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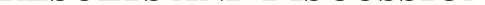
INTRODUCTION

The application of fertilizers in agriculture has contributed to improve yield of crops. However, several negative effects have been attributed to chemical compounds. This, added to serious problems such as soil salinization, has made the application of alternative soil management practices, as the use of plantgrowth promoting bacteria (PGPB), an increasingly valuable tool (Shrivastava et al., 2015). In previous analyses, strain Pseudomonas brassicacearum CDVBN10 showed its potential as PGPB by production of siderophores, cellulose synthesis, solubilisation of P, presence of PGP pathways related genes and growth promotion at five- and fifteen-days-post-inoculation rapeseed seedlings (Jiménez Gómez, 2020). In order to test the PGP ability of this strain under salinity stress conditions, we carried out a greenhouse experiment using Brassica napus as host-plant. Furthermore, we conducted a field trial in which evaluated the effect of inoculation in rapeseed plants development and analysed the alterations on endophyte microbiome diversity caused by inoculation.

METHODOLOGY

In order to carry out the greenhouse experiment, 2 days pre-germinated seeds were transferred into pots and were inoculated with a strain CDVBN10 suspension. Half of them were irrigated with a NaCl 200mM solution. After 45 days, we measured wet and dry weight of aerial portion of plants. On the other hand, in the field trial, rapeseeds were inoculated with a suspension of CDVBN10 strain in 0.9% NaCl (or with 0.9% NaCl in control plants) 27 days after seeding. After 1 month, some plants were randomly selected for amplicon sequencing on roots using the DNeasy Power Plant Pro Kit (Qiagen®, Venlo, Netherlands). Amplicons of the complete bacterial 16S rRNA gene were sequenced on a PacBio Sequel system using a SMRT Cell 1M V3 LR. The sequences were processed following QIIME (v1.9) scripts. Taxonomy assignment was obtained by using Greengenes database (v13.8). After 8 months, the rapeseed plants were collected, pods number, pods dry weight and shoot dry weight measurements were recorded and N, C, Fe, P, K content were analyzed.

RESULTS AND DISCUSSION



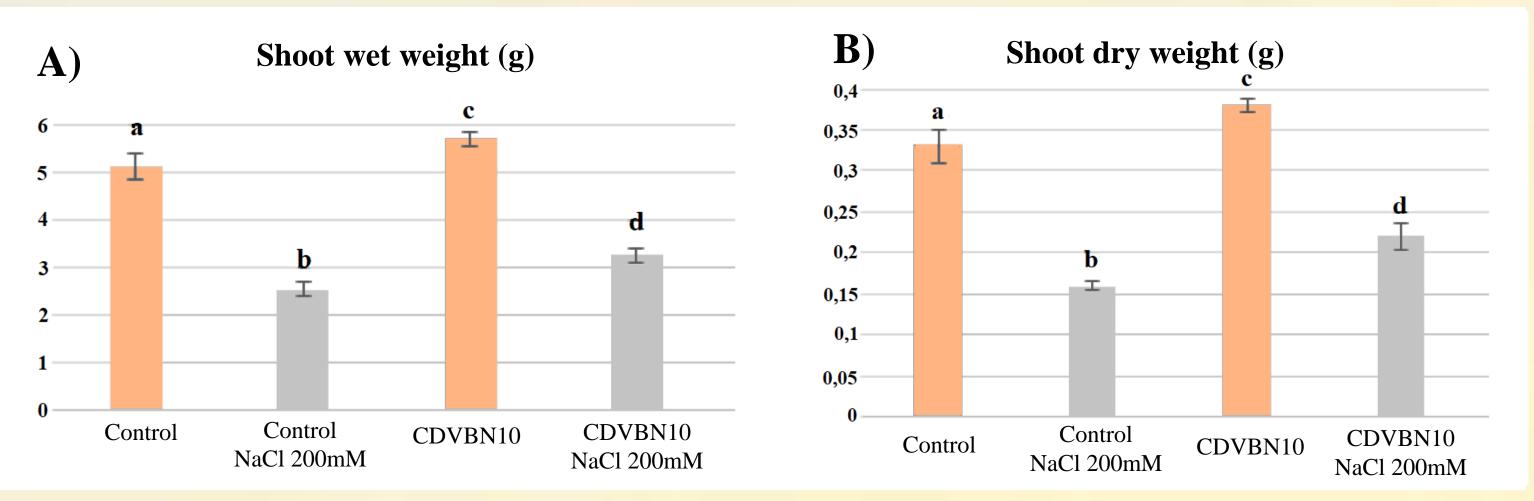
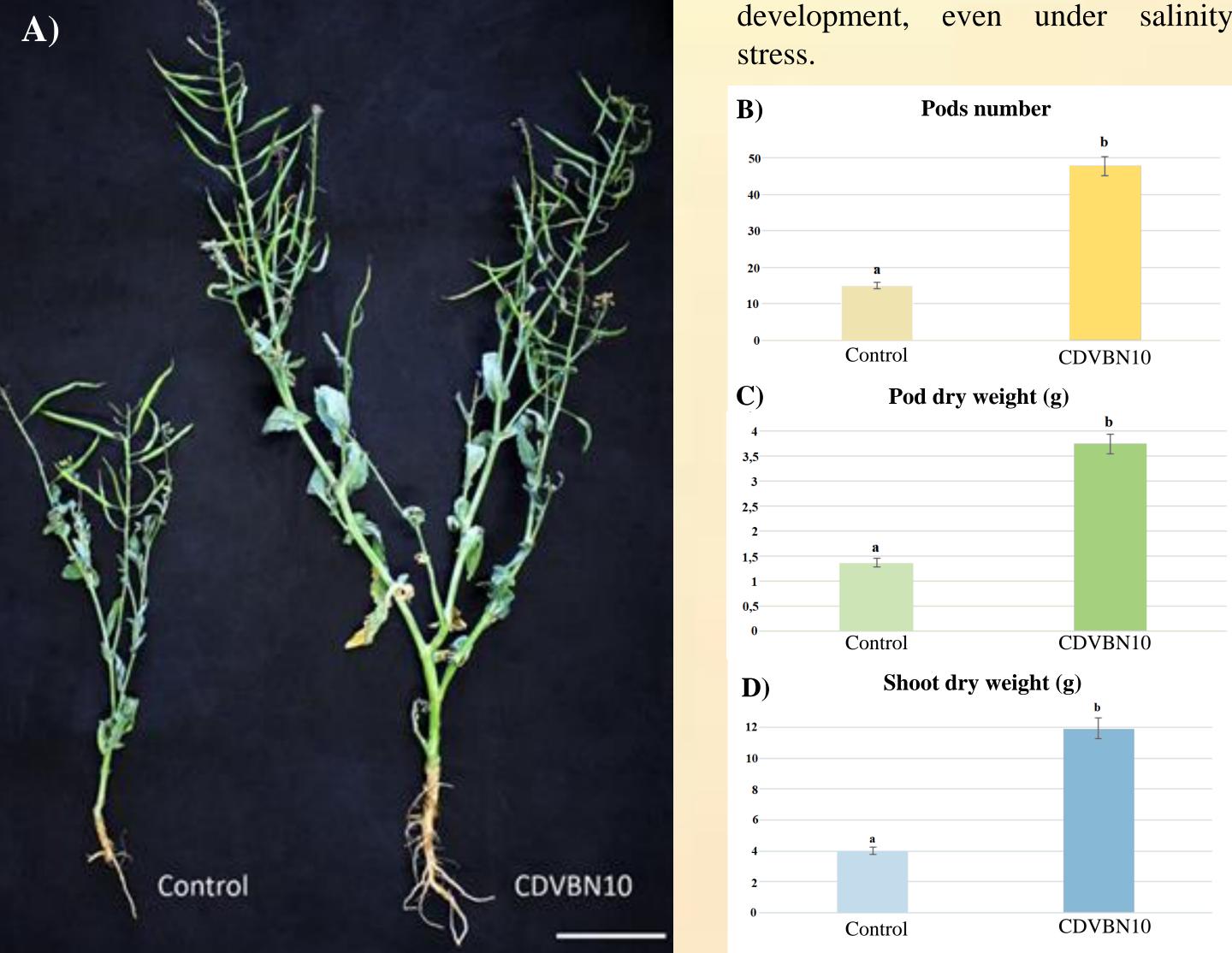


Figure 1. Representation of shoot wet weight (A) and shoot dry weight (B) values of *B. napus* plants 45 days-post-inoculation with CDVBN10 in greenhouse normal and salinity stress conditions.

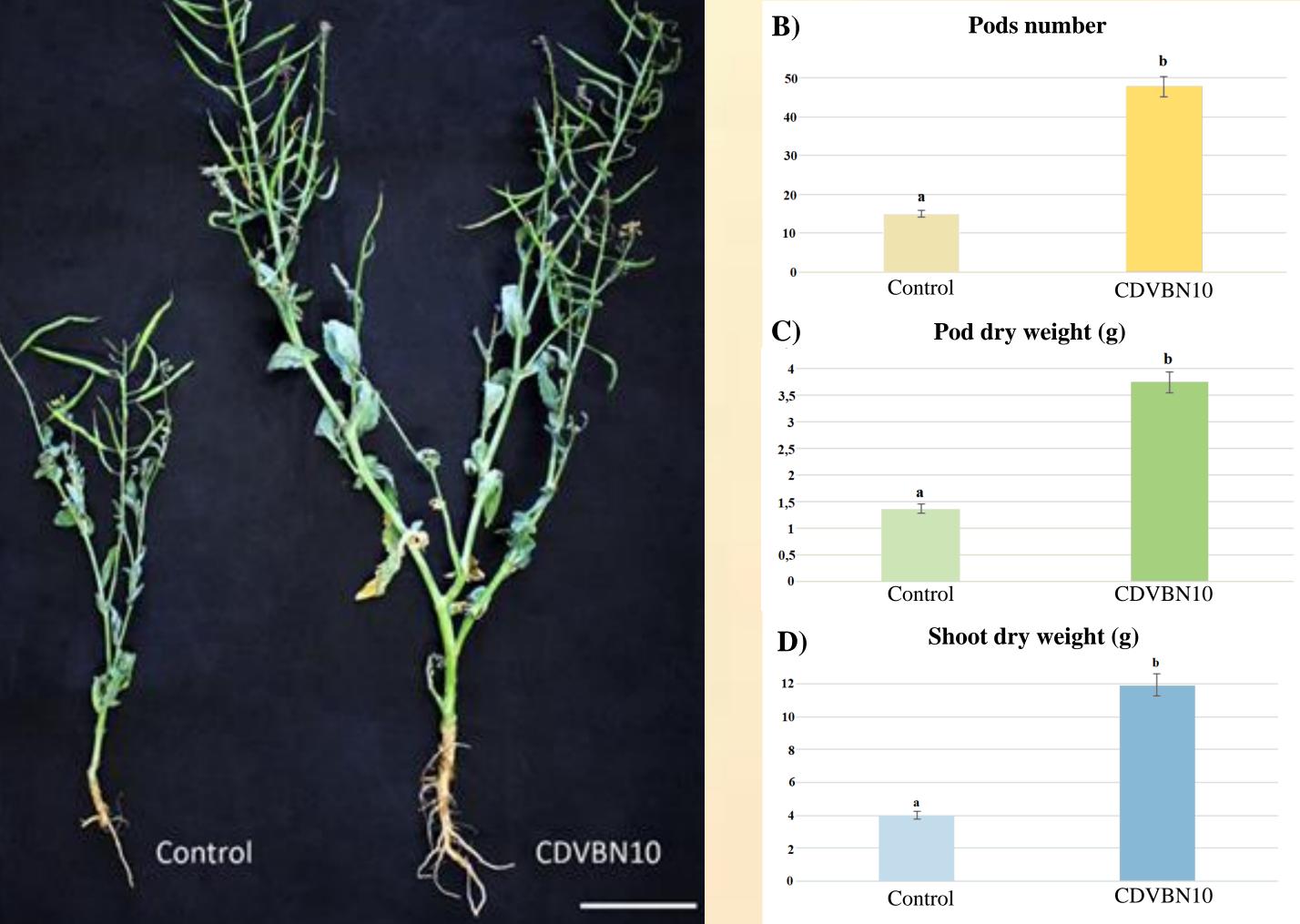
field Moreover, the assay showed significant improvements in pods number, pods dry weight and shoot dry weight in 216.0%, 174.3% and 197.3%, respectively, over the uninoculated plants (Figure 2), and resulted in a higher content of nutritional components (Table 1).

Table 1. Effects of strain CDVBN10 inoculation on nutrient contents of rapeseed plants grown in the field conditions.

Treatment	N (g/100g)	C (g/100g)	Fe (mg/kg)	K (g/100g)	P (g/100g)
Control	3.56	53.69	67.34	1.04	0.58
CDVBN10	3.82	54.89	59.60	0.99	0.65



Due to soil salinity is a major cause of reduction in agricultural productivity, we wanted to evaluate the rapeseed growth under normal and salinity stress conditions when inoculated with strain CDVBN10. The results of this greenhouse showed that assay plants inoculated had significant higher wet and dry weight of aerial portion in both normal and salinity stress conditions treatments (Figure 1), suggesting a PGP ability of strain CDVBN10 in early stages of rapeseed development, even under salinity



Finally, the 16S rRNA gene amplicon sequence analyses revealed that there are not statistically significant differences in alpha and beta diversity among and within all samples from field experiment and that Polaromonas (2.6)and 2.1 %), Janthinobacterium (3.4 and 2.9%) and Flavobacterium (11.2 and 9.6 %) were the most abundant genera in both inoculated and control treatments, respectively (Figure 3). The genus *Pseudomonas* showed a relative abundance of 0.7% in both cases.

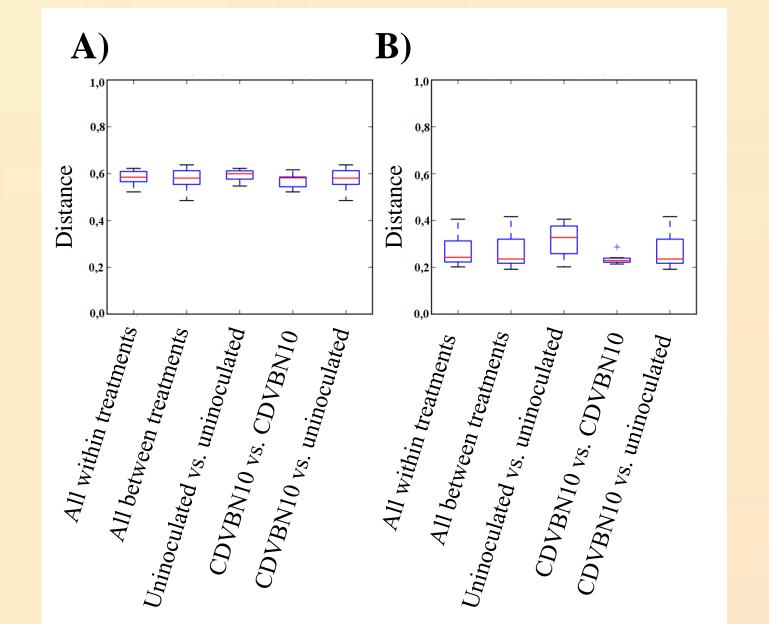
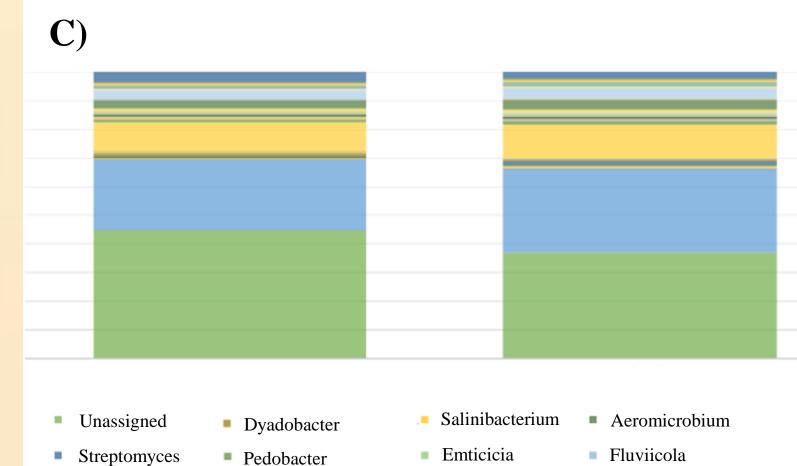


Figure 2. Comparison of uninoculated and inoculated B. napus plants grown in field conditions (A). Representation of number of pods per plant (B), pod dry weight (g) per plant (C) and shoot dry weight (D) of B. napus plants grown in field conditions during 8 months, uninoculated and inoculated with CDVBN10.



Salinibacterium	 Aeromicrobium
Emticicia	 Fluviicola
 Chitinophaga 	 Flavisolibacter
Devosia	 Mesorhizobium
1	

All these observations suggest the great potential of *P*. brassicacearum CDVBN10 to be used as a biofertilizer of rapeseed crop that, in addition, does not alter the endophyte bacterial community, considered essential for the plant fitness and development.

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References:

Jiménez Gómez, A. (2020). Estudio metagenómico y culturómico de bacterias asociadas a Phaseolus vulgaris y Brassica napus, implicaciones

Agrobacterium Polaromonas Janthinobacterium Rubrivivax Methylibium Rhodoferax Variovorax Dokdonella Methylotenera Cellvibrio Luteolibacter Pseudomonas Unclassified at genus Opitutus level

funcionales y nutricionales de su aplicación como biofertilizantes en cultivos de interés agroalimentario y bioenergético y evaluación del rol de la putrescina en la interacción bacteria-planta. Doctoral Thesis. University of Salamanca Shrivastava, P., & Kumar, R. (2015). Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi journal of biological sciences, 22(2), 123-131.

Figure 3. Comparison of beta diversity between sampling sites; representation of unweighted Unifrac distances (A) and wighted Unifrac distances (B). Representation of genera relative abundance (C) in uninoculated and inoculated field treats. No significant results were found in all these analyses.

Flavobacterium

Bosea

Phenylobacterium