ΦYTON
REVISTA INTERNACIONAL DE BOTÁNICA EXPERIMENTAL
INTERNATIONAL JOURNAL OF EXPERIMENTAL BOTANY
FUNDACION ROMULO RAGGIO
Gaspar Campos 861, 1638 Vicente López (BA), Argentina
www.revistaphyton.fund-romuloraggio.org.ar

Effects of *Rhizoglomus intraradices*, *Azospirillum brasilense* and plant growth regulators application on root architecture in barley (*Hordeum vulgare* L.)

Efecto de la aplicación de *Rhizoglomus intraradices*, *Azospirillum brasilense* y reguladores del crecimiento vegetal, sobre la arquitectura de la raíz de plantas de cebada (*Hordeum vulgare* L.)

Zepeda-Guzmán S[1], M Gómez-Romero[2], C Sosa-Aguirre[1], J Villegas[1]

Abstract. Changes in root architecture are a strategy used by plants to explore the soil for available resources. The presence of beneficial microorganisms in the rhizosphere as well as plant growth regulators can cause changes in root development and promote the availability of water and nutrients. The effect of microorganisms or growth regulators on plant growth has been tested, but little is known about the effect they have on the architecture of the root of *Hordeum vulgare* L. Therefore the objective of this study was to evaluate the effect of the application of *Rhizoglomus intraradices*, *Azospirillum brasilense*, quercetin and epibrassinolide, alone or in combination, on the root architecture and plant (shoot + root) biomass of barley. The experiment was conducted in a growth chamber and a rhizotron system was used as a case study to assess the root architecture. Each of the barley seeds that germinated in the rhizotrons was inoculated with 70 spores of *R. intraradices* or 2.5 x 10^7 CFU/mL of A. *brasilense* in a 1 mL suspension. Quercetin and/or epibrassinolide were applied in 5 mL of solution at a concentration of 10 μM, at 0, 5 and 8 days. The results showed that the growth promoter epibrassinolide affected barley root architecture by increasing the number, but decreasing the length, of seminal roots. It also promoted the early onset of lateral roots. Quercitin, applied alone or combined, had a significant effect to increase the number of lateral roots. Although changes were observed in the architecture of the root, barley biomass did not show significant differences in this evaluation period. The application of microorganisms did not produce significant changes in the variables evaluated.

Keywords: Root architecture; *Hodeum vulgare* L.; Quercitin or epibrassinolide; *Rhizoglomus intraradices*; *Azospirillum brasilense*.

Resumen. Las modificaciones en la arquitectura de la raíz son una estrategia que les permite a las plantas una mayor exploración y obtención de los recursos disponibles en el suelo. La presencia de microorganismos benéficos en la rizósfera, así como de compuestos reguladores del crecimiento, pueden producir cambios en el desarrollo radical y favorecer la disponibilidad del agua y los nutrientes del suelo. Se ha probado el efecto de microorganismos o reguladores del crecimiento sobre el desarrollo de las plantas en diferentes sistemas, pero poco se sabe del efecto que tienen sobre la arquitectura de la raíz de cebada (*Hordeum vulgare* L.). El objetivo de este trabajo fue evaluar el efecto de la aplicación de *Rhizoglomus intraradices*, *Azospirillum brasilense*, quercetina y epibrasinolida, solos o en combinación, sobre la arquitectura de la raíz y la biomasa (parte aérea + parte subterránea) de plantas de cebada. El experimento se realizó en cámara de crecimiento, y se utilizó un sistema de rizotrón como modelo de estudio para evaluar la arquitectura de la raíz. Se hicieron germinar semillas de cebada en los rizotrones. Cada semilla fue inoculada con 70 esporas de *R. intraradices* ó 2.5 x 10^7 UFC/mL de *A. brasilense* en 1 mL de solución. Además, los reguladores del crecimiento quercitina o epibrasinolida se aplicaron en una solución de 5 mL a una concentración de 10 μM, aplicándose a los 0, 5 y 8 días. Los resultados mostraron que el promotor de crecimiento epibrasinolida afectó la arquitectura de la raíz de cebada. Este regulador de crecimiento incrementó el número, pero disminuyó la longitud de las raíces seminales. Además, promovió la aparición temprana de raíces laterales. Quercitina, aplicada sola o en combinación, presentó un efecto significativo en el número de raíces laterales. No obstante los cambios observados en la arquitectura de la raíz, no se observaron cambios significativos en la biomasa de las plantas en este periodo de evaluación. Los microorganismos utilizados no produjeron cambios significativos en las variables evaluadas.

Palabras clave: Arquitectura de raíz; *Hodeum vulgare* L.; Quercitina o epibrasinolida; *Rhizoglomus intraradices*; *Azospirillum brasilense*.

[1] Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mújica s/n, Colonia Felícitas del Rio, C.P. 58030, Morelia, Michoacán, México.
[2] Cátedras CONACYT-Facultad de Biología, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mújica s/n, Colonia Felícitas del Rio, C.P. 58030, Morelia, Michoacán, México.
Address correspondence to: Dr. Javier Villegas, Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Francisco J. Mujica s/n, Colonia Felícitas del Rio, C.P. 58030, Morelia, Michoacán, México, *e-mail:* vilj4455@yahoo.com.mx
Received 23.XI.2017. Accepted 3.I.2018.

INTRODUCTION

The root architecture is defined as the spatial configuration of a root system, in a geometric display of the axes of the root that is important to acquire soil resources such as water and mineral nutrients (Lynch, 1995). It is the result of very dynamic development processes in space and time to respond to changes in the environment (Cruz et al., 2004). For example, some plants produce roots of larger diameter to facilitate penetration into dense soil and others produce roots at different angles to facilitate the exploration of mineral resources (Lynch & Van Beem, 1993).

The ability of plants to position the roots where water and nutrients are available is vital to their growth and survival (Hargreaves et al., 2009), as well as the presence of beneficial microorganisms in the rhizosphere, such as arbuscular mycorrhizal (AM) fungi or plant growth promoting rhizobacteria (PGPR). These microorganisms are capable of inducing modifications in root system to address changes in the environment (Beberidge et al., 2003; Vance et al., 2003).

Ninety five per cent of terrestrial vascular plants and AM fungi form a symbiotic mycorrhizal association, where both organisms help each other in physiological and nutritional aspects (Alarcon et al., 2004). Studies conducted by Gutjahr et al. (2009) and Wang et al. (2011) demonstrated the potential of AM fungi to induce changes in the root systems of trees and herbaceous plants. Gutjhar et al. (2009) showed that the *Rhizoglomus intraradices* mycorrhizal fungus [before *Glomus intrradices* (Sieverding et al., 2014)] led to changes in the roots of *Oryza sativa*, promoting the formation of lateral roots and increasing branching. Such changes in the architecture of the root were attributed to the increase in mycorrhizal colonization due to the high proportion of arbuscules found in the root tissue.

Another group of important microorganisms having the ability to modify root architecture through various mechanisms are PGPR (Perrig et al., 2007). Gamalero et al. (2004) documented the role of these microorganisms on root architecture in grasses. Russo et al. (2005) demonstrated that *Azospirillum brasilense* promotes the growth of side roots and adventitious roots in *Zea mays*. A study by Speapen et al. (2008) showed that *A. brasilense* modifies the root system of *Triticum aestivum*, by promoting the number of side rices and these architectural changes were attributed to the production of indole acetic acid (IAA) by the rhizobacteria.

In addition, there are compounds produced by plants that act as plant growth regulators, affecting root architecture. Such is the case of flavonoids and brassinosteroids (Walker et al., 2003). Flavonoids are considered as a group of plant hormones (Gomez-Roldan et al., 2008), a product of secondary metabolism. Recent studies have demonstrated its importance as signaling compounds in the interaction between plants and AM fungi (Akiyama et al., 2005; Yoneyama et al., 2008; Bitterlich et al, 2014). However, little is known of the effect of these compounds on the number and length of seminal roots, and the number and length of lateral roots. Under *in vitro* conditions, Imin et al. (2007) observed that the application of the flavonoids inhibited root growth of *Medicago truncatula* plants.

Brassinosteroids are considered as a group of important polyhydroxylated steroidal phytohormones that play an important role in the development and growth of plants (Nuñez & Robaina, 2000: Ding et al., 2013; Hartwig et. al., 2012; Lopez-Gomez et al., 2016). These compounds regulate development through a wide range of physiological processes such as cell elongation and differentiation in the root, responses to light, resistance to stress and senescence (Kim & Wang, 2010), and regulation of expression of xylem development (Fariduddin, et. al., 2014). Brassinosteroids also interact with other phytohormones to modulate plant development and plant responses to biotic and abiotic stresses (Zhu et al., 2013, Fariduddin el al., 2014). There are reports that have documented the effect of applying brassinosteroids on the performance of crop plants (Terry et al., 2001; Grajales & Hernández, 2005; Torres-Ruiz et al., 2007), but little information exists about the effect of brassinosteroids on the architecture of the grass root. A study by Kartal et al. (2009) documented that brassinosteroids increase the length of the seminal roots of barley (*Hordeum vulgare* L.) at very early stages of root development. Howell et al. (2007) found similar effects in *Allium cepa*. However, there are some reports showing that these compounds have an opposite effect to that described above (Roddick et al. 1993; Ozdemir et al., 2004).

Laboratory studies on root morphophysiology have been carried out in general with *in vitro* systems using gels as a growth medium (Bengough et al., 2004). Even if the roots may have rapid growth under these conditions (Van der Weele et al., 2003; Basu et al., 2007), these reduced systems limit root development. Therefore, in this study the use of rhizotron arises as a case study to determine the specific effects of compounds and microorganisms, without the interference of other biotic and abiotic variables affecting the system and without restricting growth.

Under different systems, the role of AM fungi and PGPR on root development and the effect of certain plant growth regulating compounds were documented, but little is known about the effect of these factors on root architecture. Therefore, the objective of this work was to evaluate the effect of the application of *R. intraradices*, *A. brasilense*, flavonoids and brassinosteroids such as quercetin and epibrassinolide, respectively, on the number and length of seminal roots, and the number and length of lateral roots of barley plants.

MATERIALS AND METHODS

Study system description and biological material. Rhizotron systems were used for the establishment and development of barley roots. Rhizotrons were built from four plastic

sheets of 28 x 21.5 cm, which were placed one above the other and a filter paper was placed between each sheet to allow germination of the barley seeds. Seeds of barley *Hordeum vulgare* ("*esperanza*" variety) were superficially sterilized with 75% ethanol and sodium hypochlorite 1% for 5 minutes, and then were rinsed with distilled water.

Inoculation of microorganisms and application of plant growth regulators. Once placed on the filter paper, the seeds were inoculated with the AM fungus *R. intraradices* from *in vitro* cultures, adding 70 spores per seed. Inoculation with *A. brasilense* rhizobacteria was done at a concentration of 2.5 x 10^7 CFU/mL in 5 mL of a solution of plant growth regulators at a concentration of 10 μM. Quercetin and epibrassinolide or both were applied on the seeds at 0, 5 and 8 days after seeds began to germinate.

Conditions of plant growth. Rhizotrons were placed in containers, which were added with 400 ml of Hoagland nutrient solution. They were then placed in a growth chamber under controlled conditions at 25 ˚C, 75% relative humidity and 14 hours photoperiod. They remained during 20 days under these conditions.

Variables evaluated. To determine the number and length of seminal roots, and the number and length of lateral roots, these were photographed at 20 days after planting. To quantify the lateral root emergence, photos were taken every three days. A Sony 14.1 megapixel Carl Zeiss digital camera was used. The images were analyzed with the Smart-Root-ImageJ program, version 3.42. Besides, dry weight of shoot and root was determined on each plant. The percentage of colonization of *R. intraradices* was determined as described by Vierheilig et al. (1998). Roots were cleared with KOH 10% (weight/volume) for 30 min, then stained with Black ink 10% in acetic acid 25% solution; once the roots were dyed, characteristic structures of the AM fungus (arbuscules, vesicles and / or mycelium) were photographed using a Leica MSV 266 system.

The presence of *A. brasilense* was determined as described by Pazos et al. (2000). The barley roots were washed in sterile NFB medium for 30 seconds. Then, a 50 μL aliquot of the medium was collected and added to petri plates filled with congo red medium. The plates were incubated at 37 ˚C for 24 hours and scarlet red colonies were counted.

Statistics analysis. Plant biomass and root architecture variables were analyzed in a completely randomized design with a factorial arrangement of treatments and nine replications. The factors considered were *R. intraradices* (Ri), *A. brasilense* (Ab), quercetin (Q) and epibrassinolide (E). The interactions were: Ri•Ab; Ri•Q; Ab•Q; Ri•E; Ab•E; Q•E; Ri•Ab•Q; Ri•Ab•E; Ri•Q•E; Ab•Q•E and Ri•Ab•Q•E. The percentage of AM colonization and the CFU data were analyzed using analysis of variance (ANOVA), and means were compared using the Tukey test. Analyzes were conducted with the JMP-10 statistical package.

RESULTS

Twenty days after planting, the treatments containing the brassinosteroid epibrassinolide (E) alone or in combination significantly increased the number of seminal roots (P<0.001). The only exceptions were when epibrassinolide was in combination with *R. intraradices* + *A. brasilense* (Ri•Ab•E) or *R. intraradices* + *A. brasilense* + quercitin (Ri•Ab•Q•E), where there was not a significant increase in the number of seminal roots (P=0.521 and P=0.230, respectively) (Table 1). In contrast, the epibrassinolide alone produced significant negative effects on the number of lateral roots (P=0.012). However, this effect was reversed when epibrassinolide was in combination with any of the three-way or four-way interactions (Table 1).

All tested factors containing the epibrassinolide negatively affected the length of seminal roots, compared to the treatments where the brassinosteroid was not applied (P<0.001) (Table 1).

Application of *R. intraradices, A. brasilense* and quercetin (Q) significantly increased the number of lateral roots (P<0.001). There were also increases in these effects when the interaction of either two or three of the above components was observed (Table 1).

No clear effect of tested factors alone was found on the length of lateral roots. However, the combination of *A. brasilense* with either *R. intraradices* or quercetin or the other three tested components together (i.e., the four-way interaction) showed significant decreases (P<0.05) of root length (Table 1).

The biomass growth pattern of this essay showed that most tested factors produced significant inhibitory effects on the shoot fresh and dry biomasses, and on the root fresh biomasses. None of the tested factors affected the root dry biomasses (Table 2).

At the end of the experiment, the response to the application of *R. intraradices* never surpassed 14% of AM colonization; the combined treatments containing *R. intraradices* showed a tendency to decrease the percentage of AM colonization, compared to the treatments containing the fungus alone, except in the case of Ri•E. It was in this exception when the AM percentage of colonization was significantly higher than in the former combinations (Table 3). The CFU of *Azospirillum* detected in the root system remained unchanged when the rhizobacteria was inoculated alone or in interaction, but had significant decreases at the combinations Ab•E and Ri•Ab•Q•E (Table 3). The changes detected in the root architecture among the tested factors were not associated to differences in the population of either AM FUNGI or bacteria.

Table 1. Effects of the factors *Rhizoglomus intraradices* (Ri), *Azospirillum brasilense* (Ab), quercitine (Q) y epibrassinolide (E), alone or in combination, on the variables number of seminal roots, longitude of seminal roots, number of lateral roots and longitude of lateral roots in *Hordeum vulgare* L. Bold numbers indicate significant differences. (+) means factor presence, (-) means factor absence. Means correspond to nine replicates. The numbers between parentheses indicate the standard error.

Tabla 1. Efecto de los factores *Rhizoglomus intraradices* (Ri), *Azospirillum brasilense* (Ab), quercitina (Q) y epibrasinolida (E), solos o en combinación, sobre las variables número de raíces seminales, longitud de raíces seminales, número de raíces laterales y longitud de raíces laterales en *Hordeum vulgare* L. Números en negrita indican diferencias significativas. (+) medias en presencia del factor, (-) medias en ausencia del factor. Las medias corresponden a nueve repeticiones. Los números entre paréntesis indican el error estándar.

	Seminal root number			**Seminal root length (cm)**			**Lateral root number**			**Lateral root length (cm)**		
Factor	(-)	(+)	P value	(-)	(+)	P value	(-)	(+)	P value	(-)	(+)	P value
Ri	5.639 (0.107)	5.542 (0.107)	0.521	14.482 (0.464)	13.38 (0.464)	0.095	**38.04** (3.582)	**56.06** (3.582)	**0.001**	0.722 (0.028)	0.681 (0.028)	0.311
Ab	5.667 (0.107)	5.514 (0.107)	0.314	13.987 (0.464)	13.875 (0.464)	0.866	**37.78** (3.582)	**56.32** (3.582)	**0.000**	0.746 (0.028)	0.657 (0.028)	0.127
Q	5.472 (0.107)	5.708 (0.107)	0.121	14.398 (0.464)	13.464 (0.464)	0.157	**36.21** (3.582)	**57.89** (3.582)	**0.000**	0.723 (0.028)	0.68 (0.028)	0.282
E	**5.111** (0.107)	**6.069** (0.107)	**0.000**	**20.12** (0.464)	**7.739** (0.464)	**0.000**	**53.48** (3.582)	**40.62** (3.582)	**0.012**	0.675 (0.028)	0.728 (0.028)	0.191
Ri·Ab	5.806 (0.151)	5.556 (0.151)	0.234	14.161 (0.656)	12.947 (0.656)	0.253	**25.21** (5.066)	**61.78** (5.066)	**0.000**	**0.747** (0.04)	**0.617** (0.04)	**0.027**
Ri·Q	5.611 (0.151)	5.75 (0.151)	0.234	14.96 (0.656)	12.924 (0.656)	0.973	**27.80** (5.066)	**67.51** (5.066)	**0.000**	0.763 (0.04)	0.679 (0.04)	0.334
Ab·Q	5.556 (0.151)	5.639 (0.151)	0.927	14.405 (0.656)	13.36 (0.656)	0.883	**24.42** (5.066)	**64.64** (5.066)	**0.001**	**0.801** (0.04)	**0.669** (0.04)	**0.017**
Ri·E	**5.25** (0.151)	**6.111** (0.151)	**0.000**	**20.835** (0.656)	**7.348** (0.656)	**0.000**	37.66 (5.066)	42.82 (5.066)	0.163	0.735 (0.04)	0.747 (0.04)	0.051
Ab·E	**5.222** (0.151)	**6.028** (0.151)	**0.000**	**20.023** (0.656)	**7.527** (0.656)	**0.000**	38.44 (5.066)	44.11 (5.066)	0.173	0.762 (0.04)	0.725 (0.04)	0.06
Q·E	**5.139** (0.151)	**6.333** (0.151)	**0.000**	**19.962** (0.656)	**6.644** (0.656)	**0.000**	31.22 (5.066)	40.04 (5.066)	0.231	0.749 (0.04)	0.758 (0.04)	0.084
Ri·Ab·Q	5.667 (0.214)	5.889 (0.214)	0.171	13.778 (0.928)	13.256 (0.928)	0.983	**15.61** (7.164)	**67.56** (7.164)	**0.000**	0.854 (0.056)	0.615 (0.056)	0.099
Ri·Ab·E	6.111 (0.214)	6.111 (0.214)	0.521	**19.88** (0.928)	**7.238** (0.928)	**0.000**	**18.71** (7.164)	**43.11** (7.164)	**0.018**	0.82 (0.056)	0.706 (0.056)	0.362
Ri·Q·E	**5.389** (0.214)	**6.444** (0.214)	**0.000**	**20.18** (0.928)	**6.775** (0.928)	**0.000**	**18.15** (7.164)	**40.69** (7.164)	**0.012**	0.874 (0.056)	0.751 (0.056)	0.056
Ab·Q·E	**5.222** (0.214)	**6.333** (0.214)	**0.000**	**19.28** (0.928)	**6.917** (0.928)	**0.000**	**14.45** (7.164)	**40.22** (7.164)	**0.001**	0.872 (0.056)	0.785 (0.056)	0.856
Ri·Ab·Q·E	5.444 (0.302)	6.111 (0.302)	0.230	**18.39** (1.313)	**8.529** (1.313)	**0.001**	**15.54** (10.132)	**49.94** (10.132)	**0.001**	**1.001** (0.08)	**0.68** (0.08)	**0.024**

Table 2. Effect of the factors *Rhizoglomus intraradices* (Ri), *Azospirillum brasilense* (Ab), quercitine (Q) and epibrassinolide (E) alone or in interaction, on the variables shoot fresh weight, shoot dry weight, root fresh weight and root dry weight in *Hordeum vulgare* L. Bold numbers indicate significant differences. (+) means when factor was present, (-) means when the factor was absent. Means correspond to nine replicates. The numbers between parentheses indicate the standard error.

Tabla 2. Efecto de los factores *Rhizoglomus intraradices* (Ri), *Azospirillum brasilense* (Ab), queretin (Q) y epibrassinolide (E) solos o en interacción, sobre las variables peso fresco de la pate aérea, peso seco de la parte aérea, peso fresco de raíz y peso seco de raíz en *Hordeum vulgare* L. Números en negrita indican diferencias significativas. (+) medias en presencia del factor, (-) medias en ausencia del factor. Las medias corresponden a las nueve repeticiones. Los números entre paréntesis indican el error estándar.

	Shoot fresh weight (g)			**Shoot dry weight (g)**			**Root fresh weight (g)**			**Root dry weight (g)**		
Factor	(-)	(+)	P value	(-)	(+)	P value	(-)	(+)	P value	(-)	(+)	P value
Ri	**0.216** (0.008)	**0.178** (0.008)	**0.002**	0.024 (0.001)	0.022 (0.001)	0.029	0.126 (0.004)	0.115 (0.004)	0.064	0.01 (0.001)	0.01 (0.001)	0.683
Ab	**0.219** (0.008)	**0.174** (0.008)	**0.000**	0.025 (0.001)	0.021 (0.001)	0.004	0.134 (0.004)	0.108 (0.004)	0.000	0.011 (0.001)	0.009 (0.001)	0.173
Q	0.214 (0.008)	0.18 (0.008)	0.005	0.025 (0.001)	0.021 (0.001)	0.003	0.128 (0.004)	0.114 (0.004)	0.020	0.01 (0.001)	0.01 (0.001)	0.822
E	**0.174** (0.008)	**0.22** (0.008)	**0.000**	0.024 (0.001)	0.022 (0.001)	0.031	0.141 (0.004)	0.101 (0.004)	0.000	0.01 (0.001)	0.01 (0.001)	0.897
Ri·Ab	**0.238** (0.012)	**0.155** (0.012)	**0.000**	0.026 (0.001)	0.2 (0.001)	0.004	0.135 (0.006)	0.098 (0.006)	0.000	0.01 (0.001)	0.009 (0.001)	0.199
Ri·Q	0.234 (0.012)	0.161 (0.012)	0.050	0.026 (0.001)	0.2 (0.001)	0.003	0.126 (0.006)	0.101 (0.006)	0.014	0.01 (0.001)	0.011 (0.001)	0.311
Ab·Q	0.234 (0.012)	0.155 (0.012)	0.050	0.026 (0.001)	0.019 (0.001)	0.001	0.135 (0.006)	0.096 (0.006)	0.000	0.01 (0.001)	0.009 (0.001)	0.146
Ri·E	**0.251** (0.012)	**0.166** (0.012)	0.000	0.026 (0.001)	0.021 (0.001)	0.004	0.158 (0.006)	0.108 (0.006)	0.05	0.011 (0.001)	0.011 (0.001)	0.1
Ab·E	**0.251** (0.012)	**0.16** (0.012)	0.000	0.026 (0.001)	0.021 (0.001)	0.003	0.152 (0.006)	0.086 (0.006)	0.05	0.01 (0.001)	0.009 (0.001)	0.201
Q·E	**0.244** (0.012)	**0.164** (0.012)	0.045	0.027 (0.001)	0.021 (0.001)	0.026	0.146 (0.006)	0.092 (0.006)	0.040	0.01 (0.001)	0.011 (0.001)	0.343
Ri·Ab·Q	0.246 (0.017)	0.145 (0.017)	0.050	0.026 (0.002)	0.019 (0.002)	0.001	0.126 (0.008)	0.081 (0.008)	0.361	0.01 (0.001)	0.009 (0.001)	0.883
Ri·Ab·E	**0.285** (0.017)	**0.147** (0.017)	**0.003**	0.28 (0.002)	0.019 (0.002)	0.003	0.166 (0.008)	0.088 (0.008)	0.030	0.011 (0.001)	0.009 (0.001)	0.317
Ri·Q·E	**0.274** (0.017)	**0.159** (0.017)	**0.004**	0.029 (0.002)	0.02 (0.002)	0.005	0.155 (0.008)	0.094 (0.008)	0.020	0.011 (0.001)	0.012 (0.001)	0.608
Ab·Q·E	**0.267** (0.017)	**0.153** (0.017)	**0.004**	0.028 (0.002)	0.019 (0.002)	0.004	0.136 (0.008)	0.088 (0.008)	0.018	0.01 (0.001)	0.008 (0.001)	0.616
Ri·Ab·Q·E	**0.282** (0.024)	**0.141** (0.024)	**0.002**	0.029 (0.002)	0.018 (0.002)	0.003	0.142 (0.012)	0.092 (0.012)	0.020	0.01 (0.002)	0.008 (0.002)	0.349

Table 3. Percentage of mycorrhizal colonization of *Rhizoglomus intraradices* and colony forming units (CFU mL) of *Azospirillum brasilense* associated to roots of *Hordeum vulgare* L. Different letters show significant differences according to Tukey's test at a 5% level of significance. ND: not determined.

Tabla 3. Porcentaje de colonización micorrícica de *Rhizoglomus intraradices* y Unidades formadoras de colonias (UFC mL) de *Azospirillum brasilense* asociado a las raíces de *Hordeum vulgare* L. Letras diferentes indican diferencias significativas de acuerdo a la prueba de Tukey al 5% de significancia. ND: no determinado.

	R. intraradices		***A. brasilense***	
Factor	Percentage of colonization (%)	P<0.05	CFU mL	P<0.05
Ri	10.222	ab	ND	c
Ab	ND	c	196.333	bc
Q	ND	c	ND	d
E	ND	c	ND	d
Ri·Ab	8.222	b	274.111	ab
Ri·Q	8.667	b	ND	d
Ab·Q	ND	c	153.889	cd
Ri·E	14	a	ND	d
Ab·E	ND	c	45.222	cd
Q·E	ND	c	ND	d
Ri·Ab·Q	6.444	b	408.222	a
Ri·Ab·E	6	b	137.778	bcd
Ri·Q·E	9.111	b	ND	d
Ab·Q·E	ND	c	440.667	a
Ri·Ab·Q·E	8.667	b	59.222	cd

DISCUSSION

The root system of barley has been classified into two types: (1) the seminal roots, which are those that develop from seed germination, and (2) the adventitious nodal roots or crown roots, which are those that originate from stem nodes when the plant is developed as the root system reaches various levels of branching (Varney et al., 1991). Determinations of different root structures were made in seminal roots and in the branches of these roots, because during the time of establishment of the barley root system only this type of roots reached development.

Most studies on the effect of flavonoids on root development have been performed under *in vitro* systems, which have shown that flavonoids affect the formation of roots (Imin et al., 2007; Wasson et al., 2009). Imin et al. (2007) showed that these compounds inhibit root growth in *M. truncatula*. In the roots assessed in our rhizotron system, we observed that quercetin (flavonoid) did not affect the length of the lateral and seminal roots. However, quercitin, applied alone or combined, had a significant effect to increase the number of lateral roots. This shows that certain flavonoids may have the potential to promote root development of barley.

Brassinosteroids play an important role in the growth and development of plants (& Robaina, 2000). There are few reports that documented the effect of the brassinosteroids on root systems. Terry et al. (2001) documented that *Lycopersicon esculentum* Mill showed, with foliar application of brassinosteroids, an inhibition of root development at the early stages of plant development (20 days old), that was reverted with the time. Howell et al. (2007) reported an increase in the number and length of roots of *Allium cepa* in response to the application of brassinosteroids. Müssig et al. (2003), in an *in vitro* system, reported that the exogenous supply of low doses of brassinosteroids promote the root growth in *Arabidopsis thaliana*. Supplementary to previous observations, in our experiment brassinosteroids produced a more complex pattern of root development that included an increase in the number of seminal roots, coupled with a decrease in the length of these with the addition of the epibrassinolide.

Treatment with epibrassinolide also favored the early onset of lateral roots of barley and this effect was observed after 5 days of planting. The results obtained with this type of roots match reports of Müssig et al. (2003), Bao et al. (2004) and Swami & Rao (2010), where it is mentioned that brassinosteroids increased lateral roots. In the long term, the changes in the architecture of the root observed in our rhizotron system may be advantageous; the increase in the number seminal roots, the decrease in the length of seminal roots and the early onset of lateral roots could favor the successful establishment of plants and increase the uptake of water and nutrients at an early stage of development (Manske et al., 2000; Lynch, 2005).

Previous studies have shown that AM FUNGI promotes plant growth and induce changes on root architecture, increasing the number and length of the root system of grasses (Thuler et al., 2003; Russo et al., 2005; Gutjahr et al, 2009; Yao et al., 2009). Speapen et al. (2008) documented similar effects on root architecture with *A. brasilense* colonization. At the time that the experiment was evaluated, no important effects in fresh or dry biomass were detected when the microorganisms were inoculated alone or in interaction; this could have been the consequence that both microorganisms showed low levels of root colonization and, in the case of the fungus, only the presence of internal mycelium was detected, which suggests that the fungus was in the early stages of establishment in the root. However, both *R. intraradices* and *A. brasilense*, alone or in interaction with quercetin, had a significant influence in root architecture of barley, significantly increasing the number of lateral roots (Table 1).

The decreases of plant biomass detected in response to the tested factors and their interactions, could be the result of the scarce contact between the roots and the media, produced as a consequence of the reduction in the length of seminal and

lateral roots; previous studies have suggested that poor contact between roots and solid media limit root nutrient uptake (Marschener & Timonen, 2006).

The rhizotron system used allowed us to observe qualitative changes in root architecture, difficult to detect either in soils or in *in vitro* systems. This semi-aseptic system can be a non-destructive alternative to study the effect of microorganisms and other metabolites on the roots of barley or other plants. In our soilless system, the epibrassinolide, alone or in interaction, was the factor that had the most notorious influence in affecting the barley root architecture, increasing the appearance of the seminal roots, decreasing length and inhibiting the appearance of lateral roots, either alone or in interaction, and promoting the AM FUNGI colonization.

ACKNOWLEDGMENTS

This work was supported by the Coordinación de la Investigación Científica (CIC) de la Universidad Michoacana de San Nicolás de Hidalgo. The Consejo Nacional de Ciencia y Tecnología (CONACYT) provided the scholarship to SZG for the realization of this project.

REFERENCES

Akiyama, K., K. Matsuzaki & H. Hayashi (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824-827.

Alarcón, A., S.J. Almaraz, R. Ferrera-Cerrato, M.A. González-Chavez, E.H. Lara, M. Manjarrez, L.R. Quintero & S.R. Santamaría (2004). Manual: Tecnología de hongos micorrízicos en la producción de especies forestales en vivero. In: Ferrera-Cerrato, A. Alarcón and Lara, M.E. (eds), pp. 33-73. Colegio de Posgraduados, Montecillo, SAMARNAT-PRONARE, México. 98 p.

Bao, F., J. Shen, S.R. Brady, G.K. Muday, T. Asami & Z. Yang (2004). Brassinosteroids Interact with auxin to promote lateral root development in *Arabidopsis*. *Plant Physiology* 134: 1624-1631.

Basu, P., A. Pal, J.P. Lynch & K.M. Brown (2007). A novel image analysis technique for kinematic study of growth and curvature. *Plant Physiology* 145: 305-316.

Beberidge, C.A., J.L. Weller & S.R. Singer (2003). Axillary meristem development. Budding relationships between networks controlling flowering, branching, and photoperiod responsiveness. *Plant Physiology* 131: 927-934.

Bengough, A.G., D.C. Gordon, H. Al-Menaie, R.P. Ellis, D. Allan & R. Keith (2004). Gel observation chamber for rapid screening of root traits in cereal seedlings. *Plant Soil* 262: 63-70.

Bitterlich, M., U. Krügel, K. Boldt-Burisch, P. Franken & C. Kühn (2014). The sucrose transporter SlSUT2 from tomato interacts with brassinosteroid functioning and affects arbuscular mycorrhiza formation. *The Plant Journal* 78: 877-889.

Cruz, C., J.J. Green, C.A. Watson, F. Wilson & M.A. Martins-Loução (2004). Functional aspects of root architecture and mycorrhizal inoculation with respect to nutrient uptake capacity. *Mycorrhiza* 14: 177-184.

Ding, J., L.J. Mao, S.T. Wang, B.F. Yuan & Y.Q. Feng (2013). Determination of Endogenous Brassinosteroid in Plant Tissues Using Solid-phase Extraction with Double Layered Cartridge Followed by High-performance Liquid Chromatography-Tandem Mass Spectrometry. *Phytochemical Analysis* 24: 386-394.

Fariduddin, Q., M. Yusuf, I. Ahmad & A. Ahmad (2014). Brassinosteroids and their role in response of plants to abiotic stresses. *Biologia Plantarum* 58: 9-17.

Gamalero, E., A. Trotta, N. Massa, A. Copetta, M.G. Martinotti & G. Berta (2004). Impact of two fluorescent pseudomonads and an arbuscular mycorrhizal fungus on tomato plant growth, root architecture and P acquisition. *Mycorrhiza* 14: 185-192.

Gomez-Roldan, V., S. Fermas, P.B. Brewer, V. Puech-Pagès, E.A. Dun, J.P. Pillot, F. Letisse, R. Matusova, S. Danoun, J.C. Portais, H. Bouwmeester, G. Bécard, C.A. Beveridge, C. Rameau & S.F. Rochange (2008). Strigolactone inhibition of shoot branching. *Nature* 455: 189-194.

Grajales, O. & M. Hernández (2005). Los brasinoesteroides y el desarrollo del maíz híbrido Puma 1181. *Revista FESC Divulgación Científica y Multidisciplinaria. Facultad de Estudios Superiores Cuautitlán* 4: 5-7.

Gutjahr, C., L. Casieri & U. Paszkowski (2009). *Glomus intraradices* induces changes in root system architecture of rice independently of common symbiosis signaling. *New Phytologist* 182: 829-837.

Hargreaves, C., E. Peter, J. Gregory & A. Glyn Bengough (2009). Measuring root traits in barley (*Hordeum vulgare* ssp. *vulgare* and ssp. *spontaneum*) seedlings using gel chambers, soil sacs and X-ray microtomography. *Plant Soil* 316: 285-297.

Howell, W.M., G.E. Keller & J.D. Kirkpatrick (2007). Effects of the plant steroidal hormone, 24-epibrassinolide, on the mitotic index and growth of onion (*Allium cepa*) root tips. *Genetics and Molecular Research* 6: 50-58.

Imin, N., M. Nizamidin, T. Wu & B.G. Role (2007). Factors involved in root formation in *Medicago truncatula*. *Journal of Experimental Botany* 58: 439-451.

Kartal, G., A. Temel, E. Arican & N. Gozukirmizi (2009). Effects of brassinosteroids on barley root growth, antioxidant system and cell division. *Plant Growth Regulation* 58: 261-267.

Kim, T.W. & Z.Y. Wang (2010). Brassinosteroid signal transduction from receptor kinases to transcription factors. *Annual Review of Plant Biology* 61: 681-704.

López-Gómez, M., J. Hidalgo-Castellanos, C. Lluch & J.A. Herrera-Cervera (2016). 24-Epibrassinolide Ameliorates Salt Stress Effects in the Symbiosis *Medicago truncatula–Sinorhizobium meliloti* and Regulates the Nodulation in Cross-Talk With Polyamines. *Plant Physiology and Biochemistry* 108: 212-221.

Lynch, J. (1995). Root architecture and plant productivity. *Plant Physiology* 95: 7-13.

Lynch, J. (2005). Root architecture and nutrient acquisition. In: BassiriRad, H. (ed), pp. 147-183. Nutrient Acquisition by Plants. An Ecological Perspective. Springer, Berlin/Heidelberg/New York. 347 p.

Lynch, J. & J.J. Van Beem (1993). Growth and architecture of seedling roots of common bean genotypes. *Crop Science* 33: 1253-1257.

Manske, G.B., J.I. Ortiz-Monasterio, M. Van Grinkel, R. González, S. Rajaram, E. Molina & P.L.G. Vlek (2000). Traits associated with improved P-uptake efficiency in CIMMYT's semidwarf spring bread wheat grown on an acid Andisol in Mexico. *Plant and Soil* 221: 189-204.