, *F. verticillioides*, *Pythium arrhenomanes* and *Pythium* sp.) con el objetivo de seleccionar genotipos de maíz resistentes/tolerantes y susceptibles a los diferentes patógenos. Posteriormente, se aislaron las bacterias endófitas asociadas a su semilla para evaluar su capacidad de provocar resistencia o susceptibilidad en genotipos susceptibles y resistentes respectivamente. En el presente estudio, también se incluyó la evaluación de *Rhizophagus irregularis* y su capacidad de incrementar los niveles de control de patógenos en conjunto con

las bacterias endófitas de semillas de maíz, ya que ambos forman parte del microbioma del maíz, su interacción es de particular interés pues la respuesta de las plantas a los patógenos puede verse alterada (Budi, Van Tuinen, Martinotti & Gianinazzi, 1999). Con este propósito un segundo experimento fue establecido bajo condiciones controladas en un invernadero de cristal donde genotipos tolerantes y resistentes de maíz se inocularon con *R. irregularis* y comunidades de bacterias endófitas con la finalidad de evaluar su potencial de control sobre *P. arrhenomanes*. Los resultados del primer experimento mostraron que sólo las plantas inoculadas con *P. arrhenomanes* presentaron síntomas de infección donde la arquitectura de la raíz, la biomasa de la raíz y de la parte aérea fueron negativamente afectadas. Los efectos causados en las plantas de maíz por *P. arrhenomanes* fueron dependientes del genotipos e maíz. Estadísticamente, los genotipos E. Occidentales, NB9 y PUMA no se vieron afectados. Por el contrario, H318 y R. espada fueron altamente afectados. Las comunidades de bacterias endófitas seleccionadas para el segundo experimento fueron aquellas de E. occidentales, NB9 y H318. Las dos primeras fueron catalogadas como capaces de conferir tolerancia/resistencia a plantas de H318 sobre *P. arrhenomanes*, mientras que las bacterias endófitas de H318 fueron consideradas como capaces de conferir susceptibilidad a *P. arrhenomanes* en plantas de NB9. Los resultados obtenidos mostraron que las comunidades de bacterias endófitas de NB9 y E. occidentales no contrarrestaron los efectos negativos causados por *P. arrhenomanes* en plantas de H318, y las comunidades de bacterias endófitas de H318 no incrementaron los efectos negativos de *P. arrhenomanes* en plantas de NB9, de esta manera se rechazó nuestra hipótesis principal, donde se estableció que la salud de las raíces de plantas de maíz dependía de la asociación que establece que con las bacterias endófitas de semillas. De la misma manera, *R. irregularis* no contrarrestó los efectos negativos causados por *P. arrhenomanes* en

plantas de H318 cuando se inoculó sólo o en conjunto con las bacterias endófitas de NB9 y E. occidentales. Sin embargo, tanto *R. irregularis* como las bacterias endófitas de semillas de maíz afectaron la biomasa de las plantas y la arquitectura de la de la raíz por si solos y en interacción. Sin embargo, estos efectos fueron diferentes según le genotipo de maíz.

Palabras clave: Endófitos, *Zea mays* L. ssp. *mays*, *Rhizophagus irregularis*, Fitopatología, raíz.

III. INTRODUCTION

In the present study, the role of maize endophytic seed bacteria in maize root health was investigated. An endophyte is defined as a bacterium or fungus inhabiting any plant tissue with healthy appearance (Schulz & Boyle, 2005). The role of endophytes in host plant health and performance still remains to be further examined though several traits has been identified including plant defense induction in terms of both induced resistance and tolerance as well as direct antibiosis and/or niche competition against pathogens (Johnston-Monje & Raizada, 2011a). In maize, seeds are the main source of microbial inoculum (Johnston-Monje, Lundberg, Lazarovits, Reis & Raizada, 2016). Maize seed endophytic bacteria colonize plant endosphere and rhizosphere (McInroy & Kloepper, 1994; Rijavec et al., 2007; Rai, Dash, Prasanna & Singh, 2007). Indeed, maize rhizosphere is mainly composed of bacteria from seed origin and form close associations with roots (Johnston-Monje et al., 2016). Overall, plant-microbial interactions have been demonstrated to be crucial for plant survival under disadvantageous conditions (Larsen et al., 2015). Maize endophytes are of particular interest since are the major group of plant interacting microbes at least during the first life stages, moreover the major part of this group is vertically transmitted from mother plants suggesting that these bacteria may have major implications in *Zea mayz* varieties performance (Philippot et al., 2013; Johnston-Monje & Raizada, 2011a). Community composition endophytes vary in

relation to maize genotype, which may result in different responses to biotic stress caused by plant pathogens (Johnston-Monje et al., 2016).

Endophytic communities with health improvement traits may be well represented in Mexican landraces (Dalton & Kramer, 2007). In Mexico, landraces represent 75% of the maize cultivated area, which still are selected by farmers for desirable agronomical traits (Polanco-Jaime & Flores-Méndez, 2008). Preference for maize landraces over hybrid maize genotypes is attributed to cultural and dietary preferences such as higher nutritional value, flavor and properties for tortilla preparation and above 600 hundred traditional dishes prepared from maize (Vázquez-Carrillo, García-Lara, Salinas-Moreno, Bergvinson & Palacios-Rojas, 2011; Echeverría & Arroyo, 1983). Moreover, landraces are better adapted at regional level and reasonable yield is achieved under low input agricultural systems (Macrobert, Kosina & Jones, 2007). Additionally, a major part of Mexican farmers live in poverty and hybrid genotypes adoption implies higher costs that they cannot afford (Zeven, 1998; Macrobert et al., 2007).

Other common maize root endophytes and rhizosphere associated microorganisms involved in plants health improvement are Arbuscular Mycorrhiza Fungi (AMF) (Bofante & Anca, 2005), recruitment of microorganisms with antibiosis traits by AMF are suggested as one of the mechanisms involved to suppress root pathogens (Azcón-Aguilar & Barea, 1996; Barea, Azcón & Azcón-Aguilar, 2002). Changes in root exudates lead to changes in rhizosphere microflora structure and dynamics of plants that form symbiosis with AMF, this particular environment is defined as Mycorrhizophere (Liderman, 1988), which conform the soil under AMF influence, microbes settle in Mycorrhizophere influence AMF- plant symbiosis having effects on plant development, but whether this tripartite symbiosis affect host plant health is

poorly explored (Larsen et al., 2015). In this study, *R. irregularis* was also investigated for its capability to reach higher root pathogen biocontrol in association with maize seed endophytic bacteria, both are common inhabitants of maize roots, their interaction is of particular interest since plant response to root pathogens may be altered (Budi, et al., 1999).

IV. BACKGROUND

Maize (*Zea mays* L. ssp. *mays*) from the Poacea family is an annual plant domesticated around six thousand years ago in Mesoamerica, now Mexico, where the predecessor, teosintle (*Zea mays* ssp. *parviglumis*), could be found growing as a weed (Wang, Stec, Hey, Lukens & Doebley, 1999). Crop diversified into different maize races, which long after were dispersed across the American continent (Matsuoka, Vigouroux, Goodman, Sanchez, Buckler & Doebley, 2002). Trough natural and artificial selection new races surged in each geographical region supporting cultural and nutritional requirements (Tenaillon, Sawkins, Long, Gaut, Doebley & Gaut, 2001). Approximately, three hundred fifty races cultivated are now recognized (Vigouroux, Glaubitz, Matsuoka, Goodman, Sánchez & Doebley, 2008), with conspicuous phenotypical differences between them (Wellhausen, Fuentes, & Hernández-Corzo, 1957).

Maize grain quality was the principal target of selection (Whitt, Wilson, Tenaillon, Gaut & Buckler, 2002). In Mesoamerica, maize became the most prevalent and the principal subsistence crop of native Americans whom transformed whole grains through nixtamalization process into tortillas, the main source of protein and carbohydrates. Maize shaped culture, economy and diet of pre-Columbian people (Caballero-Briones, Iribarren, Peña, Castro-Rodríguez & Oliva, 2000).

Today, maize is consolidated as a high yield crop, cultivated in 163 countries and the third most important grain as staple food (FAOSTAT, 2009). Maize is also used for industry, fiber and as bioenergy and to a high extent for livestock feeds (Ram, 2011). The growing grain demand has led to breeding of high yielding varieties, bred for particular ends, uses and regions. Hybrid genotypes that have accomplished these necessities, are extensively adopted by small and big landholders worldwide and mainly cultivated under conventional agriculture schemes (Heisey, Morris, Byerlee & López-Pereira,1998).

High yielding potential of hybrid genotypes is achieved under favorable environmental conditions hardly ever found in agrosystems, where major crop limitations include water and nutrient deficiency. Pests such as diseases, arthropod herbivores and weeds represent another important limitation for maize growth (Boyer, 1982).

Conventional agriculture heavily relies on irrigation systems, chemical inputs, monoculture, tillage and mechanized machinery to improve the crop growth environment in order to reach higher yields. However, these practices are hardly criticized due to the negative impacts on agroecosystems (Cook, 2006). Long term effects include pest and weed persistence, crop health decline, loss of biodiversity, soil erosion, soil compaction and salinity (Chen, 2006; Blevins, Thomas, & Cornelius, 1977; Pitman & Läuchli, 2002, Cook, 2006). Hence, sustainable crop production practices are now emerging (Broders, Lipps, Paul, & Dorrance, 2007a; Broders, Lipps, Paul, & Dorrance, 2007b).

Crop rotation, cover crops, no tillage and integrated pest management have been implemented within a sustainable farming scheme (Cook, 2006). These practices promote higher diversity of microbial communities and natural enemies capable to reduce pest incidence (Aguilar, Carreón-Abud, López-Carmona, & Larsen, 2017), protect soil from water loss, erosion, allow nutrient recycling, control weeds and promote soil aggregation (Ortiz, 2015).

However, neither conventional nor sustainable agricultural practices have resulted in efficient control of plant pathogens (Cook, 2006). Farmers often fail in rotate crops in time and space by growing the same crop in the same field each year (Cook, 2006). In this way, soil propagules of pathogens survive on crop residue, or even more concern colonize crop residues from previously uninfected tissue waiting for the appropriate host (Sutton, 1982; Nyvall & Kommedahl, 1968). Nevertheless, even when not related crops are employed generalist pathogen are able to colonize weeds or not related plants to host becoming a persistent inoculum (Cotten & Munkvold, 1998). Under these circumstances chemical treatments are inevitably employed, though pest resistance to pesticides is continuously reported (Broders et al., 2007a; Broders et al., 2007b). Hence, plant genetic resistance is considered the most successful tool to avoid pathogen infection (Rodríguez-del Bosque, 1996; White, 1999).

Around 100 diseases are reported for maize though different causal agents may cause the same symptoms (White, 1999). Environmental conditions joint with intrinsic characteristics of either maize genotype and pathogen strain determine the disease severity. Plant breeding programs have focused efforts in develop resistant maize genotypes to common and devastating plant pathogens (Rodriguez del Bosque, 1996; White, 1999).

The pathogens *Pythium* spp. and *Fusarium* spp. are important causal agents of maize root rot (Broders et al., 2007a; Broders et al., 2007b). Root pathogens affect water and nutrient uptake to higher plant parts, resulting in decline in plant vigor and may even result in crop death (Deep & Lipps, 1996).

Pythium spp. causing root rot, commonly spoils root tips and rootlets without showing symptoms of infection on aboveground plant parts (Agrios, 1988). However, when older roots are affected necrosis and lesions are visible, higher plant parts turn chlorotic and then wilt. If the infection advances to the vascular system, plant collapse and death occur (White, 1999), provoking high yield losses (Reyes-Tena, Vallejo-González, Santillán-Mendoza, Rodríguez-Alvarado, Larsen & Fernández-Pavía, 2018).

Fusarium spp. include some of the major maize pathogens such as *F. graminearum* and *F. verticillioides* (Leslie & Summerell, 2006). Roots infected with *Fusarium* spp. present light to dark brown discoloration and pink to red pigmentation (Programa de Maíz del CIMMYT, 2004). Aboveground symptoms are chlorosis and wilting. However, *Fusarium* spp. can also easily be isolated from asymptomatic plants. Commonly, *Fusarium* spp. causing root rot spread to aboveground plant parts causing crown, stalk and ear rot (White, 1999). Its incidence is of concern since contamination of grains and plant tissue with mycotoxins is a serious human and animal health problem (Pestka &Smolinski, 2005; Ueno, 1983; Rocha, Ansari & Doohan 2005).

High resistance hybrid maize genotypes to *Pythium* spp. or *Fusarium* spp. are not available (Munkvold. 2003b, Cook, 2006). Selection was not possible since breeding was commonly carried out in the same locations wherein no epidemics took place (Mesterházy, Lemmens & Reid, 2012). Nowadays, infection assays are sometimes included in breeding programs. However, resistance is apparently not related to high yielding traits (Robertson-Hoyt, Kleinschmidt, White, Payne, Maragos & Holland, 2007). Considering maize as a global crop, the development of resistant genotypes to several pathogen populations is of major importance. Also improving the knowledge of the basic ecology of *Pythium* spp. and *Fusarium* spp. seems to important when developing alternative crop heath strategies. Both pathogens present complex epidemiology in agrosystems that has resulted complicated to accurately study natural populations (Munkvold, 2003a; Rodriguez-del Bosque, 1996; Flett & Wehner, 1991). Transgenic maize is also proposed as a promising solution, but no resistant genes have been identified yet (Cook, 2006; Mesterhazy et al., 2012). Apparently, resistance is conferred by many genes and its expression is sensitive to environmental conditions (Nankam & Pataky, 1996).

Maize landraces constitute a reservoir of plant pathogen resistant genes. In view that landraces were taking as starting breeding material for hybrid genotypes development, resistance may result in undesirable agronomical traits, which is important to consider (Robertson-Hoyt et al., 2007). Hence, new insights on health improvement of valuable agronomic plants have surged highlighting the importance of the maize microbiome in plant health (Johnston-Monje & Raizada, 2011b).

The maize microbiome occurs in different niches offered by the host plant. Soil surrounding a germinating seed (spermosphere) (Nelson, 2004), plant organs interior (endosphere) (Wilson, 1995), surface of stems, leaves (phyllosphere) and roots (rhizoplane) (Whipps, Hand, Pink & Bending, 2008) and soil surrounding root system (rhizosphere). Bacteria represent the bulk of maize microbiome, though fungi are also abundant (Philippot, et al., 2013).

Bacteria inhabiting the endosphere are of particular interest in root health (Hallmann, Quadt-Hallmann, Mahaffee & Kloepper, 1997). Interestingly, maize seeds of modern hybrid genotypes, landraces and teosinte predecessor share a core group of endophytic bacteria vertically transmitted from mother plants. During seed germination, they are stimulated to proliferate and allocate in the different organs with some being able to leave plants interior and settle in the rhizosphere (Johnston-Monje & Raizada, 2011b). In majority, rhizosphere of maize juvenile plants is conformed of bacteria from seed origin. In this way, seeds represent the principal microbial inoculum of maize plants at least during the first stages of the crop growth cycle. It is assumed that such bacteria conform a founder population that assist seedlings during establishment, the most vulnerable plant stage, having major implications on pathogen suppression and nutrient allocation. These effects are attributed to a co-evolutionary mutualism with serious repercussions over host fitness (Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2016).

The endosphere is a stable niche to live in, wherein nutrients and water are easily acquired (Rasche, Lueders, Schloter, Schaefer, Buegger, Gattinger & Sessitsch, 2009). Presumably, endophytes are better adapted to plants life, colonize plant niches and respond to plant stimuli more effectively than non-endophytic microorganisms (Nelson, 2004; Cankar et al., 2005). Plant endophytic associations have positive or neutral effects on host plants, but never

negative (Hallman et al., 1997).

Bacteria community structure varies according to maize genotype (Johnston-Monje et al., 2014). Besides the core microbiota, the endosphere is composed by other bacteria (Johnston-Monje & Raizada, 2011b). In some cases, specific strains are harbored (Johnston-Monje & Raizada, 2011b). For instance, Rijavec et al., (2007) found that seeds with *Lecanicillium aphanocladii* as endophyte*,* the human and plant pathogen *Pantoea ananatis* was absent and vice versa, a relation that was maize genotype dependent. This finding suggests that microbiome community composition may determine host plant health.

Life history, biotic or abiotic pressure on which maize genotypes were subjected may have been regulating the microbiome composition (Dalton & Kramer, 2007). Taking into account this hypothesis, hybrid genotypes may lack effective associated microorganisms capable to ameliorate unfavorable conditions. Since they were breed under high amounts of synthetic fertilizers and pesticides it was not necessary to recruit microbes to mitigate plant stress (Germinda & Siciliano, 2001; Smith & Goodman, 1999). On the contrary, for centuries, landraces were produced without chemical inputs, microorganisms are assumed to be of great importance for plant survival under stressful conditions (Estrada-De Los Santos, Bustillos-Cristales, & Caballero-Mellado, 2001; Gutierrez-Zamora, & Martínez-Romero, 2001). Hence, among *Zea mays* genotypes, landraces may support endophytic communities with biocontrol traits against root pathogens. However, whether endophytic bacteria contribute to host defense or resistance to pathogens remains unexplored (Philippot et al., 2013).

Studies have been conducted mainly to compare endophytic bacteria communities and structure among maize genotypes. The most abundant phyla of maize endosphere are Proteobacteria. Predominant classes are α proteobacteria, β proteobacteria and γ proteobacteria (Roesch, Camargo, Bento & Triplett, 2008; Johnston-Monje & Raizada, 2011b; Peiffer, Spor, Koren, Jin, Tringe, Dangl, Buckler & Ley, 2013). Other phyla such as Bacteroidetes, Actinobacteria, Firmicutes, Acidobacteria, Verrucomicrobia, Planctomycetes, Armatimonadetes, Gemmatimonadetes, Chloroflexi and SPAM have been also identified in lower frequencies with Bacilli, Actinobacteria, Clostridia, Deinococci as representative classes (Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2016). The identified genera on the different reports are listed in Table 1.

Table 1. Reported genera of maize bacteria endophytes.

Genera	Reference
Agrobacterium	Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Alcaligenes	Roesch et al., 2008
Arthrobacter	Johnston-Monje & Raizada, 2011b
Azoarcus	Roesch et al., 2008
Azotobacter	Roesch et al., 2008
Azohydromonas	Roesch et al., 2008
Azonexus	Roesch et al., 2008
Azoarcus	Roesch et al., 2008
Azospirillum	Johnston-Monje & Raizada, 2011b
Bacillus	Roesch et al., 2008; Rijavec et al., 2007; Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2014
Bradyrhizobium	Roesch et al., 2008; Chabot, Antoun & Cescas, 1996; Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2016;
Brevibacillus	Johnston-Monje & Raizada, 2011b
Burkholderia	Estrada de los Santos et al., 2001; Roesch et al., 2007; Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Cellulomonas	Johnston-Monje & Raizada, 2011b
Citrobacter	Johnston-Monje & Raizada, 2011b
Clostridium	Johnston-Monje & Raizada, 2011b
Chloroflexi	Johnston-Monje & Raizada, 2011b
Cohnella	Johnston-Monje et al., 2014

Cupriavidus	Johnston-Monje et al., 2014
Curtobacterium	Johnston-Monje et al., 2014
Deinococcus	Johnston-Monje & Raizada, 2011b
Delftia	Roesch et al., 2008; Johnston-Monje et al., 2014
Derxia	Roesch et al., 2008
Enterobacter	Berge, Heulin, Achouak, Richard, Bally & Balandreau, 1991; Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Enterococcus	Johnston-Monje & Raizada, 2011b
Escherichia	Johnston-Monje & Raizada, 2011b
Flexibacter	Johnston-Monje et al., 2014
Frigobacterium	Rijavec et al., 2007
Hafnia	Johnston-Monje & Raizada, 2011
Herbaspirillum	Roesch et al., 2008; Baldani et al., 1986; Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2014
Janthinobacterium	Johnston-Monje et al., 2014
Klebsiella	Roesch et al., 2008; Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2014
Kytococcus	Johnston-Monje et al., 2014
Luiteibacter	Johnston-Monje & Raizada, 2011b
Lysobacter	Johnston-Monje et al., 2014
Mesorhizobium	Roesch et al., 2008; Johnston-Monje et al., 2014
Methylobacterium	Roesch et al., 2008, Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2014
Methylocystis	Roesch et al., 2008
Methylosinus	Roesch et al., 2008
Microbacterium	Roesch et al., 2008; Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2014
Micrococcus	Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2014
Mycobacterium	Johnston-Monje et al., 2014
Paenibacillus	Roesch et al., 2008; Rijavec et al., 2007; Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2014
Pantoea	Rijavec et al., 2007; Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Pandoraea	Johnston-Monje et al., 2014
Pedobacter	Johnston-Monje et al., 2014
Pelomonas	Roesch et al., 2008
Pseudomonas	Roesch et al., 2008; Berge et al., 1991; Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2016
Raoultella	Roesch et al., 2008; Berge et al., 1991
Rhizobium	Roesch et al., 2008; Gutiérrez-Zamora & Martínez-Romero, 2001; Rosenblueth & Martínez-Romero, 2004; Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Rodococcus	Johnston-Monje & Raizada, 2011b

Rhodoblastus	Roesch et al., 2008
Sediminibacterium	Johnston-Monje & Raizada, 2011b
Sinorhizobium	Roesch et al., 2008
Sphingobacterium	Johnston-Monje & Raizada, 2011b
Sphingobium	Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Sphingomonas	Rijavec et al., 2007; Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Staphilococcus	Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2014
Stenotrophomonas	Johnston-Monje & Raizada, 2011b; Johnston-Monje et al., 2014; Johnston-Monje et al., 2016
Streptomyces	Johnston-Monje & Raizada, 2011b
Xanthobacter	Roesch et al., 2008
Xanthomonas	Johnston-Monje & Raizada, 2011; Johnston-Monje et al., 2014

Diazotrophy is a common trait of members of the genera listed in table 1 (Estrada de los Santos et al., 2001; Roesch et al., 2008; Fouts, Tyler, DeBoy, Daugherty, Ren, Badger, Durkin, Huot, Shrivastava, Kothari, Dodson, Mohamoud, Khouri, Roesch, Krogfelt, Struve, Triplett & Methé, 2008) Phosphorous solubilization, auxin, gibberellin, siderophore, acetoin, lumichrome and ACC deaminase production are also exhibited by some species (Bashan, Holguin & de-Bashan, 2004; Johnston-Monje & Raizada, 2011b). In terms of health, some species have shown excellent antagonistic activity against plant pathogens (Dobbelaere et al., 2003; Kennedy, Choudhury & Kecskés, 2004). In a study conducted by Johnston-Monje et al., (2014), several endophytic strains isolated from two maize genotypes suppressed *Fusarium graminearum* and *Aspergillus flavus* growth. Overall, many bacteria have been found to effectively suppress root pathogens (Philippot et al., 2013; Pal, Tilak, Saxcna, Dey & Singh, 2001). The mechanisms involved include secretion of lytic acid, release of nonspecific volatile inhibitors, production of siderophore and antibiotic compounds as well as induction of the plant defense system (Raaijmakers, Leeman, Van Oorschot, Van der Sluis, Schippers & Bakker, 1995; Handelsman & Stabb, 1996; Whipps, 2001; Van Loon, Bakker & Pieterse, 1998).

The root system and associated rhizosphere are carbon rich substrates that promote microbial proliferation and activity. Indeed, both represent the richest and most diverse microbial plant habitat. Microorganisms residing in soil, organic debris, or neighboring rhizospheres are attracted by rhizodeposits, some settle in the rhizosphere, others enter to the root endosphere through cracks, wounds, root tips, mycorrhizae or by secreting cellulose and pectinase (Starr and Chatterjee 1972; Reis, Urquiaga, Paula & Döbereiner, 1990; Hallmann et al., 1997; Kovtunovych, Lar, Kamalova, Kordyum, Kleiner & Kozyrovska, 1999). Both plant pathogens and mutualistic symbionts respond to plant signals. However, indigenous plant endophytes are more competitive in colonizing maize niches than exogenous microbes, being the first in respond to plants stimuli (Rosenblueth & Martínez-Romero, 2004). Suppressive microbial communities to soil borne pathogens may be well represented in roots. Firstly, because it is a microbial hot spot niche, and secondly in response to biotic pressure during evolution as site of main entrance and direct contact to soil borne pathogens. For instance, diazotrophic endophytes diversity is higher in maize roots and rhizosphere than in stems (Roesch et al., 2008).

Another group that respond rapidly to maize rhizodeposits are arbuscular mycorrhiza fungi (AMF) (Larsen et al., 2015). AMF symbiosis is considered an ancient microbial-plant interacting system with serious implications in land plant evolution (Remy, Taylor, Hass & Kerp, 1994). AMF are ubiquitous soil borne microbes, propagated by spores, of obligate biotrophy, capable to either colonize roots endosphere and rhizosphere (Smith & Read, 2008). Commonly, AMF establish symbiosis with maize plants, but genotype affinity is often

documented (Sawers, Gutjahr & Paszkowski, 2008). The overall consensus is phosphorus translocation as major contribution of AMF symbiosis to host plant. In turn, plants pay with 4% to 20% of carbon rich compounds gained from photosynthesis (Wright, Read & Scholes, 1998; Bago, Pfeffer & Shachar-Hill, 2000). Another plant improvement gained from AMF symbiosis is plant pathogen suppression, there are reports that AMF symbiosis reduce soilborne pathogens propagules such as *Aphanomyces*, *Fusarium*, *Phytophtora* and *Pythium* species (Slezack, Dumas-Gaudot, Paynot & Gianinazzi, 2000; Filion, St-Arnaud & Jabaji-Hare 2003; Cordier, Gianinazzi & Gianinazzi-Pearson, 1996; St-Arnaud, Hamel & Fortin, 1994; Larsen, Graham, Cubero & Ravnskov, 2012). The evidence shows that AMF may act as plant disease suppressors through *i*) direct competition or inhibition of plant pathogens; (*ii*) enhanced or altered plant growth, nutrition, and morphology; (*iii*) promote biochemical changes associated with plant defense mechanisms and induced resistance; and (*iv*) recruit microorganisms with antibiosis traits against plant pathogens (Whipps, 2004). The last mechanism is of particular interest in the study of maize microbiome as potential source of biocontrol agents of plant pathogens. Azcón-Aguilar & Barea (1996) showed that composition of microbes residing in rhizosphere change under arbuscular mycorrhiza formation and suggested that bacteria with plants health improvement traits may be favored to settle. Indeed, Vázquez, César, Azcón & Barea (2000) reported that *Azospirillum*, *Pseudomanas* and *Trichoderma,* all suppressors of plant disease, succeed in stablish in maize mycorrhizal plants. In a study conducted by Li, Ravnskov, Xie & Larsen (2007), the biocontrol of *Pythium* damping-off by *Paenibacillus* spp. isolated from mycorrhizosphere was demonstrated. Association between some AMF and bacteria with biocontrol disease traits is expected to show a greater plant disease suppression (Budi, Van Tuinen, Martinotti & Gianinazzi, 1999),

though this synergic effect depend on AMF strain, pathogen strain, soil, host, bacteria strain, time and environmental conditions (Whipps, 2004).

Taking in to account that maize endophytic bacteria are a key part of the maize microbiome and that AMF commonly form symbiosis with maize plants, interaction between both agents most be considered as highly frequent and may play a significant role in maize root health (Hallman & Berg, 2006).

V. GENERAL HYPOTHESIS

Maize root health depends on associated seed endophytic bacteria resulting in improved root health.

VI. GENERAL OBJECTIVE

Main objective is to investigate the role of maize seed endophytic bacterial communities in root health of non-mycorrhizal and mycorrhizal maize.

VII. PHASE 1

SUSCEPTIBILITY OF MAIZE LANDRACES AND HYBRIDS GENOTYPES TO ROOT PATHOGENS

HYPOTHESIS

-Maize genotypes are differentially affected by pathogens

-Maize landraces are resistant or more tolerant to root pathogens than maize hybrid genotypes

OBJECTIVE

To identify maize genotypes resistant and susceptible to *Pythium arrhenomanes*, *Pythium* sp., *F. graminearum* and *F. verticillioides*.

MATERIAL AND METHODS

Experimental design

The experiment was performed with a two-way factorial design with maize genotypes (10 genotypes) and pathogens (five levels; without and with four different pathogens) as main factors. Each of the 50 treatments had 4 repetitions resulting in 200 experimental units. The factor maize (*Zea mays* spp. *mays*) genotype included ten genotypes with commercial importance in the midwest area of Mexico. Five hybrid genotypes: DK-2061 (DK2061) from Dekalb®, H-318 (H318) from Milpal®, PUMA from Asgrow®, NB9 from Novasem® and CRM-52 (CRM52) from CB®, and five landraces: Elotes occidentales (E. occidentales or E. occi), Azul, Rojo espada (R. espada), Dulce and Ancho. The factor pathogen included a non-inoculated control treatment and the four pathogens *Pythium arrhenomanes*, *Pythium* sp., *Fusarium verticillioides* amd *F. graminearum* (Table 2).

Table 2. Fully factorial design (10x5), with 50 treatments, 4 replicates per treatments and 200 experimental units.

Factors	
Maize genotype	Pathogen
DK2061	Without
H318	*P. arrhenomanes*
PUMA	*Pythium* sp.
NB9	*F. verticillioides*
CRM52	*F. graminearum*
E. occidendatales	
Azul	

R. espada

Dulce

Ancho

The experiment was performed as a greenhouse pot experiment at the Autonomous National University of Mexico, Morelia, Mexico (19°39′03″N, 101°14′06″W), from October to November 2016, under greenhouse conditions. Plants were grown with natural day/night photoperiod and temperature. Pots were arranged in a complete randomized block design of four blocks and fifty pots per block with one repetition from each treatment on each block.

Maize genotypes

Ten maize genotypes were employed, five hybrids, NB9, DK2061, PUMA, CRM52 and H318, and five landraces, Dulce, E. occidentales, Ancho, Azúl and R. espada. Seeds were stored at 4 °C ± 1 °C. Prior use DK2061, PUMA, CRM52 and R. espada seeds were immersed in tap water and scraped with a scouring pad in order to remove fungicide, insecticide and color coatings.

Seed surface disinfestation

Under an optical microscope, seeds with no cracks or visible deformation were selected, immersed in 50% commercial chlorine (Clorox®) and placed in an orbital shaker (200 rpm) for 10 min, then drained, finally rinsed three times in distilled sterile water for 5 minutes in an orbital shaker. To test the efficiency of surface disinfestation, 100 µl of the final wash was plated out on Triptic Soy Agar (TSA), in five replicates. Plates were incubated at 27 °C, after

five days plates were checked and no bacteria growth was detected.

Pathogens

The pathogens employed were *P. arrhenomanes* CPV-669, *Pythium* sp., *F. verticillioides* and *F. graminearum*. All strains were isolated from maize diseased plants. Until their use, mycelia discs (5 mm Ø) were stored in distilled sterile water at 4 °C ± 1 °C.

Pathogen inoculum

Agar discs (3mm Ø) with mycelium of an actively growing pathogen culture were inoculated on cucumber discs (7 mm Ø), then incubated in a Petri dish in the dark at room temperature for 48 h. For treatments without pathogen (-P), agar discs with no pathogen were applied to the cucumber discs.

Plant growth substrate

The growth substrate consisted on a mixture of fertilized sterile soil and sand 1:1 (w:w). The soil employed was of clayish texture, composed of 53.2% clay, 27.3% silt and 19.5% sand. The chemical composition reported was: 2.7% organic matter, 23.2 mg/kg inorganic nitrogen, 5.8 mg/kg available phosphorus (Olsen P) and pH (H_2O) 7.3. Soil was collected from the experimental field of the Chapingo Autonomous University located in Morelia, Michoacán, Mexico in Summer 2016.

Soil:sand mix (1:1, w/w) applied to plastic bags was sterilized by autoclaving at 121 °C ± 1 °C, 15 Lb for 1 h. Then, bags were left opened for 24 h, and autoclaved again at 121 °C ± 1

°C, 15 Lb for 1 h. Once sterile, 200 g soil and 200 g sand were mixed homogeneously and transferred to a plastic bag. On the surface of the soil sand mix six rows were made, on each row the following nutrient solutions were added (mg kg^{-1} growth substrate): (I) K_2SO_4, 370.31. (II) $CaCl_2$ x $2H_2O$, 75.0. (III) $CuSO_4$ x $5H_2O$, 2.1; $ZnSO_4$ x $7H_2O$, 5.4; $MnSO_4$ x H_2O,10.5; Na_2MoO_4 x $2H_2O$, 0.18. (IV) NH_4NO_3, 285.71. (V) $MgSO_4$ x $7H_2O$, 405.43. (VI) KH_2PO_4, 30.0. Finally, it was left to dry for 24 h. Hereafter the nutrients were mixed carefully into the soil:sand mix.

Plant bioassay

Each plastic bag containing sterile fertilized soil-sand mix was placed inside a plastic cup. Then, a sterile seed was sown, and growth substrate was watered at 65% of the water holding capacity when needed. Seventeen days after sowing, pathogens were inoculated by placing one infested cucumber disc next to the stem at 3 cm depth. In treatments without pathogen a non-infected cucumber disc was applied. Every second day the growth substrate was watered at 85% of their water holding capacity and plants rotated. Twenty-five days after pathogen inoculation, plants were harvested.

Harvest and analyses

The entire plant was removed from the plastic bag. Then soil was removed by washing roots thoroughly under running tap water. Once roots were cleaned, shoots and root were separated. Shoots were placed in paper bags and dried in an oven at 50°C, 72 h. Then, weighed out and recorded as shoot dry weight.

Roots were cut in 1 cm pieces and homogenized in a container with water. Then, filtered through a sieve, roots were collected and hand squeezed to remove excess water. Hereafter root fresh weight was recorded. A root sample of around 1 g was taken and stored in 85% glycerol. The rest was placed in a paper bag, dried in an oven at 50°C for 72 h, and the root dry weight was recorded.

The root sample was used to calculate total root length, measured by microscopy at 16x magnification, using the point intersect method, over 1 cm field of view. In a Petri dish (8 cm ⌀), fifteen random points were drawn and root sample was uniformly in the Petri dish. Roots intersecting the hair line corresponding to 1 cm were recorded. Root length was calculated (Newman, 1966). Total root length was estimated multiplying root length from the subsample with the total root fresh weight. Root length density was calculated dividing total root length by soil volume on each pot. Specific root length was calculated dividing total root length by rot dry weight.

Statistics

Statistical analysis and figures were made by using R software (v. 3.3.2, R Core Team 2013). Results were analyzed in their original scale of measurement with a generalized linear model (GLM). Distribution was fit as gamma, Gaussian or quasibinomial. Post-hoc analysis for mean comparison at a 95 % confidence level was performed in terms of Tukey´s test.

RESULTS

Significant "Maize genotype x Pathogen" interactions were obtained for the variables root dry weight, total root length and root length density (Table 3), where differential response of maize genotypes to root pathogens was observed.

For shoot dry weight significant factor effects for both "Maize genotypes" and "Pathogens" were obtained, but no interaction between factors was observed (Table 3). Specific root length significantly differed between maize genotypes independent of pathogen treatment (Table 3).

Table 3. Levels of significance of main factors and their interaction assessed by generalized linear model analysis of the different variables examined.

	Shoot dry weight (g)	Root dry weight (g)	Plant total root length (m)	Root length density ($m\ g^{-1}$)	Specific root length ($m\ g^{-1}$)
Maize genotype (M)	***	***	***	***	***
Pathogen (P)	***	***	***	***	0.104
M X P	0.528	***	***	***	0.070

*, 0.05; **, 0.01; ***, 0.001

Shoot dry weight

Shoot dry weight varied between maize genotypes. CRM52 and NB9 produced the lowest shoot dry weight being statistically different from H318, PUMA, E. occidentales and Ancho. CRM52 was also different to R. espada (Fig. 1). In hybrids, significant differences in shoot dry weight were shown, where no differences were found between landraces (Fig. 1). In general, variation in shoot dry weight of the individual maize genotype was higher in landraces than in hybrids (Fig. 1).

In the pathogen treatments, the shoot dry weight was significantly suppressed by *P. arrhenomanes* CPV-669 (Fig. 2) independent of maize genotypes, whereas no effects were observed with *Pythium* sp., *F. verticillioides* and *F. graminearum* treatments (Fig. 2).

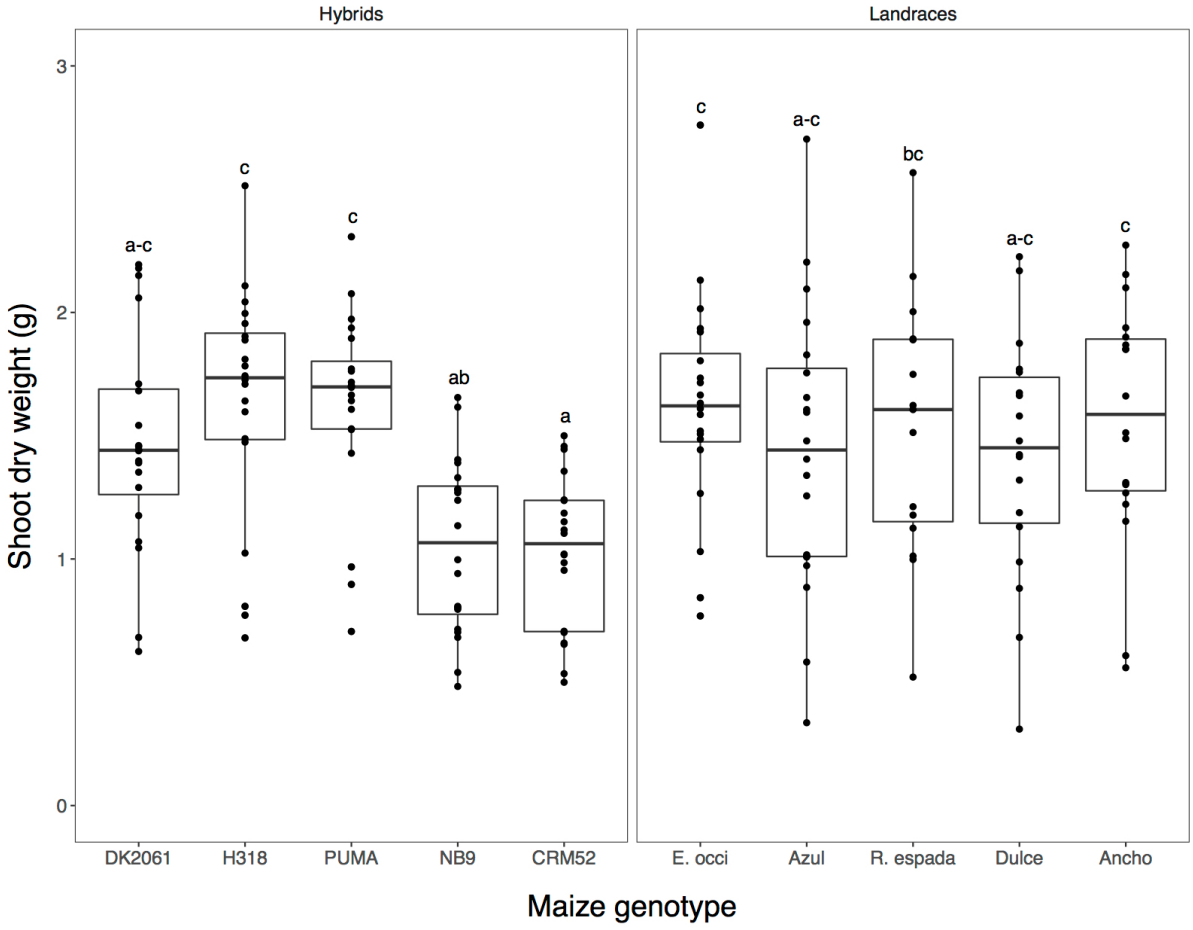

Figure 1. Box-plot showing factor treatments means for shoot dry weight of 42 days old maize plants of the maize hybrids DK2061, H318, PUMA, NB9 and CRM52 and the landraces E. occi, Azul, R. espada, Dulce and Ancho grown under greenhouse conditions. Upper extreme is maximum observation, lower extreme is minimum observation, top of the box is upper or third quartile, bottom of the box is lower or first quartile, middle bar is median value, points are data of twenty maize plants, outside points are possible outliers. Treatments with same letters are not significantly different by Tukey´s test ($p < 0.05$).

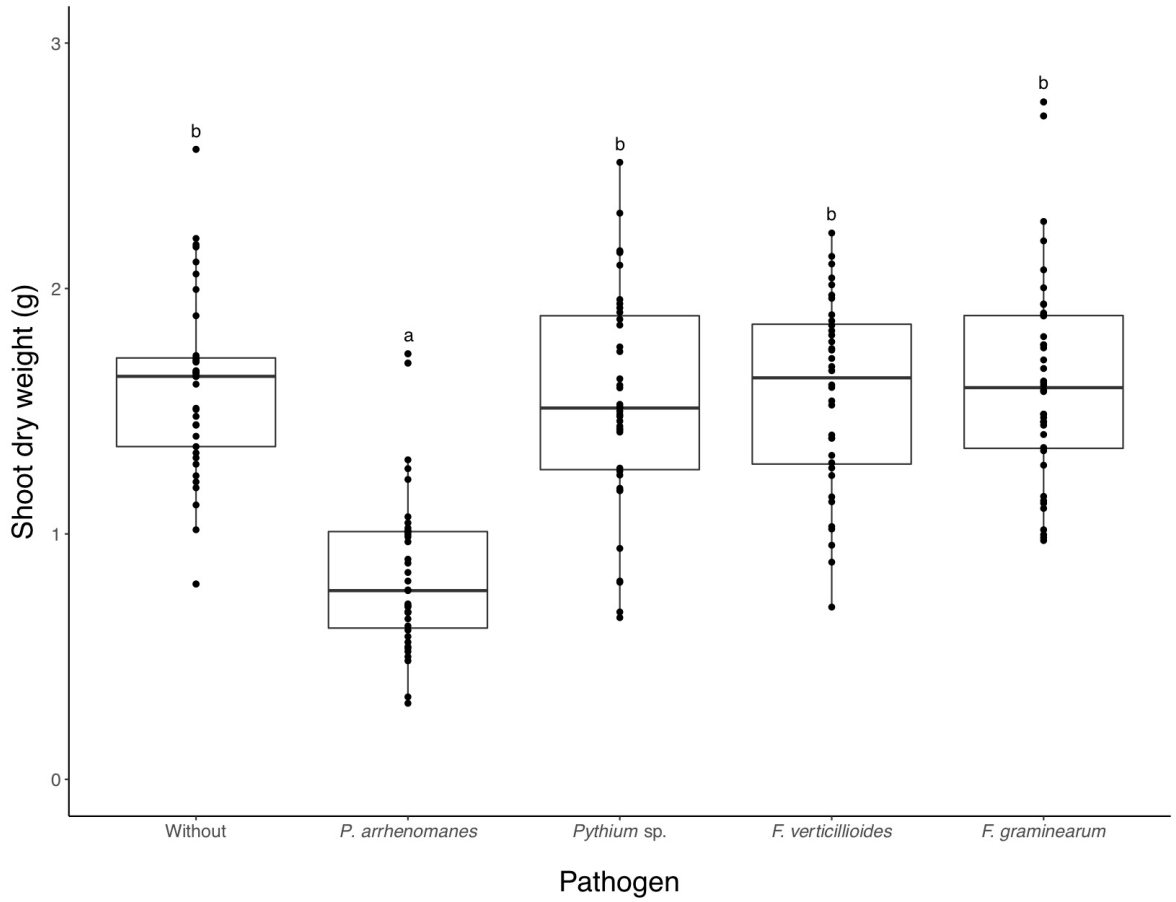

Figure 2. Shoot dry weight factor treatment means of the factor "Pathogen"of maize plants infected with four pathogens (*P. arrhenomanes*, *Pythium* sp., *F. verticillioides* and *F. graminearum*) or without (-P) seventeen days after sowing. Plants were harvested forty-two days after sowing. Upper extreme is maximum observation, lower extreme is minimum observation, top of box is upper or third quartile, bottom of box is lower or first quartile, middle bar is median value, points are data of around twenty maize plants, outside points are possible outliers. Same letters are not significantly different by Tukey´s test ($p < 0.05$).

Root dry weight

In all *P. arrhenomanes* treatments, root dry weight decreased. However, only DK2061, H318, CRM52 and Azul showed statistical differences with treatments without pathogen inoculation (Fig. 3). For the other pathogens *Pythium* sp, *F. verticillioides* and *F. graminearum* no effects were observed (Fig. 3).

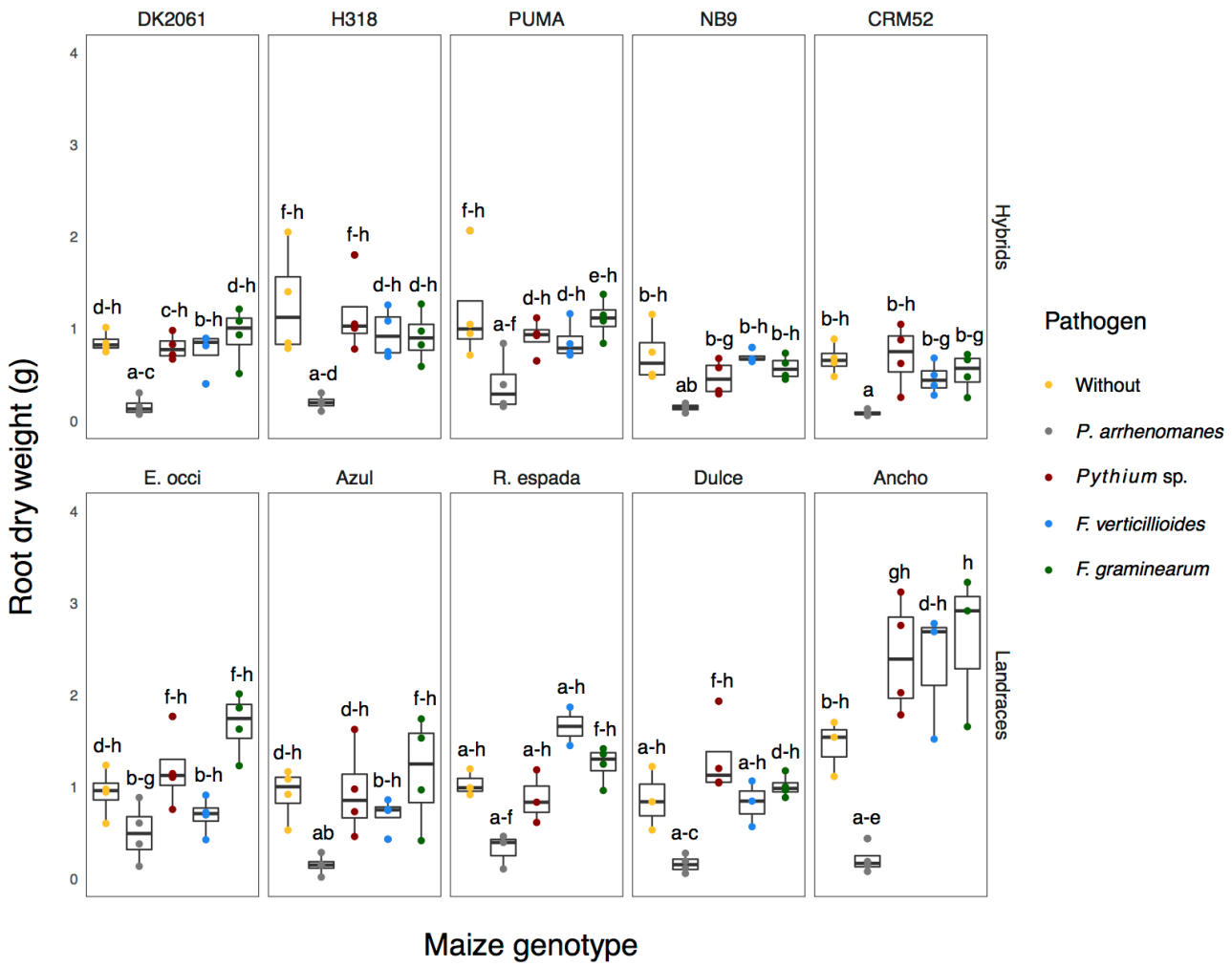

Figure 3. Box-plot showing root dry weight of five maize hybrid genotypes (DK2061, H318, PUMA, NB9 and CRM52) and five landraces (E. occi, Azul, R. espada, Dulce and Ancho), grown under greenhouse conditions, inoculated with four pathogens (*P. arrhenomanes* CPV-669, *Pythium* sp., *F. verticillioides* and *F. graminearum*) or without (-P). Plants were harvested forty-two days after sowing. Upper extreme is maximum observation, lower extreme is minimum observation, top of box is upper or third quartile, bottom of box is lower or first quartile, middle bar is median value, points are data of around four maize plants, outside points are possible outliers. Treatments with the same letters are not significantly different by Tukey´s test ($p < 0.05$).

Plant total root length

Total root length of all genotypes decreased in *P. arrhenomanes* treatments, however, only significantly for the genotypes DK2061, H318, NB9, CRM52, Azul, R. espada and Ancho

(Fig. 4). No effects were observed with the other pathogens *Pythium* sp., *F. verticillioides* and *F. graminearum* (Fig. 4).

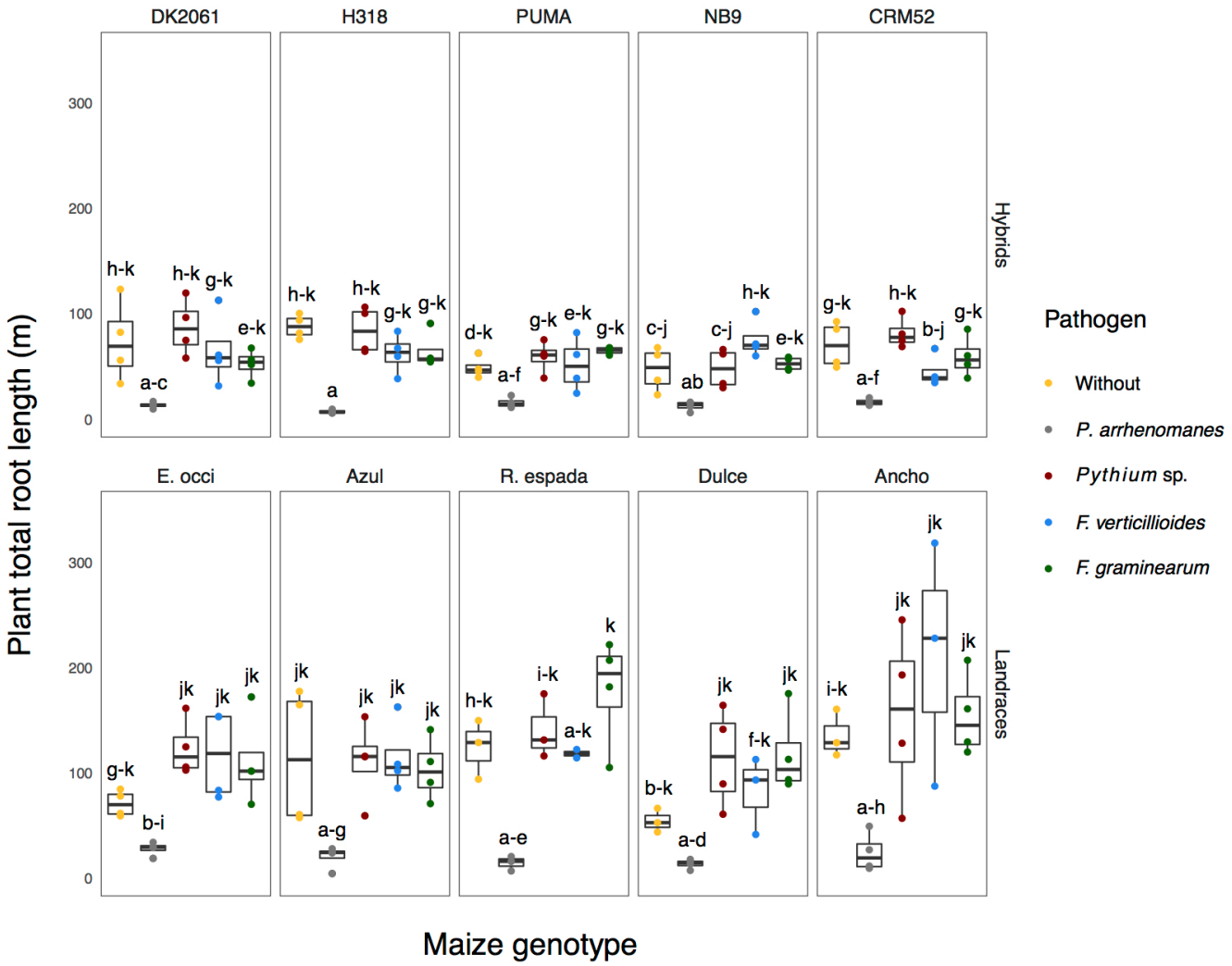

Figure 4. Box-plot showing plant total root length of five maize hybrid genotypes (DK2061, H318, PUMA, NB9 and CRM52) and five landraces (E. occi, Azul, R. espada, Dulce and Ancho), grown under greenhouse conditions, inoculated with four pathogens (*P. arrhenomanes* CPV-669, *Pythium* sp., *F. verticillioides* and *F. graminearum*) or without (-P). Plants were harvested forty-two days after sowing. Upper extreme is maximum observation, lower extreme is minimum observation, top of box is upper or third quartile, bottom of box is lower or first quartile, middle bar is median value, points are data of around four maize plants, outside points are possible outliers. Treatments with the same letters are not significantly different by Tukey´s test ($p < 0.05$).

Root length density

Root length density of all genotypes decreased in *P. arrhenomanes* CPV-669 treatments, however, only significantly for the genotypes DK2061, H318, NB9, CRM52, Azul, R. espada and Ancho (Fig. 5). No effects of the other pathogens were observed.

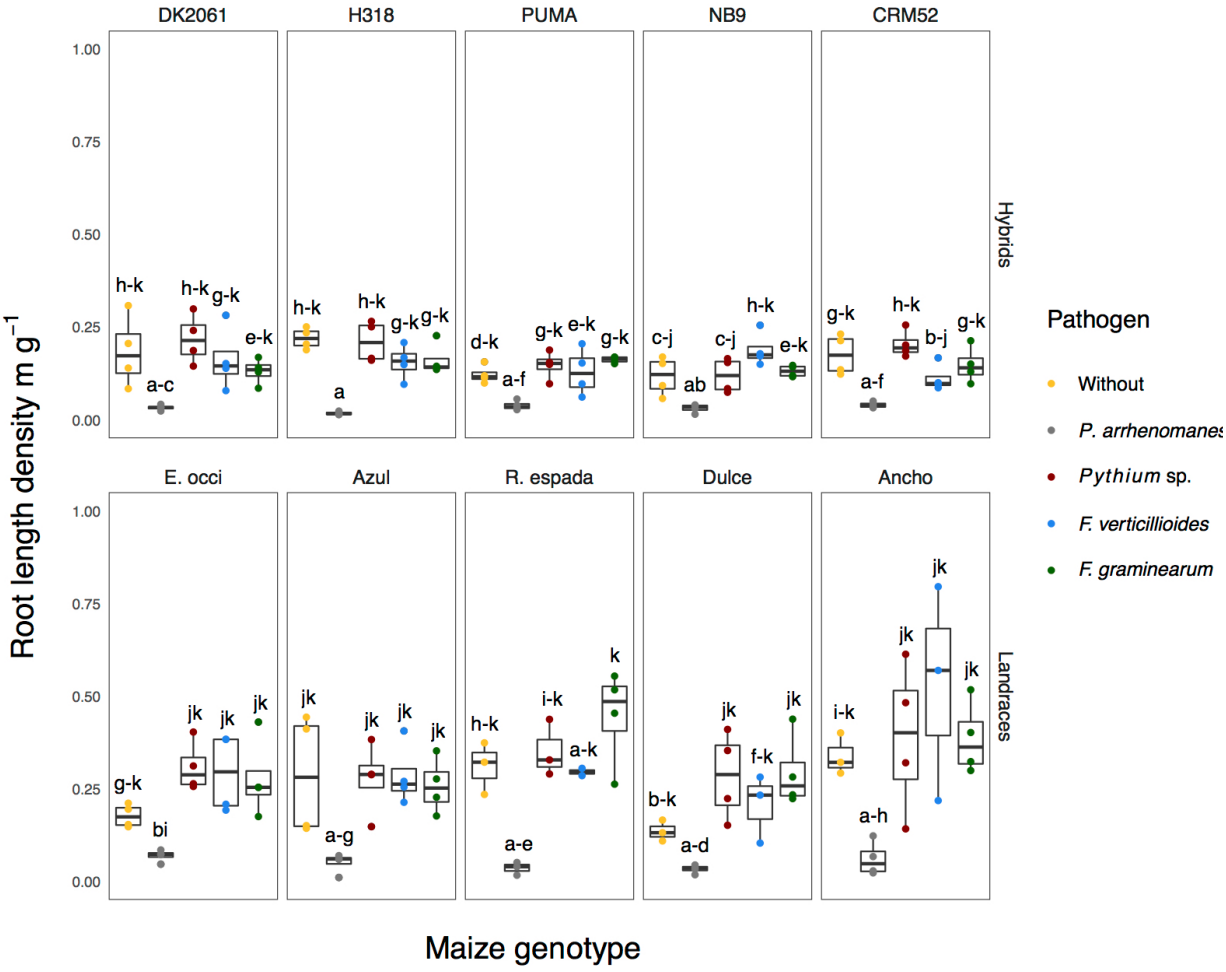

Figure 5. Box-plot showing root length density of five maize hybrid genotypes (DK2061, H318, PUMA, NB9 and CRM52) and five landraces (E. occi, Azul, R. espada, Dulce and Ancho), grown under greenhouse conditions, inoculated with four pathogens (*P. arrhenomanes* CPV-669, *Pythium* sp., *F. verticillioides* and *F. graminearum*) or without (-P). Plants were harvested forty-two days after sowing. Upper extreme is maximum observation, lower extreme is minimum observation, top of box is upper or third quartile, bottom of box is lower or first quartile, middle bar is median value, points are data of around four maize plants, outside points are possible outliers. Treatments with the same letters are not significantly different by Tukey´s test ($p < 0.05$).

Specific root length

Specific root length of each maize genotype was different. In H318 and PUMA SRL was low comparing with CRM52, Azul and R. espada, among them statistical differences were noted (Fig. 6). In CRM52, Azul and R. espada, SRL was higher, in particular on Azul (Fig. 6). Nonetheless, neither PUMA and H318 nor CRM52, Azul and R. espada were different to the rest of genotypes (Fig. 6). Excepting Azul or CRM52 with Ancho (Fig. 6). In hybrids, CRM52 produced the highest SRL. On the contrary, H318 and PUMA produced the lowest SRL. Nonetheless, CRM52, H318 and PUMA did not were significative different with DK2061 and NB9 SRL (Fig. 6). As for landraces, only statisticall differences were noted between Ancho and Azul SRL, the highest and lowest respectively (Fig. 6). But, both were not different to E. occidentales, R. espada and Dulce. However, hybrids and landraces did not show particularities on SRL (Fig. 6).

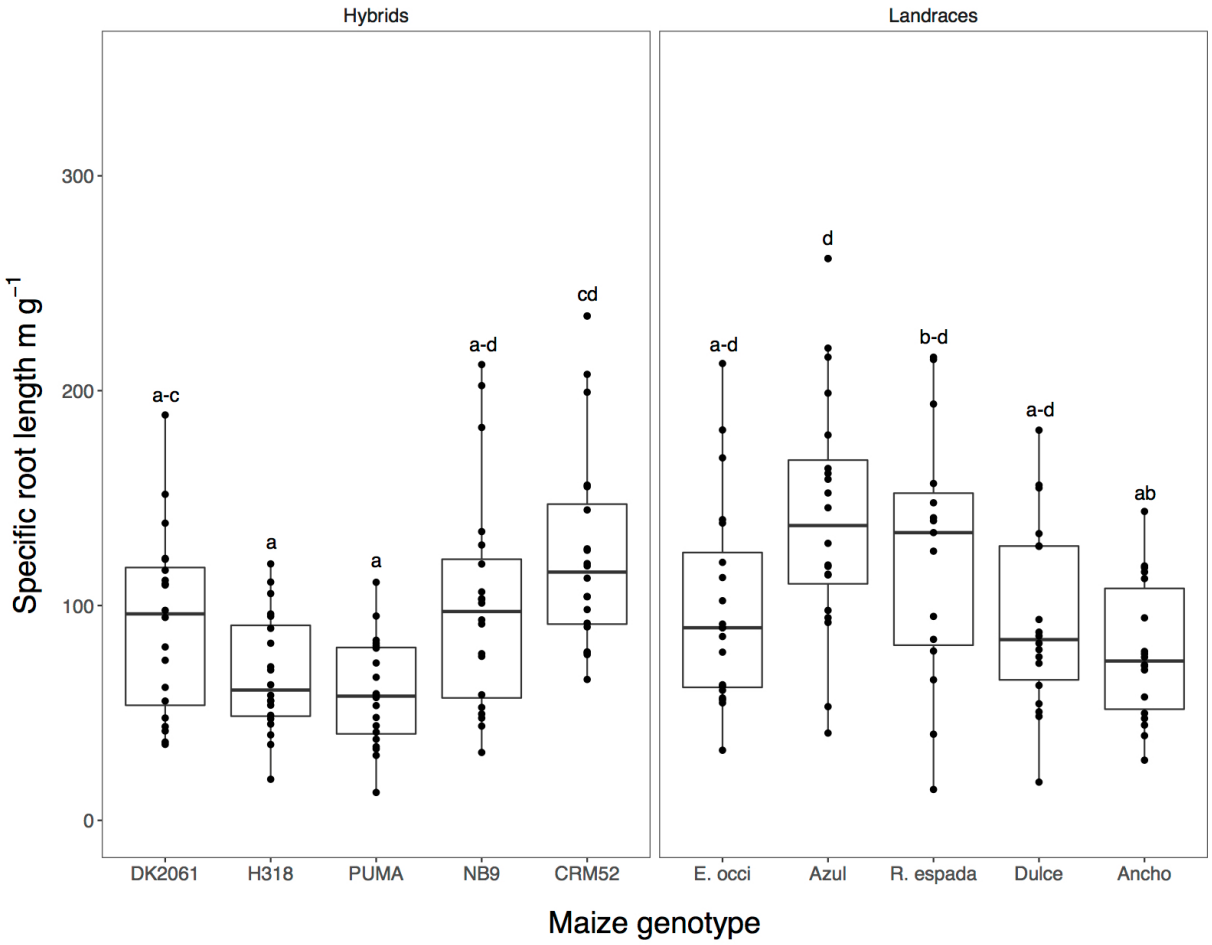

Figure 6. Box-plot showing specific root length of five maize hybrid genotypes (DK2061, H318, PUMA, NB9 and CRM52) and five landraces (E. occi, Azul, R. espada, Dulce and Ancho), grown under greenhouse conditions, inoculated with four pathogens (*P. arrhenomanes* CPV-669, *Pythium* sp., *F. verticillioides* and *F. graminearum*) or without (-P). Plants were harvested forty-two days after sowing. Upper extreme is maximum observation, lower extreme is minimum observation, top of box is upper or third quartile, bottom of box is lower or first quartile, middle bar is median value, points are data of around four maize plants, outside points are possible outliers. Treatments with the same letters are not significantly different by Tukey´s test ($p < 0.05$).

VIII. PHASE 2

INTERACTION MAIZE-ROOT ENDOPHITIC BACTERIA COMMUNITIES-*R. irregularis* - *P. arrhenomanes*

HYPOTHESES

-Maize seed bacterial communities from pathogen resistant maize genotypes have biocontrol traits against root pathogens

-The AMF *R. irregularis* promote plant resistance to *P. arrhenomanes* and modify the biocontrol effect of maize seed bacterial communities against *P. arrhenomanes*

-The effects of seed bacterial communities, *P. arrhenomanes* and *R. irregularis* on maize plant growth depend on maize genotype

OBJECTIVE

Examine the effects of *R. irregularis* and maize seed endophytic bacteria communities on maize plants growth and biocontrol against *P. arrhenomanes*

MATERIAL AND METHODS

Experimental design

Treatments consisted in maize plants (*Zea mays* ssp. *mays*) of susceptible (H318) and partial resistant (NB9) genotypes to *P. arrhenomanes,* inoculated with AMF (+ AMF) or not (- AMF), infected with *P. arrhenomanes* (+ P) or not (- P), and inoculated with bacteria (BNB9, BEocci and BH318) or not (- B) in a full factorial design (2x2x4x2) with 4 replicates per treatment (Table 4). BNB9 and BEocci were isolated from partial resistant maize genotypes (NB9 and E. occidentales) and BH318 from a susceptible maize genotype (H318) to *P. arrhenomanes.*

Table 4. Full factorial design (2x2x4x2), with 32 treatments, 4 replicates per treatment and 120 experimental units (eu).

Factors			
Maize	*P. arrhenomanes*	Bacterial community	AMF
NB9	With (+ P)	BNB9	With (+ AMF)
H318	Without (- P)	BH318	Without (- AMF)
		BEocci	
		Without (- B)	

The experiment was performed under greenhouse conditions at Aarhus University, Denmark (55°19'N,11°24'E), from February to March 2017. Plants were grown in pots with day/night

photoperiod 18/20 h, 24/20 °C set in a complete randomized block design of four blocks and thirty-two plants per block.

Pathogen

P. arrhenomanes CPV-669 were stored at 4 °C ± 1 °C in sterile distilled water until used. To conduct the experiments the pathogen was reactivated on corn meal agar (CMA) and maintained through periodically plating a single hyphae from the edge of a growing culture, and maintained in the dark at room temperature.

Pathogen inoculum

Corn meal agar (CMA) discs with 48 h old mycelium (5 mm Ø), were inoculated on cucumber discs (1.0 cm Ø), and maintained at room temperature in the dark for 72 h. Cucumber discs colonized with mycelium were used as pathogen inoculum in +P treatments. On -P treatments CMA discs with no mycelium colonization were used.

Maize genotypes

Tree maize genotypes, two hybrids (NB9 and H-318) and one land race (E. occidentales) were employed.

Seed surface disinfestation

Under an optical microscope, seeds with no cracks or visible deformation were selected, then surface disinfested with 2.5% NaClO and placed in an orbital shaker (200 rpm) for 10 min, then drained, following two washing steps in 2% $Na_2S_2O_3$ for 5 min under orbital shaking

(Miché & Bandndreau 2001). Finally, seeds were rinsed two times in distilled sterile water for 5 minutes in an orbital shaker. To test the efficiency of surface disinfestation, 100 µl of the final wash was plated out on Triptic Soy Agar (TSA), in five replicates. Plates were incubated at 27 °C, after five days plates were checked and no bacterial growth was detected.

Seedlings

Surface disinfested seeds were placed tip cap down in rows with alternate arrangement between a rolled humid paper sheet. Rolls were placed inside a sealed Petri dish (15 cm Ø) and maintained in a growth chamber at 27 °C, 72 h.

Bacteria inoculum

Tree seedlings with same root length were dissected, macerated with 5 mL 0.9% NaCl in a sterile mortar using a sterile ceramic pestle. The homogenate was poured into a flask containing Tryptic Soy Broth (TSB) (150 mL) at 7.2 pH, with cycloheximide (200 mg L^{-1}), and incubated at 27 °C under orbital shaking (200 rpm). After 24 h bacteria cells were harvested by centrifugation for 10 min at 4500 rpm, 20°C. Resulted bacteria pellet was rinsed three times in 0.9% NaCl by vortex and centrifugation (10 min, 4500 rpm, 20°C). Finally, bacteria pellet was resuspended in sterile distilled water, the resulted suspension was used as inoculum in + B treatments. Three replicates per inoculum were prepared. For - B treatments, the inoculum was sterilized in an autoclave and let to cool to room temperature. To ensure no contamination, tree additional flasks with TSB without roots homogenate were employed, where no bacteria growth was recorded.

Bacteria cells viability was calculated by preparing serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}), an aliquot (100 µL) of each dilution was spread on TSA and incubated at 27 °C, 72 h. Every 24 h, CFU were counted. Five replicates per dilution were done. In fuchs-rosenthal chamber, bacteria cells were calculated and final suspension adjusted to 10^{8} CFU mL^{-1}.

Growth substrate

Soil

Soil was sieved. Then, heat sterilized at 80 °C for 48 h, left to cool for 24 h at room temperature and again heat sterilized at 80 °C for 48 h.

Sand

Sand was spread in plastic trays, on the surface, five rows were made, in each row the following nutrient solutions were added (mg kg^{-1} growth substrate): (I) K_2SO_4, 370.31.(II) $CaCl_2X2H_2O$, 75.0. (III) $CuSO_4X5H_2O$, 2.1; $ZnSO_4X7H_2O$, 5.4; $MnSO_4XH_2O$,10.5; $Na_2MoO_4X2H_2O$, 0.18. (IV) NH_4NO_3, 285.71. (V) $MgSO_4X7H_2O$, 405.43. Finally, the sand was left to dry overnight in an oven at 30°C.

Substrate preparation

In a blender, sand and sterile soil were mixed 1:1 w/w (100:100 kg). The resulted mix was employed as a growth substrate for maize plants.

AMF Inoculum

In +AMF treatments a commercial inoculum provided by Simbiom Ltd., (Lanskroun, Czech Republic) was employed. It contained expanded clay as a carrier of *Rhizophagus irregularis* spores (250^3 spores 250 g^{-1}). In -AMF treatments, carrier expanded clay without inoculum was obtained.

Plant assay

Each pot was filled out with two layers. On + AMF treatments the bottom layer contained a mixture of 750 g of growth substrate and 1.5 g of AMF inoculum and top layer 750 g of growth substrate. In -AMF treatments, the bottom layer was a mixture of 750 g of growth substrate and 1.5 g of carrier expanded clay without inoculum. Growth substrate was then watered, two rounded wood sticks (8 cm x 0.7 mm ⌀) were buried in the humid growth substrate (4 cm depth) and 1 cm beside the stick two sowing holes were made (5 cm depth). On + B treatments 1 mL of bacteria suspension was added to each sowing hole just before pre-germinated seeds were sown. On - B treatments 1 mL of the sterile suspension was added. Two seedlings were sown in each pot and seven days after emergence seedlings were thinned out to one plant per pot. Eighteen days after sowing, pathogen was inoculated in + P treatments by removing wood sticks and burying in the hole one infested cucumber disc next to the roots. In - P treatments none infested cucumber discs were placed next to the roots. Pots were rotated and watered to 85% of the WC with tap water every second day. Once a week, plants were fertilized with 4.5 mL of NH_4NO_3 (N:100 mg kg^{-1}). Thirty-five days after sowing plants were harvested.

Harvest

The entire plant was removed from the plastic pot. Then soil was thoroughly washed off the roots under running tap water. Once roots were cleaned, shoots and roots were separated.

Shoots were placed in paper bags and dried in an oven at 80°C, 48 h. Then, weighed out and recorded as shoot dry weight.

Roots were cut in 1 cm pieces, and mixed in a container with water. Then, filtered through a sieve, roots were collected and hand squeezed to remove water. Finally, roots were dried out between filter paper by pressing to remove excess water. A 1 g root sample was taken for staining. The rest were placed in a paper bag and dried in an oven at 80°C for 48 h, weighed and recorded as root dry weight. The sum of shoot and root dry weights were recorded as total plant dry weight.

Root staining

Root samples were first cleared by immersion in 10% KOH, in a water bath at 90 °C for 30 min and then, rinsed tree times with tap water. After that, roots were stained by immersion in 5% ink-blue in water bath at 90 °C for 5 min, adapted from (Vierheilig, Coughlan, Wyss & Piché, 1998). Finally, stained roots were stored in 85% glycerol.

Root length and AMF colonization

Root length and colonization were measured by microscopy at 16x magnification, using the point intersect method, over 1 cm field of view. In a Petri dish (20 cm ⌀), fifteen random points were drawn and root samples spread over. Roots with or without AMF structures (intraradical hyphae, arbuscules and vesicles) intersecting points and hair line were recorded. Root length and AMF colonization were calculated (Newman, 1966; Giovannetti & Mosse, 1980). Using total root length, root length density was estimated by dividing total root length by soil volume on each pot.

Statistics

Statistical analysis was performed by using the R software (v. 3.3.2) (R Core Team 2013) and figures were made using GraphPad Prism (v. 7.0 c) (GraphPad Software, Inc., San Diego, CA, USA). Results were analyzed in their original scale of measurement with a generalized linear model (GLM). Distribution was fit as gamma, Gaussian or quasibinomial. Post-hoc analysis for mean comparison at a 95 % confidence level was performed in terms of Tukey´s test.

RESULTS

Table 5. *P* values obtained from GLM of all variables measured in terms of the individual factors maize genotypes (NB9 and H318), *P. arrhenomanes* (with (+ P) and without (–P)), bacterial communities (BEocci, BH318 and BNB9 or without (-B)), arbuscular mycorrhizal fungi (with (+ AMF) and without (- AMF)) and their interactions.

	Shoot dry weigth (g)	Root dry weight (g)	Total dry weight (g)	Plant total root length (m)	Specific root length (m g^{-1})	AMF colonization (%)
Maize genotype (M)	***	***	***	0.491	**	0.536
P. arrhenomanes (P)	*	0.237	*	0.048	0.134	0.794
Bacterial community (B)	0.628	*	0.232	0.470	**	**
Arbuscular Mycorrhiza Fungi (AMF)	**	0.276	**	***	***	NA
M x P	0.308	0.495	0.458	0.739	0.449	0.163
M x B	0.878	0.156	0.534	0.325	0.354	0.068
P x B	0.969	0.854	0.900	0.758	0.755	0.048
B x AMF	0.758	0.483	0.741	0.424	0.362	NA
P x AMF	0.783	0.530	0.820	0.219	0.576	NA
M x AMF	0.947	0.631	0.501	0.914	0.095	NA
M x B x AMF	0.594	*	0.290	0.592	**	NA
P x B x AMF	0.810	0.424	0.611	0.220	0.535	NA
M x P x AMF	0.107	0.126	*	0.380	0.449	NA
M x P x B	0.191	0.478	0.077	0.078	0.494	0.278
M x P x B x AMF	0.808	0.121	0.429	0.349	0.987	NA

Levels of significance of main factors (M, P, B and AMF) and their interaction assessed by generalized linear model analysis on the different explanatory variables. Significative codes: 0 '***' 0.001, '**' 0.01, '*' 0.05. *NA*: Not analyzed.

Shoot dry weight

Shoot dry weight was differentially affected among maize genotypes, *P. arrhenomanes* and AMF treatments (Table 5). However, no interactions were shown. Shoot dry weight was significantly different in maize genotypes where NB9 plants produced less shoot biomass than H318 (Fig. 6A). On the other hand, shoot dry weight was lower in plants infected with *P. arrhenomanes* compared to the ones without pathogen inoculation (Fig. 6B), but higher in plants inoculated with AMF compared to plants without AMF inoculation (Fig. 6C).

Root dry weight

A significant "Maize genotype x Bacterial community x AMF" interaction was observed for root dry weight (Table 5), however according to the post-hoc GLM treatment mean comparison no differences were observed (Fig. 7). Nevertheless, the response of the two maize genotypes to AMF and bacteria seems to differ. While the genotype NB9 did not respond to AMF and bacteria, increased root growth was observed in H318 after inoculation with both AMF and bacteria (Fig. 7).

Total dry weight

A significant "Maize genotype x AMF x *P. arrhenomanes*" interaction was observed for total plant dry weight (Table 5). The maize genotype H318 produced more total biomass than NB9 in treatments without AMF, whereas with AMF no difference in biomass between genotypes was observed (Fig. 8). Though not significantly different according to the post hoc GLM treatment means comparison the effect of inoculation with *P. arrhenomanes* seemed to differ between maize genotypes.

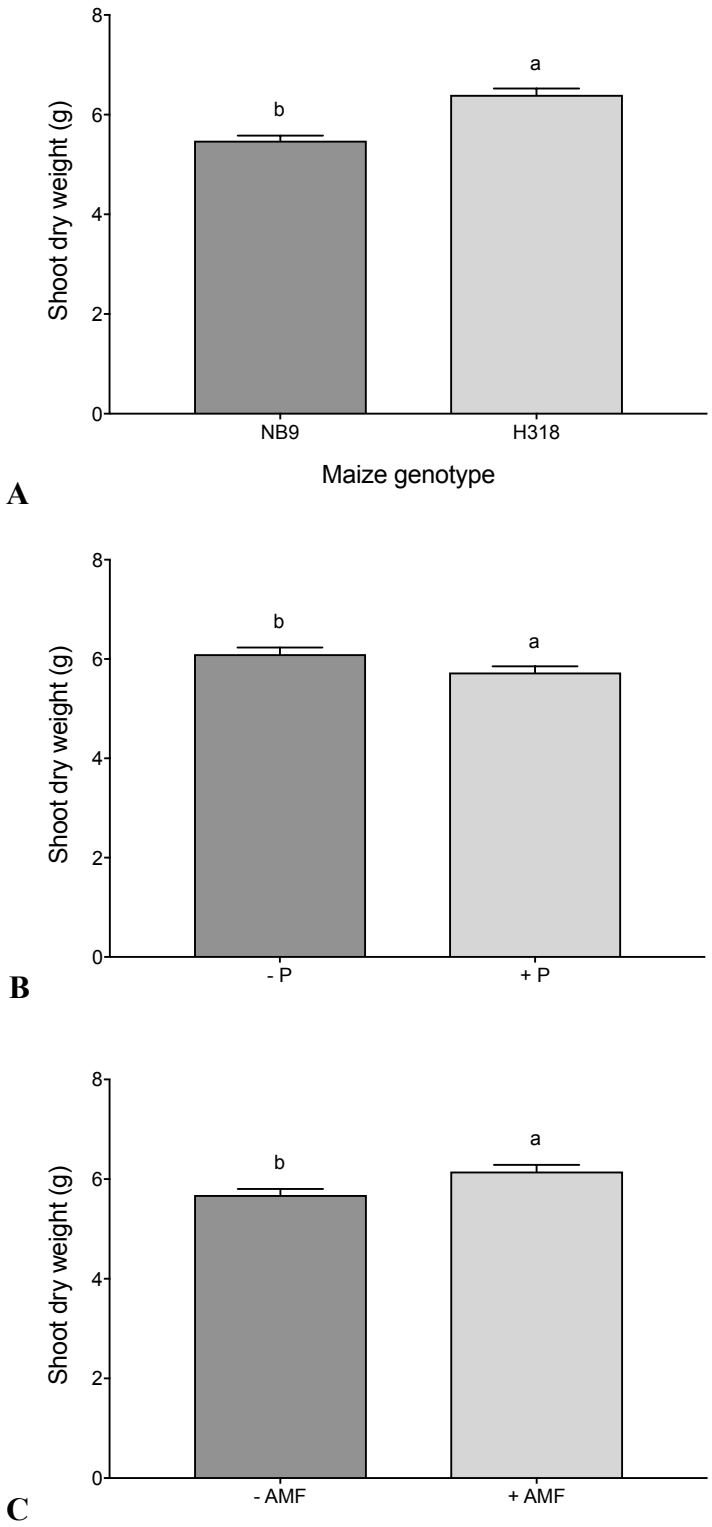

Figure 7. Shoot dry weight of maize plants of two genotypes, NB9 and H318 **(a),** infected with *P. arrhenomanes* (-P) or not (+P) eighteen days after sowing **(b**), inoculated with arbuscular mycorrhiza (+AMF) or not (-AMF) **(c)**, harvested thirty-five days after sowing.

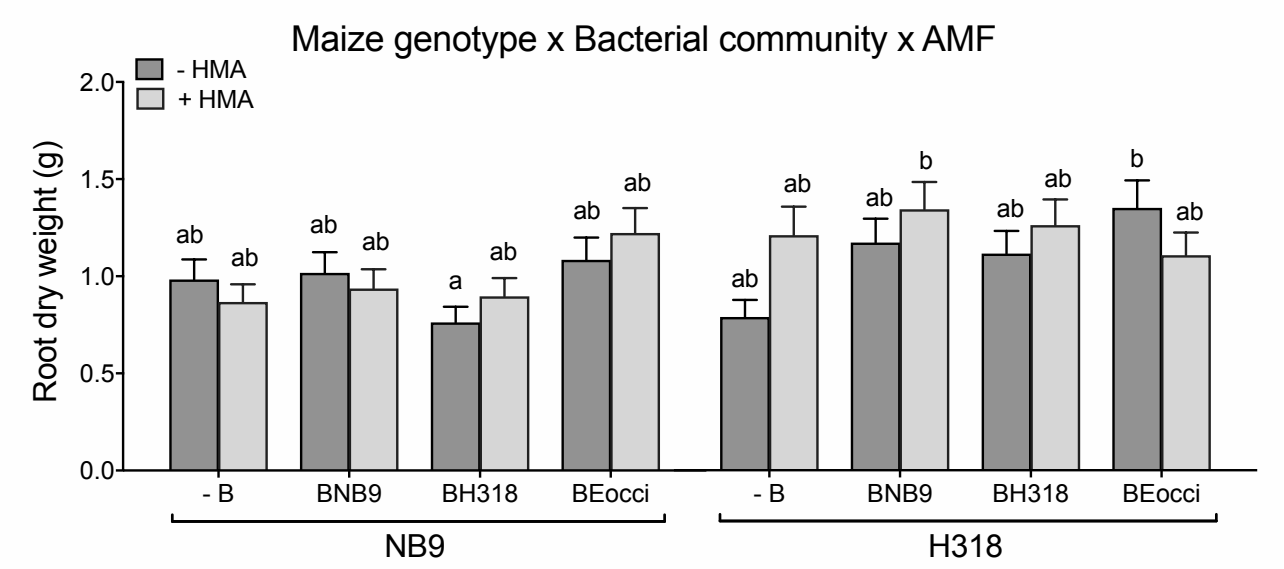

Figure 8. Root dry weight of maize plants of two genotypes (NB9 and H318), inoculated with arbuscular mycorrhiza (+AMF) or not (-AMF), and with three bacterial communities (BNB9, BH318 and BEocci) or not (-B). Harvested thirty-five days after sowing.

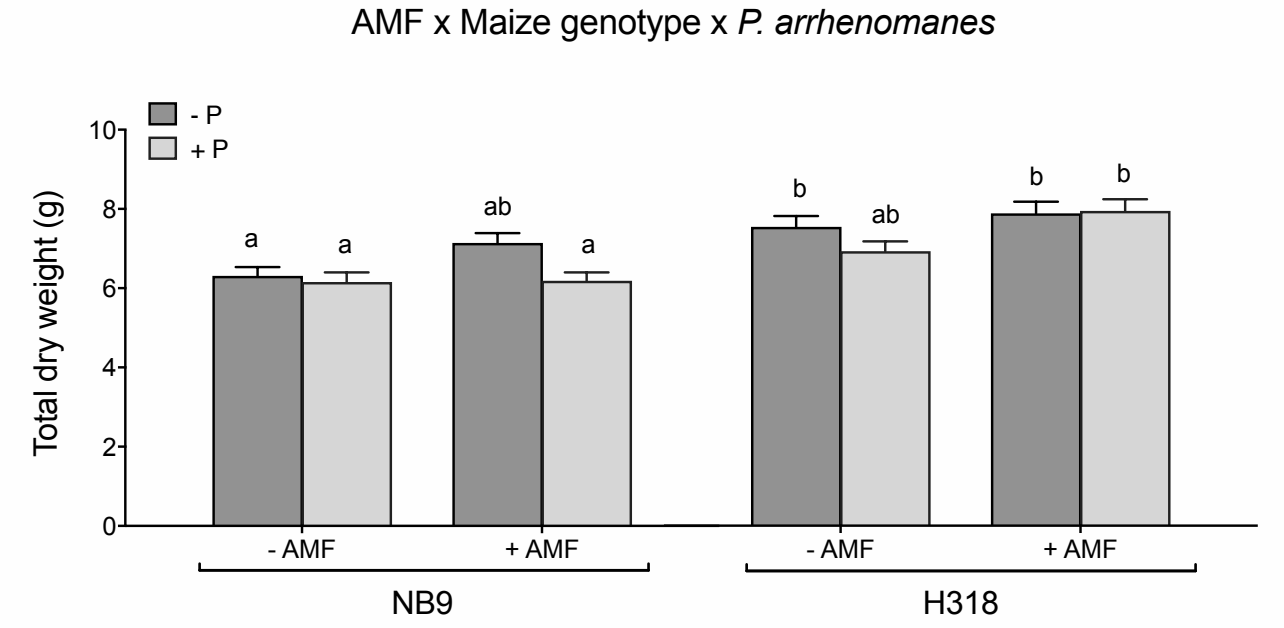

Figure 9. Total dry weight of maize plants of two genotypes (NB9 and H318), inoculated with arbuscular mycorrhiza (+AMF) or not (-AMF), infected with *P. arrhenomanes* (+*Pythium*) or not (-*Pythium*). Harvested thirty-five days after sowing.

For the maize genotype NB9 inoculation with *P. arrhenomanes* only reduced plant dry weight in combination with AMF inoculation, whereas in the genotype H318 inoculation reduced biomass on plants only without AMF (Fig. 8).

Plant total root length

Plant total root length was only affected by AMF inoculation (Table 5). Maize plants inoculated with AMF produced less root length than plants without AMF inoculation (Fig. 9).

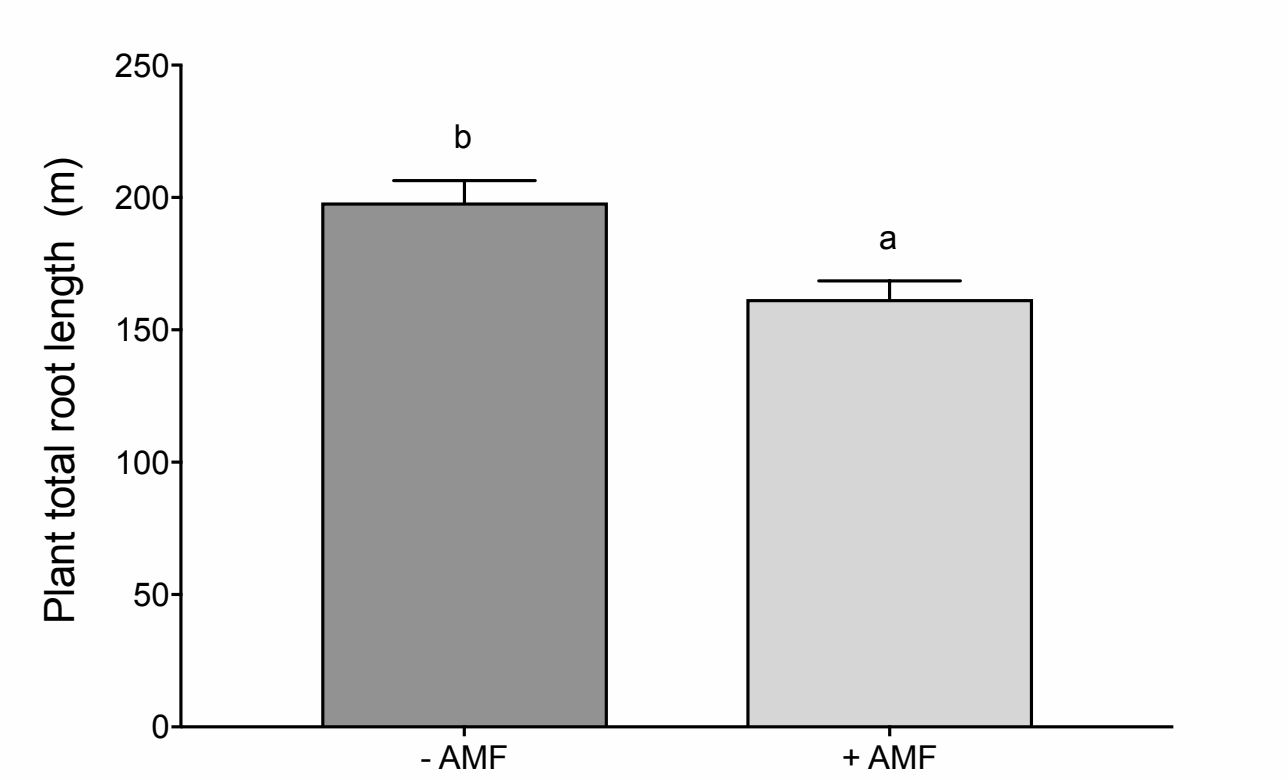

Figure 10. Plant total root length of maize plants of two genotypes (NB9 and H318), inoculated with arbuscular mycorrhiza (+AMF) or not (-AMF). Harvested thirty-five days after sowing.

Specific root length

A significant "Maize genotype x AMF x Bacterial community" interaction was observed for specific root length (Table 5). In the maize genotype NB9 no effect of AMF and bacterial communities were observed (Fig. 10). In contrast in the maize genotype H318 inoculation with AMF reduced the specific root length. Also, inoculation with bacteria reduced the specific root length in H318, but only without AMF inoculation and only significantly for BEocci (Fig. 10).

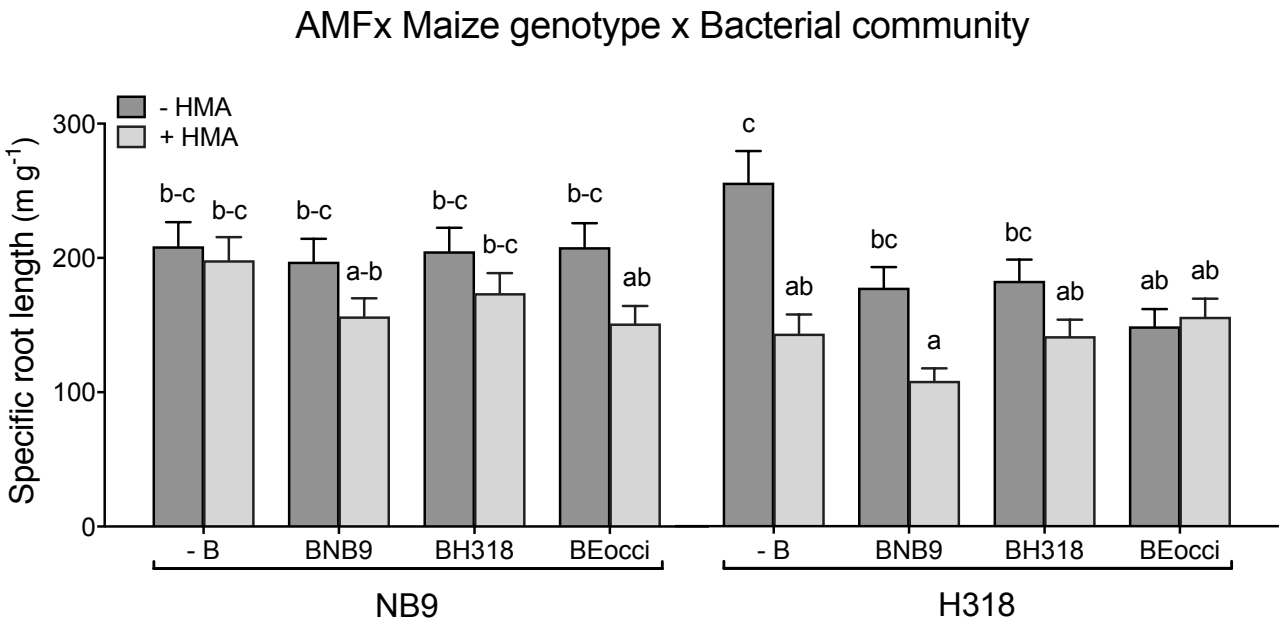

Fig 11. Specific root length of maize plants of two genotypes (NB9 and H318), inoculated with arbuscular mycorrhiza (+AMF) or not (-AMF), and with three bacterial communities (BNB9, BH318 and BEocci) or not (-B). Harvested thirty-five days after sowing.

AMF root colonization

AMF root colonization was significantly affected by inoculation with the bacterial communities (Table 5), which all promoted AMF root colonization (Fig. 11).

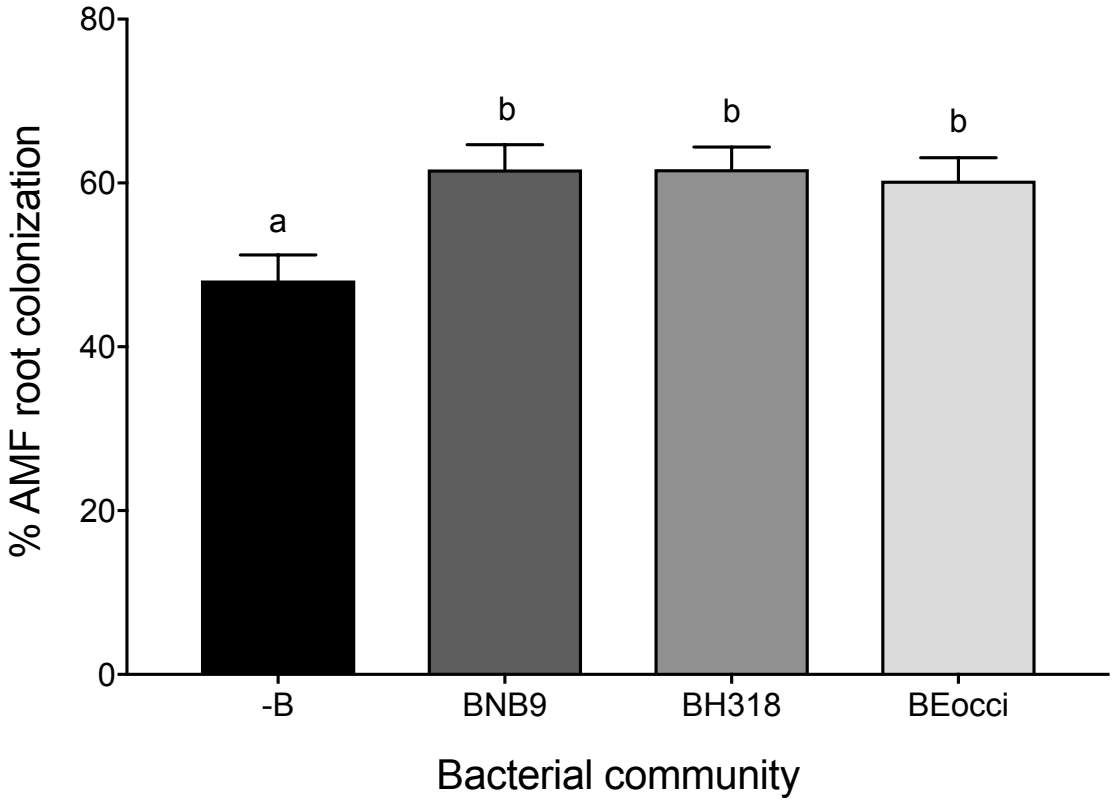

Figure 12. Arbuscular mycorrhizal fungi root colonization percentage. Maize plants inoculated with AMF and three bacterial communities (BNB9, BH318 and BEocci) or not (-B). Harvested thirty-five days after sowing.

IX. DISCUSSION

Plant growth parameters of maize genotype

Results from experiment one showed contrasting performance between maize genotypes in terms of shoot and root biomass as well as root architecture, these results confirm the high phenotypic and genetic diversity in maize (Wellhausen et al., 1957; Matsuoka et al., 2002). Higher variation in plant growth parameters was observed in landraces compared with hybrids, which was expected since landraces are more genetically diverse than hybrids (Altieri & Merrick, 1987). In the field landraces vary in plant height, maturity and cob shape, yet still being part of the same cultivar (Macrobert et al., 2007).

Effects of root pathogens on plant growth parameters

The observed difference in the effects caused by the root pathogens *P. arrhenomanes, Pythium* sp., *F. verticillioides* and *F. graminearum* in maize plants seems to be linked to variability in pathogenicity and virulence between pathogen species and strains, which have also been reported in similar studies (Van Buyten et al., 1996; Vanterpool & Truscott, 1932; Kommedahl, Windels & Stucker, 1979). Environmental conditions may have been affected the virulence and pathogenicity of the root pathogens (Briones-Reyes, González, Servia, Rincón, García-de Alba, & Hernández., 2015). *F. verticillioides* and *F. graminearum* root rot infection is related to temperatures rating from 22°C to 25°C (Reid, Nicol, Ouellet, Savard, Miller, Young, Stewart & Schaafsma, 1999), while many root rot *Pythium* spp. frequently occur in cool temperatures 21°C to 5°C (Zhang, Chen, & Yang, 1998; Pieczarka, & Abawi, 1978; Sippell & Hall, 1982). In this study, temperatures of 4°C prevailed at night in the green

house from September to December 2016, these conditions may have given advantage to *Pythium* spp. over *Fusarium* spp. to cause disease. Nevertheless, many root rot *Fusarium* species stablish as endophytes in roots without causing symptoms of infection, environmental conditions, host and pathogen genetics may be involved (Bacon & Yates, 2006). However, among the *Pythium* pathogens, *P. arrhenomanes* was the only that caused detrimental effects in maize plants. Differences in virulence among *Pythium* spp. are related to root exudates or aminoacid profile utilization during colonization (Van Buyten & Höfte, 2013; Mao, Lumsden, Lewis & Hebbar, 1998), but it must be taken into account that many other *Pythium* plant pathogens are also related to warm temperatures and in many cases temperature is crucial to cause disease (Nelson & Craft, 1991). When conditions are not favorable for *Pythium* pathogens to cause disease, they could die, stablish as saprophytes, or may form survival structures waiting for optimum conditions or a suitable host, but in this study mycelium was employed as inoculum and is considered a short-lived structure in soil (Peethambaran & Singh, 1977). Anyway, we did not conduct any further study to ensure the presence of *Pythium* sp. mycelium neither in host tissue nor in host substrate.

Nevertheless, caution must be taken since other authors have detected loss of virulence in *Pythium* spp. and *Fusarium* spp. after being sub-cultured several times (Martin & Lopper, 1999). In this study, all isolates were sub-cultured from single hypha to avoid loss of virulence (Nelson, 1992). Another important aspect, is that besides *P. arrhenomanes*, the other root pathogens *Fusarium* spp. and *Pythium* sp. were obtained from external laboratories without knowledge of previous culture management practices.

In accordance to our results Reyes-Tena et al., (2018) reported high virulence of *P. arrhenomanes* in maize plants. Plant growth depression is a common symptom of root rot caused by *P. arrhenomanes* in maize (Yanar, Lipps, & Deep, 1997), as well as reduction of total root length and root length density (Van Buyten & Höfte, 2013).

Plant growth suppression caused by *P. arrhenomanes* differed between maize genotype, confirming our hypothesis that maize genotypes are differentially affected by pathogens (Aguilar et al., 2017). Apparently, PUMA, E. occidentales and Dulce maize genotypes were not affected by *P. arrhenomanes* in none of the plant variables measured in this work. Other maize genotypes such as NB9, R. espada and Ancho were not affected by *P. arrhenomanes* in terms of root dry weight, albeit root length density and specific root length were reduced. Overall, H318 was highly suppressed by *P. arrhenomanes* in all variables measured. In this study, landraces were not less affected than hybrid genotypes by *P. arrhenomanes*, rejecting the hypothesis that landraces are less susceptible to plant pathogens than hybrid genotypes. Apparently, neither plant breeding nor environmental pressure have selected pathogen resistant genes.

Overall, in experiment two, *P. arrhenomanes* had limited plant growth suppressive effect independent of maize genotype. The different response to *P. arrhenomanes* may be related to different environmental conditions such as temperature and soil characteristics (Pieczarka, & Abawi, 1978; Sippell & Hall,1982; Martin & Loper, 1999). Root rot in maize caused by some pathogenic *Pythium* spp. are favored by low temperatures (Zhang, Chen & Yang, 1998), as was the case for experiment 1, where night temperatures reached 4°C. Cold temperatures are

related to high infection rates in maize caused by *Pythium* root pathogens. Another important factor to be consider is that in experiment one maize roots were exposed eight more days to *P. arrhenomanes* than in experiment two.

Effects of AMF and seed bacteria on root rot caused by P. arrhenomanes

In experiment 2 neither *R. irregularis* nor the root endophytic bacterial communities had any effect on the plant growth suppression caused by *P. arrhenomanes,* rejecting our main hypothesis. However, biocontrol traits of rhizosphere bacteria and fungi such as *Burkholderia cepacia*, *Gliocladium virens* and *Trichoderma viride* have been reported (Mao, Lewis, Hebbar & Lumsden, 1997; Lumsden & Locke, 1998; Dissanayake & Hoy, 1999). Also, biocontrol traits of *Pseudomonas* and *Paenibacillus* have been reported for other *Pythium* spp. root pathogens (Martin & Loper, 1999; Li et al., 2007). Other studies have also shown the biological potential of *R. irregularis* as a biocontrol agent against *Pythium* spp. in maize (Sarabia, 2012) and tomatoes (Larsen et al., 2012).

Effects of AMF and seed bacteria on maize plant growth

Plant growth performance was altered from inoculation with *R. irregularis* and root endophytic bacterial communities. The observed shoot growth promotion after individual inoculation with *R. irregularis* is a common maize growth response (Sarabia, 2012; Sarabia, Cornejo, Azcón, Carreón-Abud & Larsen, 2017; Sarabia, Jakobsen, Grønlund, Carreon-Abud & Larsen 2018), which however depends on maize genotype and other agricultural practices such as tillage (Gavito & Miller, 1998; Hao, Zhang, Christie & Li, 2008) and fertilization (Gavito & Varela, 1995; Aguilar et al., 2016). On the other hand, *R. irregularis* reduced the plant total root length and also the specific root length, which are a common mycorrhiza

growth response in maize (Kothari, Marschner & George, 1990; Hetrick, 1991; Hao, Zhang, Christie & Li, 2008; Sarabia et al., 2017). Instead of allocating energy in root production, plants allocate energy to the AMF symbiosis, which allow mycelial growth and expansion to explore nutrient resources in the soil.

Bacteria have also been shown to promote maize plant growth both as endophytes (Johnston-Monje & Raizada, 2011a) or rhizosphere associated (Lee, Gray, Mabood, Jung, Charles, Clark, Ly, Souleimanov, Zhou & Smith, 2009). In our study, maize root dry weight increased in treatments with E. Occidentales bacterial community. In the same way, Gutierrez-Zamora & Martinez-Romero (2001) reported that maize endophytic bacteria highly increased maize root weight; apparently the mechanism involved was through lumichrome production (Gutiérrez-Zamora & Martínez-Romero, 2001). Promotion of root growth by bacteria has also been linked to improved root hair production and elongation of primary and secondary roots allowing the root system to expand providing greater area for microbial proliferation (Roesch et al., 2008). However, in the present work the increased root dry weight by E. Occidentales bacterial community was observed producing more compact roots with poor branching. The same response was found with maize root architecture caused by *Azospirillum brazilensis* and *Brayrhizobium japonicum* endophytes (Cassan, Perrig, Sgroy, Masciarelli, Penna & Luna, 2009), where apparently secretion of gibberellin played a major role. Since bacterial communities employed in this study were of root endophytic origin, the effect on roots may be a response of its re-establishment in maize root endosphere wherein energy was allocated to increase root size for their proliferation and activity. Other authors have demonstrated that maize endophytic bacteria inoculated to maize seeds were capable to establish in host endosphere (Rosenblueth & Martínez-Romero, 2004). However, in this study its re-

establishment in root endosphere was not explored.

Effect of bacteria on AMF root colonization

Our finding that bacterial communities promoted AMF root colonization, is in accordance with Vosátka & Gryndler (1999) reporting that *Pseudomonas putida* increased root colonization by *Glomus fistulosum* in maize plants. Indeed, it is well known that bacteria can promote mycorrhiza formation, which has led to the concept of mycorrhiza helper bacteria (MHP) (Frey-Klett, Garbaye, & Tarkka, 2007). Possible mechanisms for this helper effect include production of bioactive compounds such as flavonoids (Xie, Staehelin, Vierheilig, Wiemken, Jabbouri, Broughton, Vögeli-Lange & Boller,1995), plant pathogen inhibition compounds or the production of enzymes to facilitate AMF plant root colonization. However, mechanisms may vary between bacteria species and strain (Aspray, Eirian Jones, Whipps & Bending, 2006; Mosse, 1962; Lehr, Schrey, Bauer, Hampp & Tarkka, 2007). Promotion of plant-*R. irregularis* interaction by bacteria may also be related to enhanced host nutrient and health status. Indeed, the bacterium *Rahnella aquatilis* has been shown to improve organic P minerization and P availability for *R. irregularis* and in turn *R. irregularis* transfer carbon rich exudates and improve *R. aquatilis* activity and growth (Zhang, Xu, Liu, Zhang, Hodge & Feng, 2016).

Our results revealed differential maize genotype response to microbial inoculation, accepting our hypothesis that the effect at least for seed bacteria communities and *R. irregularis* on maize plants would depend on maize genotype. Interestingly, single inoculation with the seed endophytic bacteria community (BEocci) was as effective as dual inoculation with *R. irregularis* in reducing specific root length of the H318 maize genotype, though this effect was not observed in the NB9 maize genotype. Other studies have also shown that maize

response to AMF colonization, root pathogens or endophytic bacteria differ between maize genotypes (Aguilar et al., 2017; Montañez, Blanco, Barlocco, Beracochea & Sicardi, 2012; Rodríguez-Blanco, A., Sicardi, M., & Frioni, 2015).

X. CONCLUSSION

Maize root health is not improved by root endophytic bacteria community, rejecting our main hypothesis which state that maize root health depends on associated seed endophytic bacteria resulting in improved root health. However, our results showed that maize genotype played a major role in the effects of *R. irregularis* and maize root endophytic bacteria communities in plant response which must be a major consideration either in future experiments and in agronomical practices.

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