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CENTRO HISPANOLUSO DE INVESTIGACIONES AGRARIAS

Evaluation of the potential of a *Rhizobium* sp. strain to improve the productivity of *Lactuca sativa* L. under salinity

Miguel Ayuso-Calles, Alejandro Jiménez-Gómez, José David Flores-Félix, Raúl Rivas

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INTRODUCTION

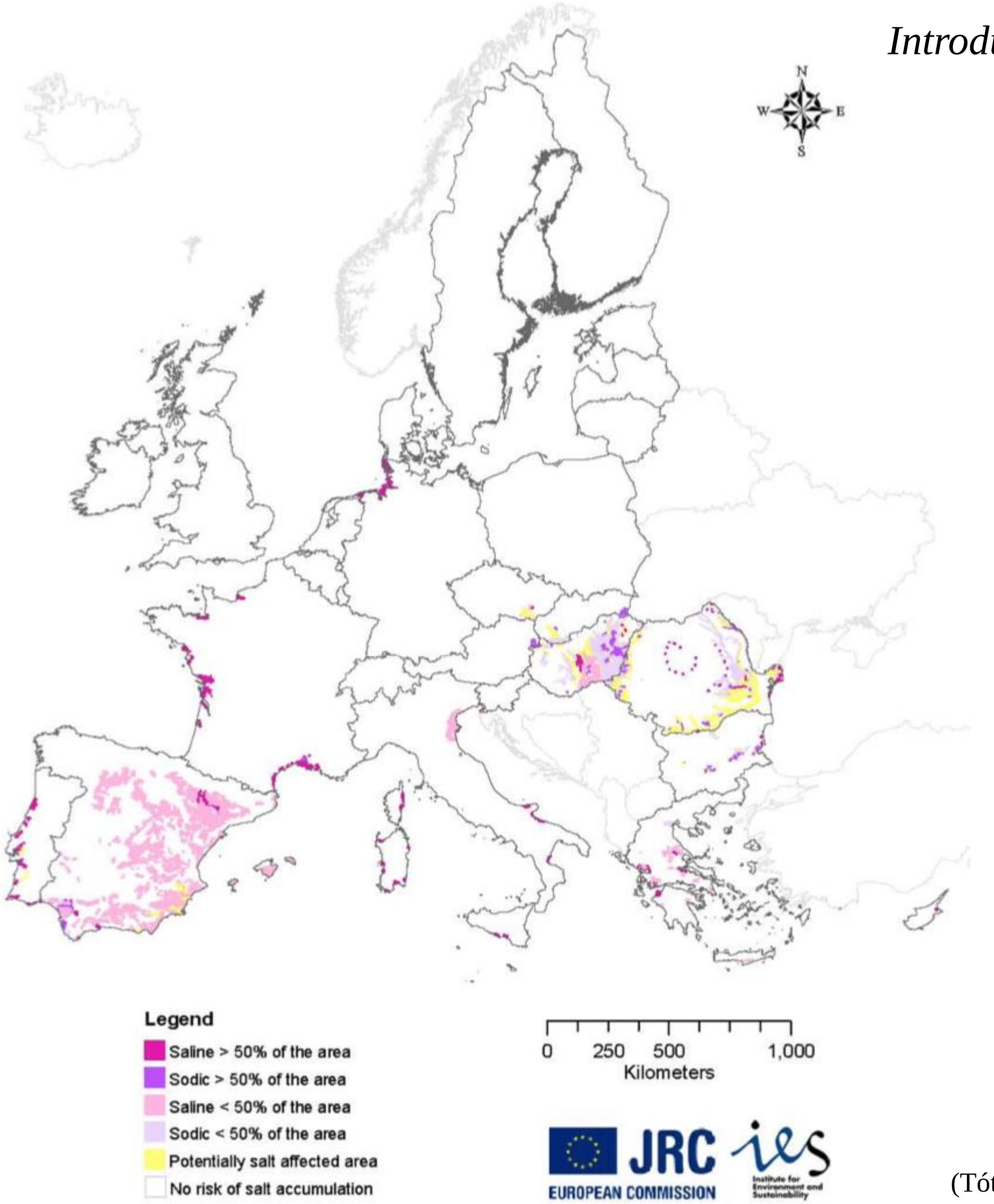
Adverse environmental conditions

- Nutrient deficiency
- Extremes temperatures
- Hydric stress
- UV Radiation
- Salinity

Decrease in crop yield and production

Saline soil: 40 mM (NaCl)

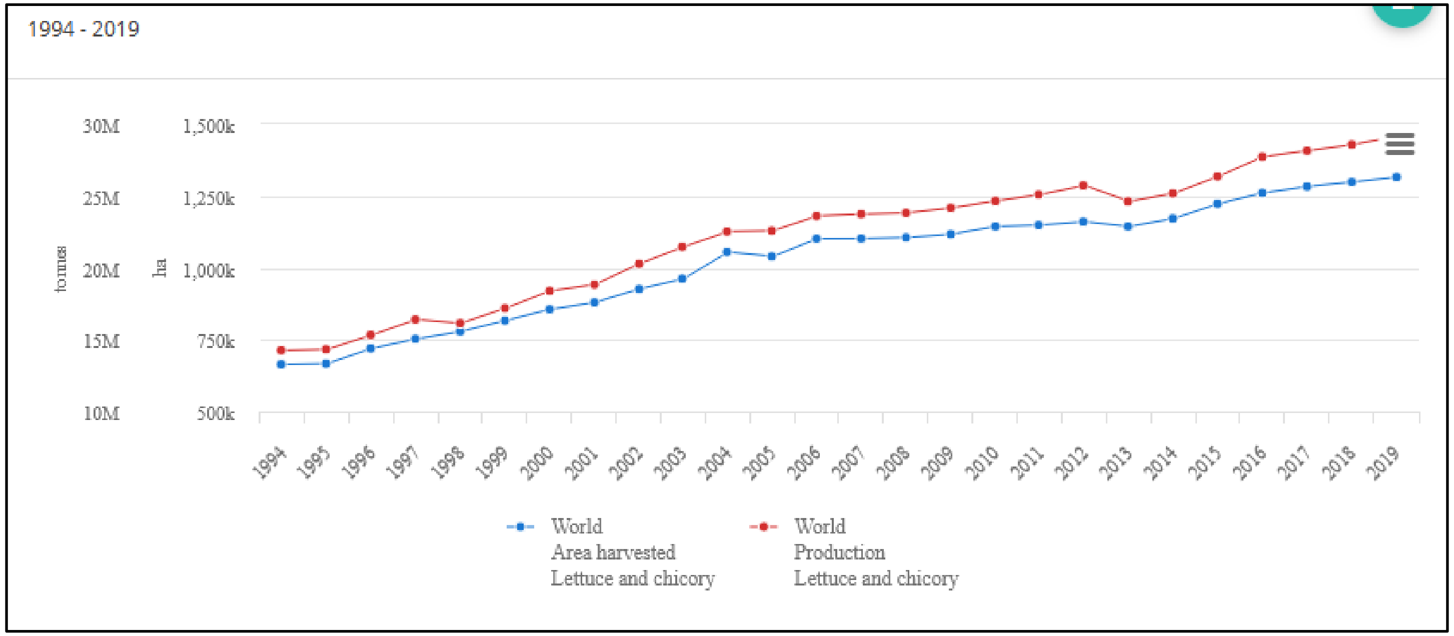
Increased rate of salinization→ 10 % annual



(Tóth *et al.*, 2008)

Lactuca sativa L.

Lettuce and chicory Production/Yield quantities worldwide



FAOSTAT, http://www.fao.org/faostat/en/#data/QC/visualize

Salt stress afection

Synergistic Action of a Microbial-based Biostimulant and a Plant Derived-Protein Hydrolysate Enhances Lettuce Tolerance to Alkalinity and Salinity

Youssef Rouphael[1], Mariateresa Cardarelli[2], Paolo Bonini[3] and Giuseppe Colla[4*]

[1]Department of Agricultural Sciences, University of Naples Federico II, Portici, Italy
[2]Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di Ricerca per lo Studio delle Relazioni tra Pianta e Suolo, Rome, Italy
[3]NGA Laboratory, Tarragona, Spain
[4]Department of Agricultural and Forestry Sciences, Tuscia University, Viterbo, Italy

Varied tolerance to NaCl salinity is related to biochemical changes in two contrasting lettuce genotypes

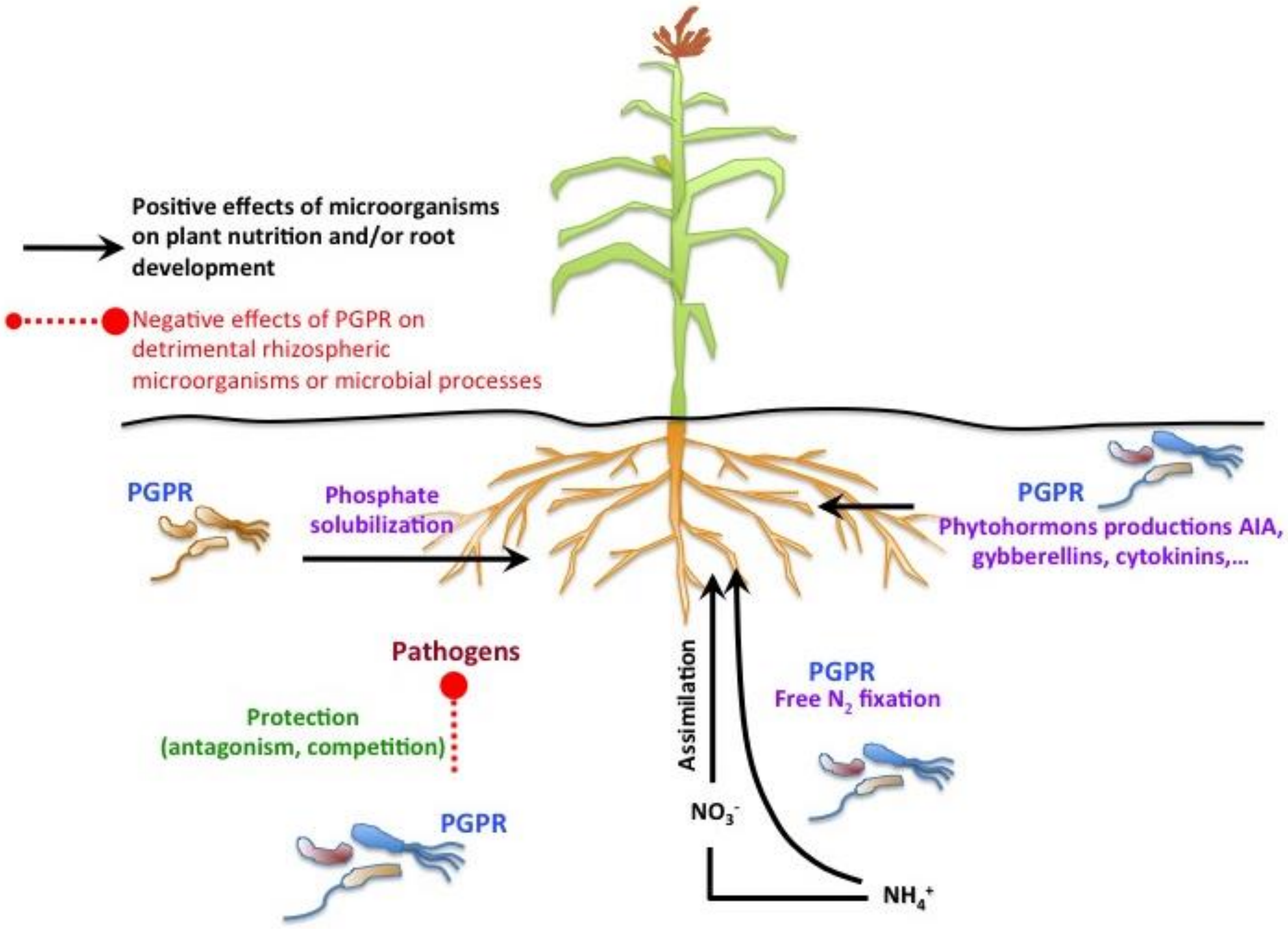
Héla Mahmoudi, Rym Kaddour, +6 authors Zeineb Ouerghi • Published 2010 in Acta Physiologiae Plantarum •
DOI: 10.1007/s11738-010-0696-2

Salt stress perturbs a multitude of physiological processes such as photosynthesis and growth. To understand the biochemical changes associated with physiological and cellular adaptations to salinity, two lettuce varieties (Verte and Romaine) were grown in a hydroponics culture system supplemented with 0, 100 or 200 mM NaCl. Verte displayed better growth... (More)

Plant Growth-Promoting Rhizobacteria
PGPR

Rhizobium genus

- Biological Nitrogen Fixation
- Promotion of plant growth in horticultural crops
- *Rhizobium* helps mitigate salinity (Ayuso-Calles *et al.*, 2020)

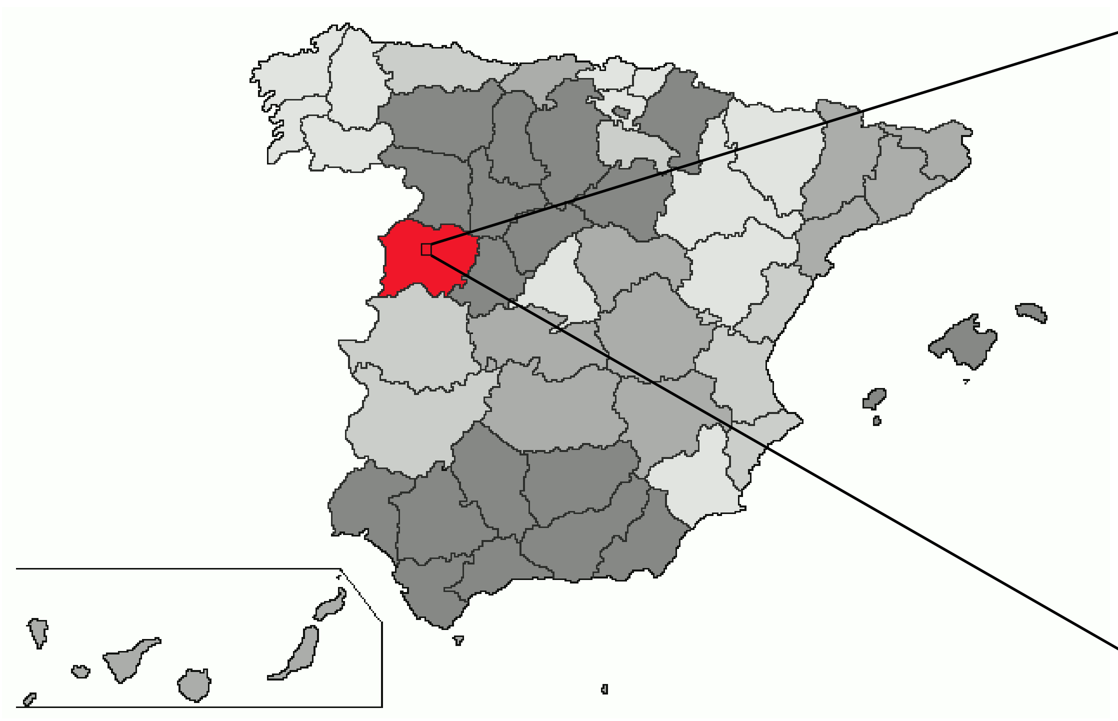


Comte *et al.* (2008)

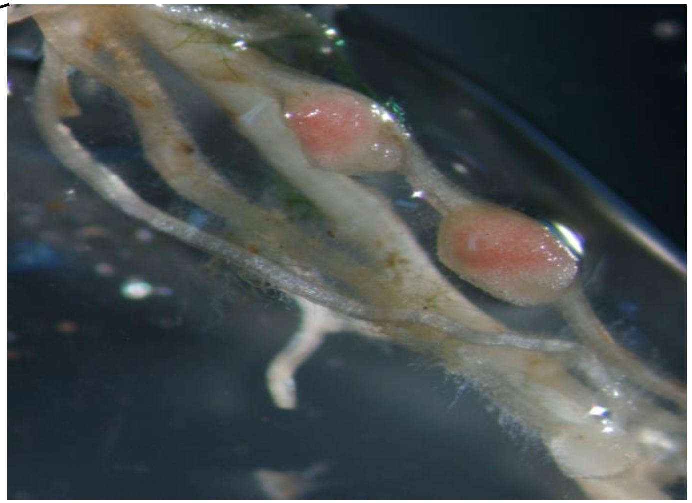
- Pepper and tomato (García-Fraile *et al.*, 2012)
- Spinach (Jiménez-Gómez *et al.*, 2018)

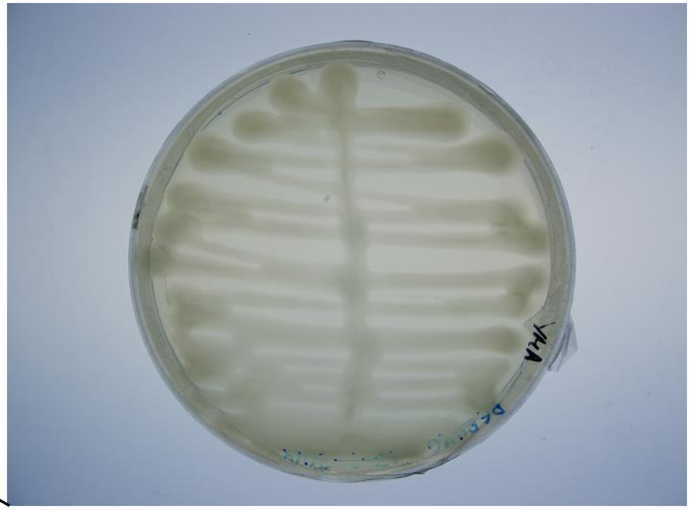
RESULTS AND DISCUSSION

Microorganism isolation



White clover plants were grown in a soil obtained from Golpejas (Salamanca, Spain)





Trifolium repens L. nodules and microorganisms isolated from the inside

Amplification and sequencing of 16S rRNA gene

Strain	Similarity (%)	Closest Type Strain
GPTR29	99.6 %	*Rhizobium laguerreae* FB206^T

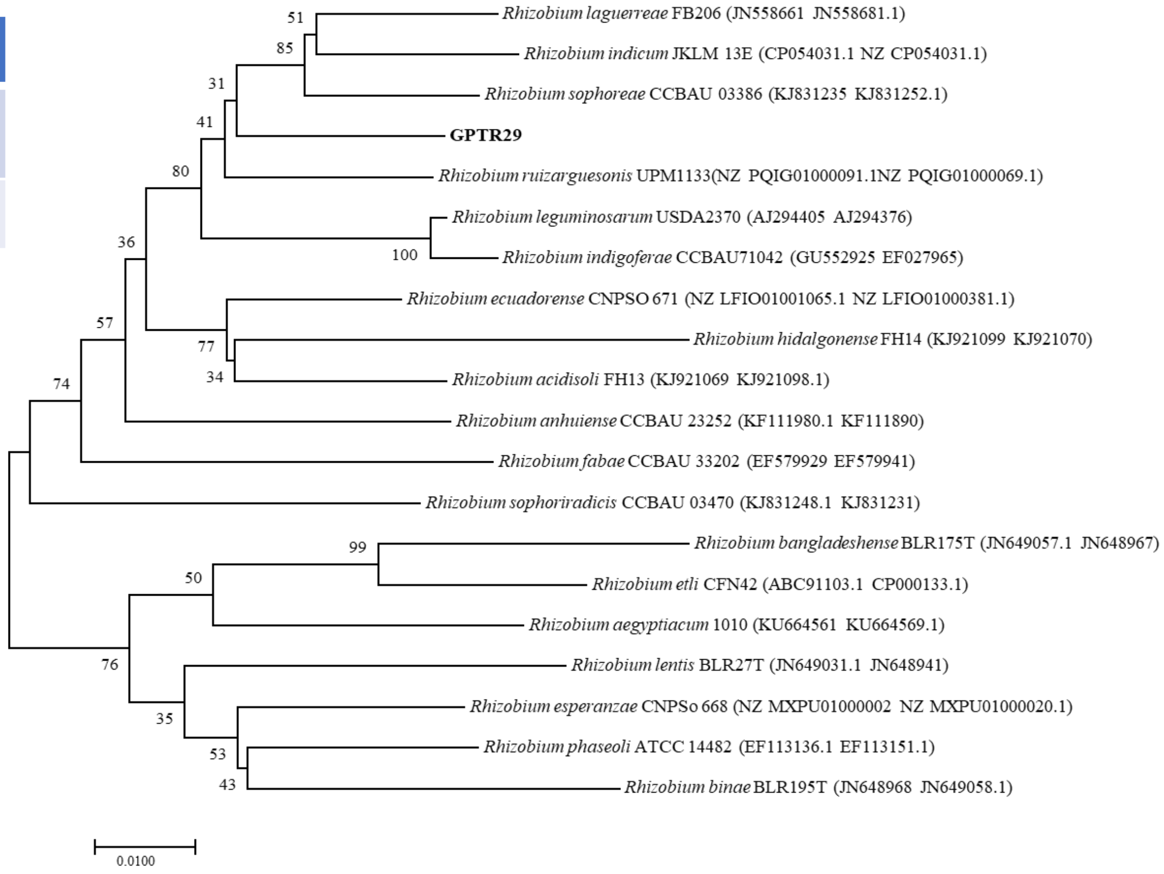
Percentage of similarity of the strain isolated in the study with respect to those present in the public databases

Amplification and sequencing of housekeeping genes

Strain	*atpD*		*recA*	
	Closest Type Strain	% similarity	Closest Type Strain	% similarity
GPTR29	*Rhizobium leguminosarum* (TC40)	99.8%	*Rhizobium leguminosarum* (LMR577)	99.7%

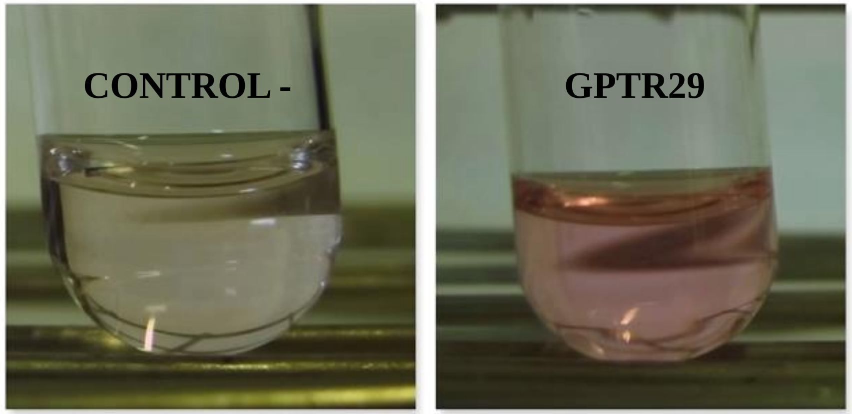
Percentage of similarity of the strain isolated in the study with respect to those present in the public databases

MEGA Molecular Evolutionary Genetics Analysis



Phylogenetic tree obtained from *atpD* and *recA* genes concatenation of the studied strain and its closest species described in the *Rhizobium* genus

Auxins production capacity

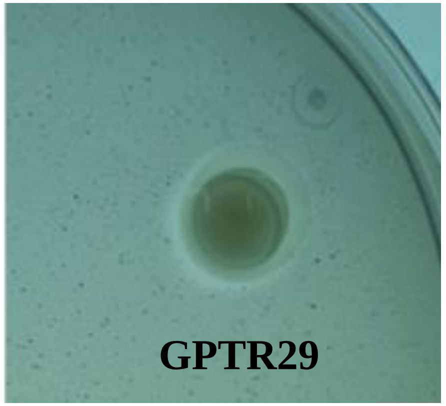


Comparison of the auxin production capacity between the selected strain and the negative control

Strain	Auxinas (colorimetry)		IAA (HPLC)
	Abs (550 nm)	Concentration (mg/L)	Concentration (mg/L)
Control -	0	0	0
GPTR29	0.188	74.29	0.773

Auxins concentration measured by colorimetry and IAA measured by HPLC

Phosphate solubilization capacity



Pikovskaya Agar plate showing the solubilization halo produced by the selected isolate

Strain	Pikovskaya medium	NBRIP medium
GPTR29	++	+

(-) negative production, (+) weak production, (++) high production

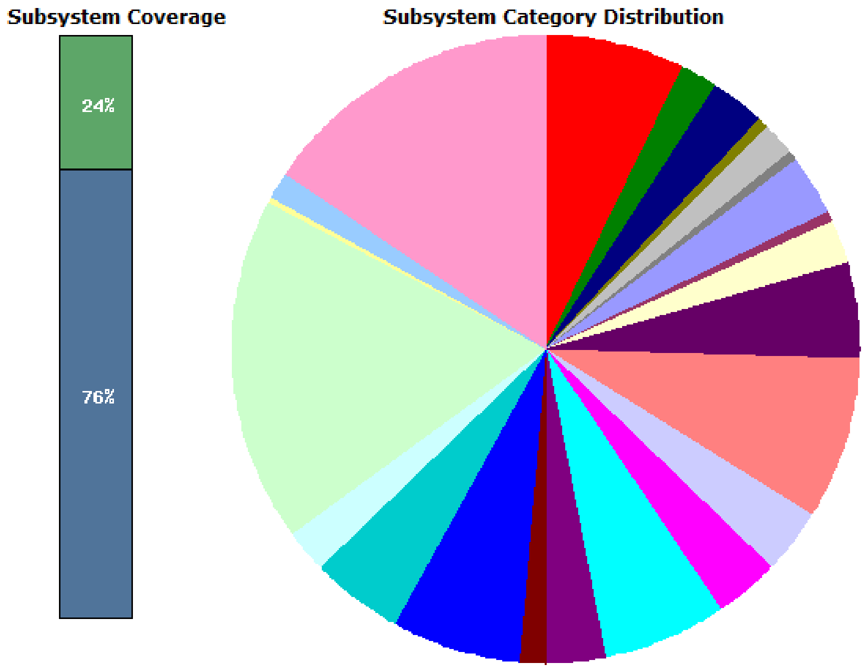
Siderophores production capacity

M9-CAS-Agar plate showing the siderophores production halo generated by the selected isolate

Strain	M9-CAS-AGAR
GPTR29	++

(-) negative production, (+) weak production, (++) high production

Genome *in silico* analysis



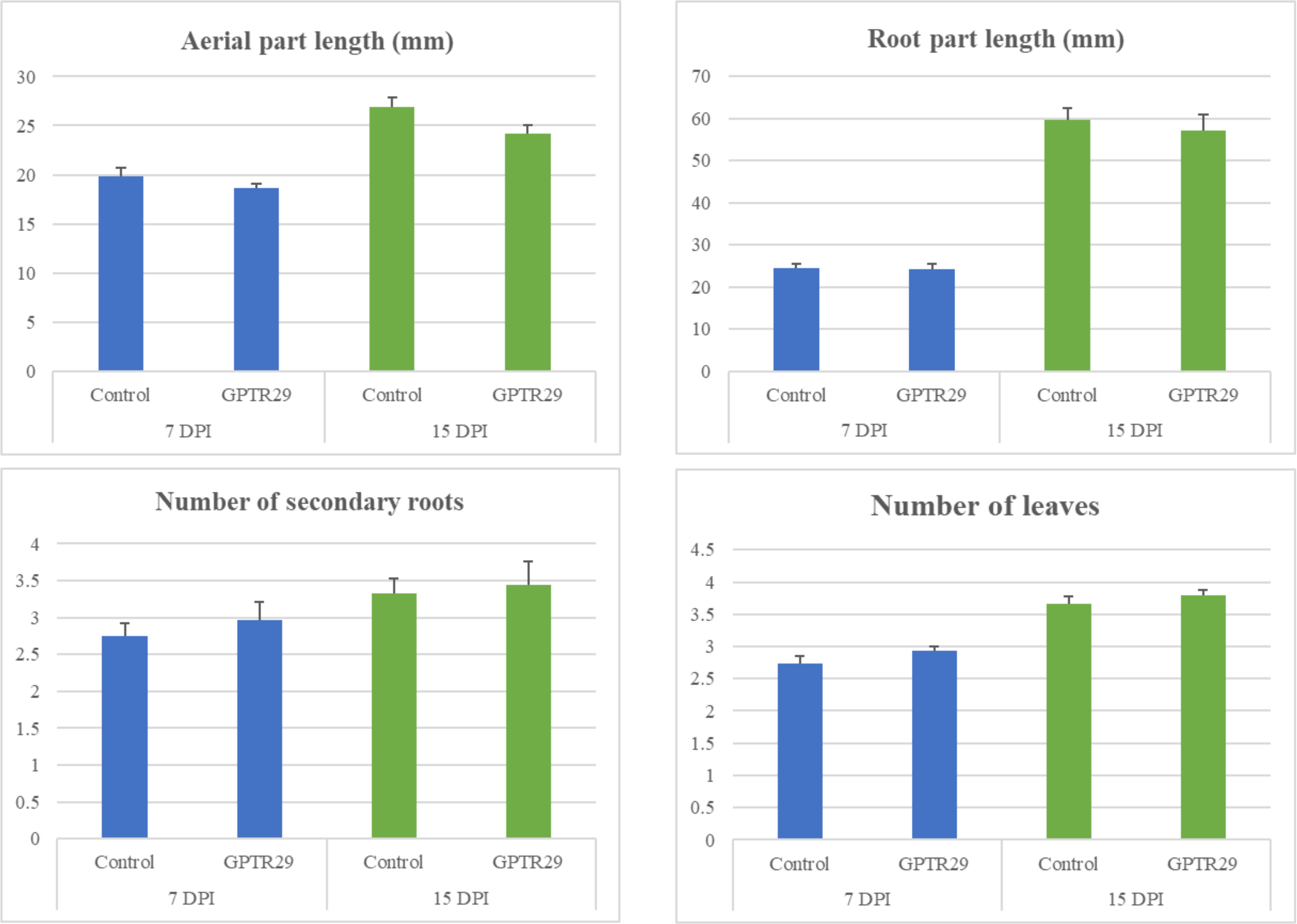
GPTR29 genome	
Contigs	70
Size	6,934,675
GC Content	60.7
CDS	7,202
rRNAs	50

SYSTEMS	GPTR29
Cofactors, Vitamins, Prosthetic Groups, Pigments	169
Cell Wall and Capsule	43
Virulence, Disease and Defense	63
Potassium metabolism	12
Photosynthesis	0
Miscellaneous	34
Phages, Prophages, Transposable elements, Plasmids	13
Membrane Transport	69
Iron acquisition and metabolism	16
RNA Metabolism	46
Nucleosides and Nucleotides	112
Protein Metabolism	193
Cell Division and Cell Cycle	0
Motility and Chemotaxis	70
Regulation and Cell signaling	79
Secondary Metabolism	6
DNA Metabolism	140
Fatty Acids, Lipids, and Isoprenoids	71
Nitrogen Metabolism	30
Dormancy and Sporulation	1
Respiration	153
Stress Response	103
Metabolism of Aromatic Compounds	54
Amino Acids and Derivatives	400
Sulfur Metabolism	8
Phosphorus Metabolism	31
Carbohydrates	339

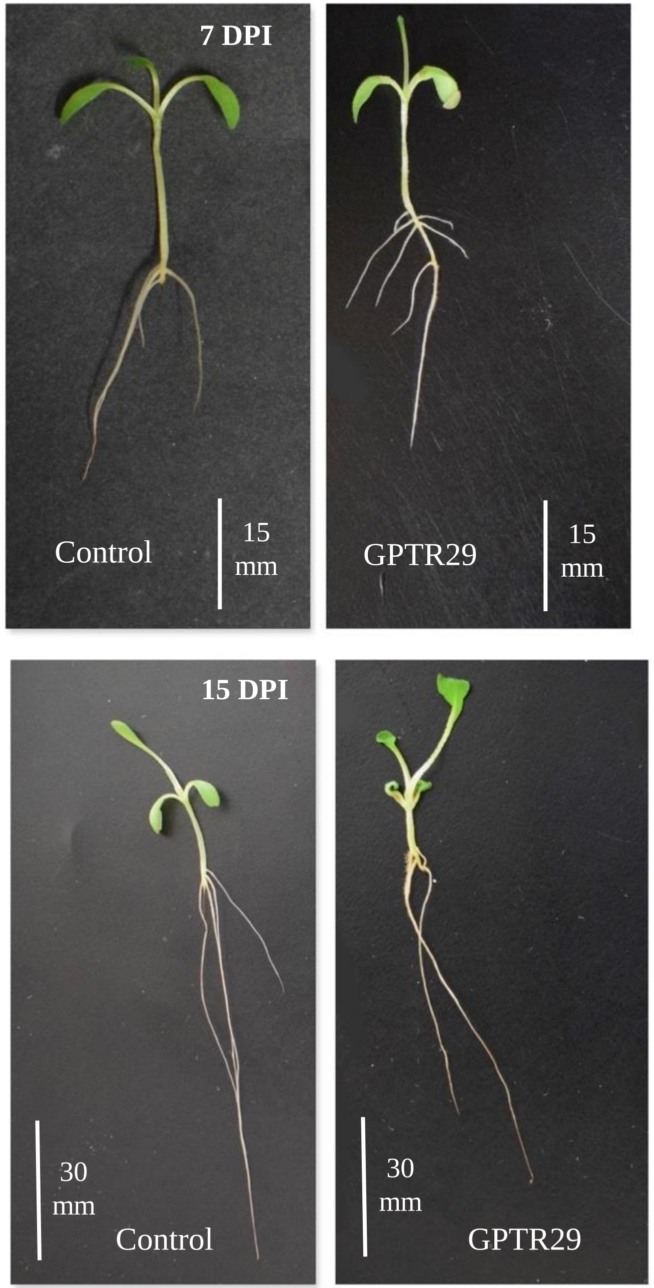
Genome *in silico* analysis

Phosphorus metabolim	PHO operon
	Phosphatases (EC 3.6.1.11; EC 3.6.1.40; EC 3.6.1.1; EC 3.1.3.18; EC 3.1.3.1; EC 2.7.4.1; EC 2.7.1.63)
Siderophores production	fhu system (*fhuA*, *fhuB*, *fhuC* and *fhuD*)
	piaABC, *pitABC*, *piuABC* and FeABC transporters
	iucABCD and *vatBCD* operons (aerobactin)
	siderX123456 operon (anthrachelin)
Phytohormone production	EC 4.1.1.48 (indole-3-glycerol-phosphate synthase), EC 4.2.1.84 (nitrile hydro-lyase), EC 3.5.1.4 (aliphatic amidase), EC 4.1.1.74 (indolepyruvate decarboxylase)
Response mechanisms against osmotic stress	Genes related to OPGs synthesis (*MdoBCDGH*, *NdvAB*, *CgmB* and *OpcG*)
	betABC operon (glycine betaine synthesis)
	Enzymes related to trehalose synthesis (EC 5.4.99.16; EC 2.4.1.15; EC 3.1.3.12; EC 5.4.99.15; EC 3.2.1.141)
Colonization mechanism	*exoFQZ*, *wadC*, *lptABC* (exopolysaccharide and lipopolysaccharide biosynthesis)
	celC and *bcsC* (cellulose biosynthesis)
Bioactive compounds	Enzymes related to phenylalanine synthesis (EC 4.2.1.51; EC 2.6.1.9)
	Naringenin-chalcone synthase (EC 2.3.1.74)

In vitro inoculation of *Lactuca sativa* L. roots

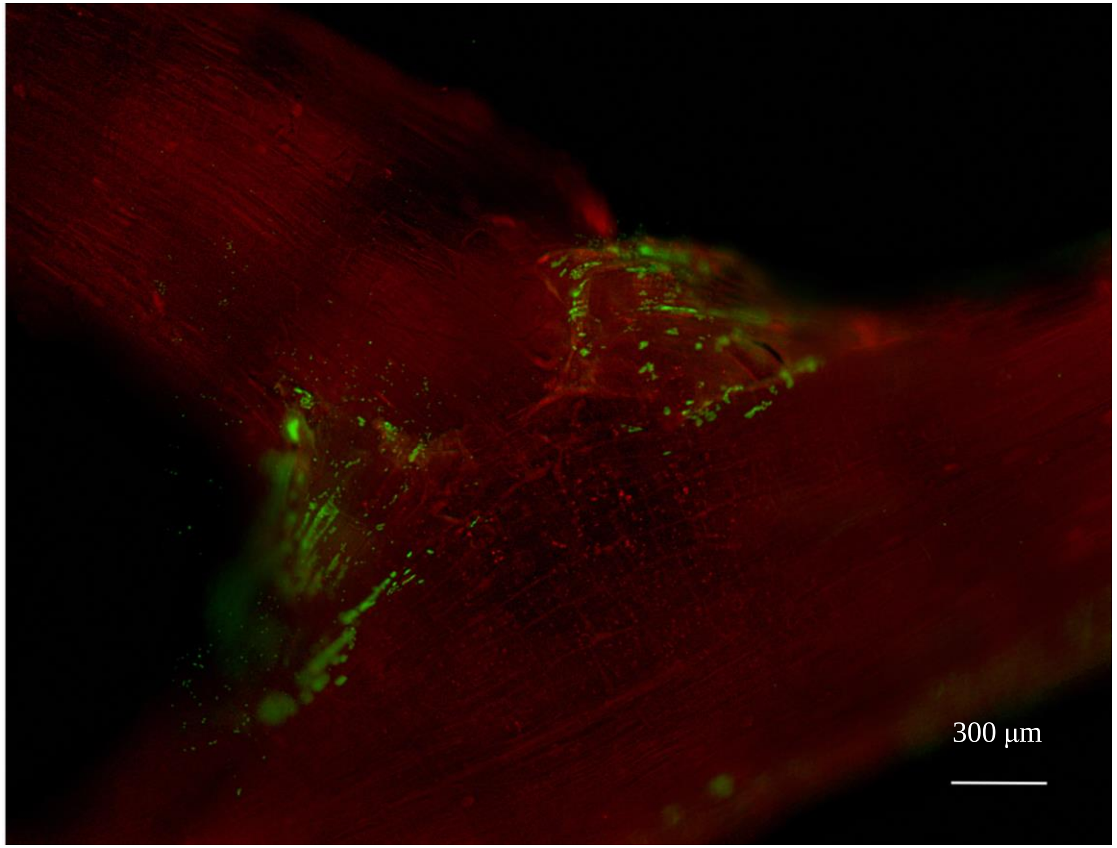


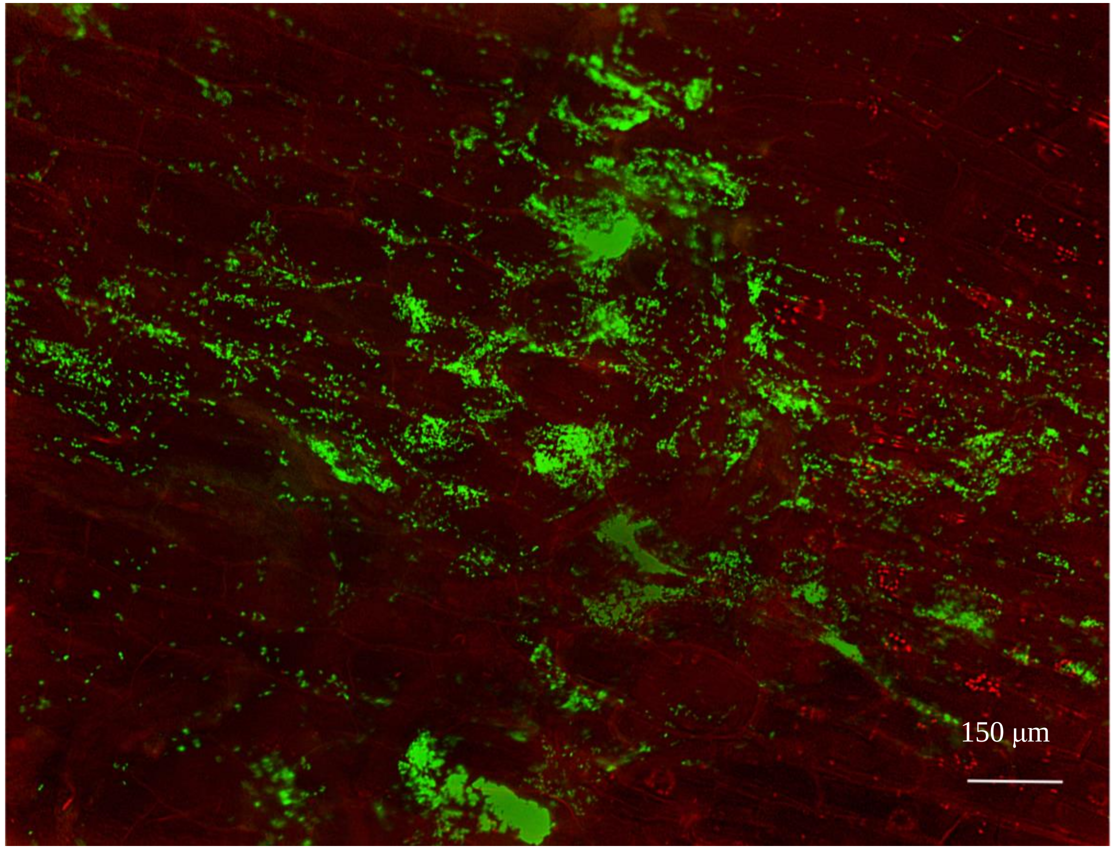
Parameters analyzed in the *in vitro* seedlings assay of *Lactuca sativa* L.



Comparison between lettuce plants grown *in vitro*

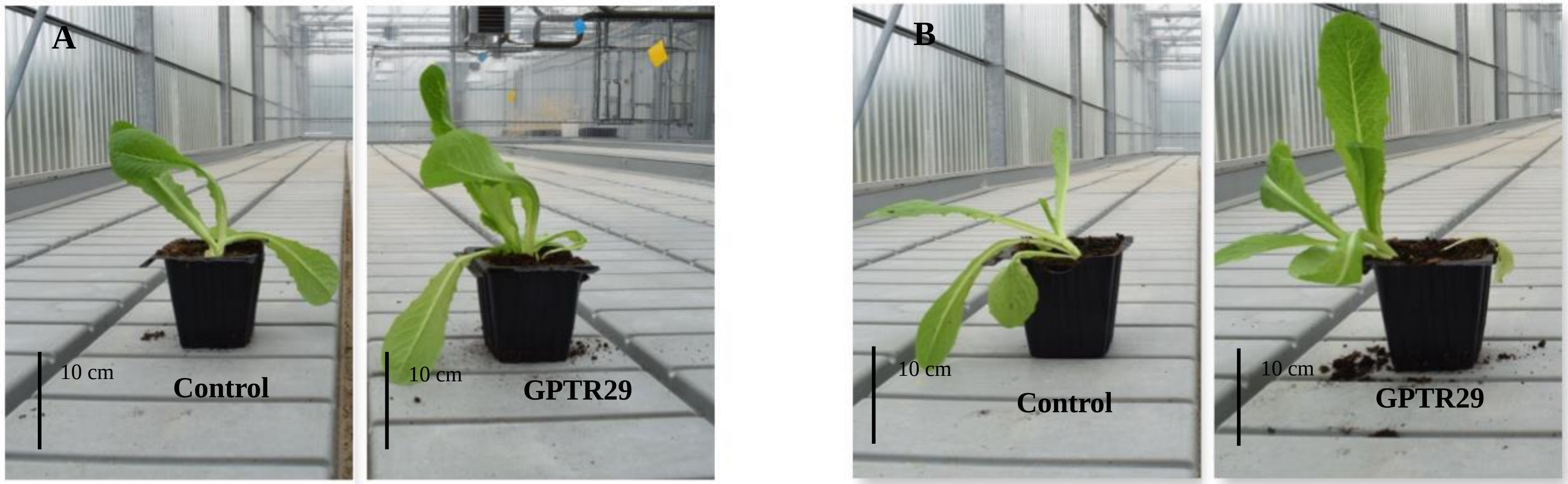
In vitro colonization of GPTR29 strain in *Lactuca sativa* L. roots and monitoring by fluorescence microscopy



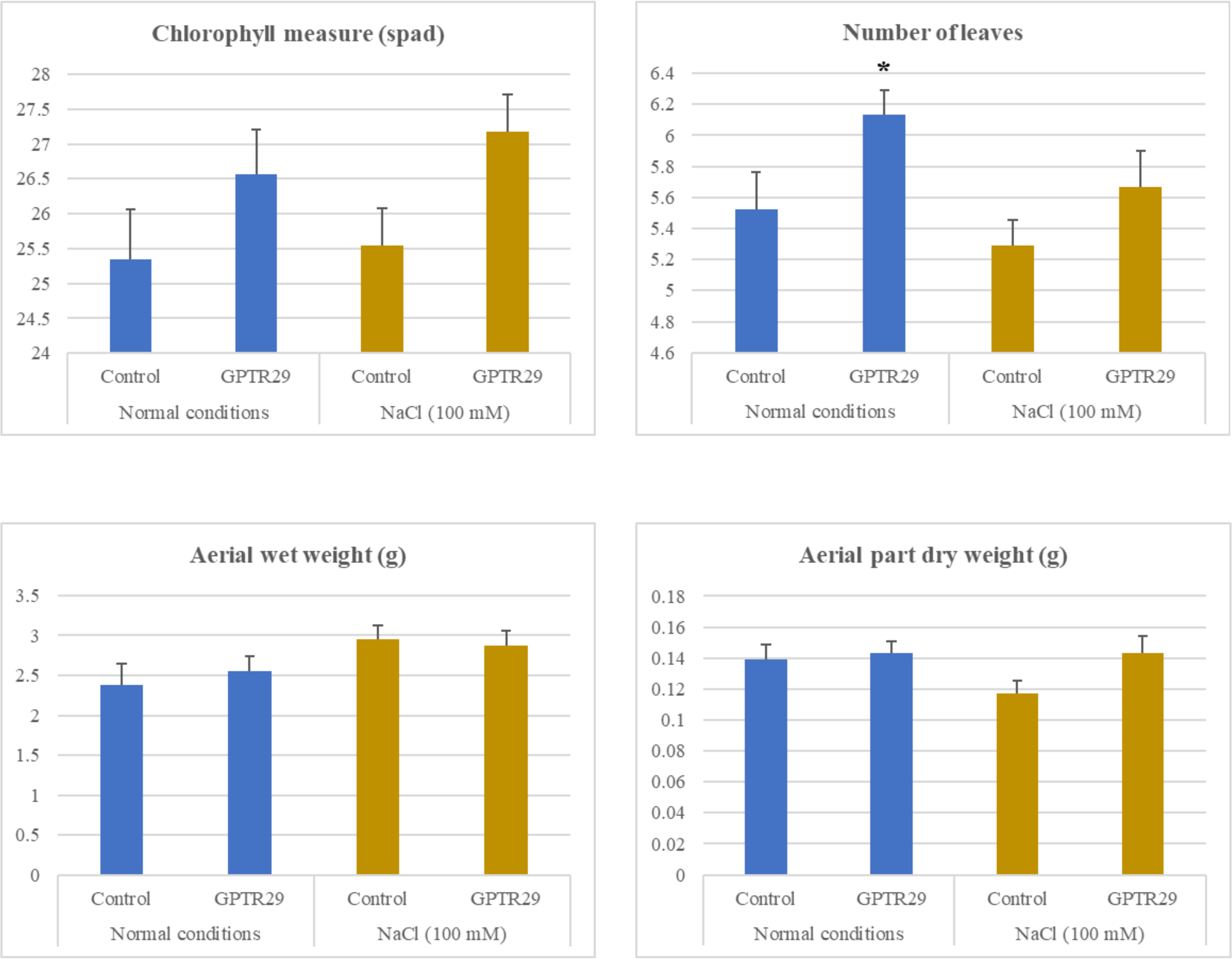


Red stain with propidium iodide (10 µM) → plant structures
Green stain with GFP → bacteria

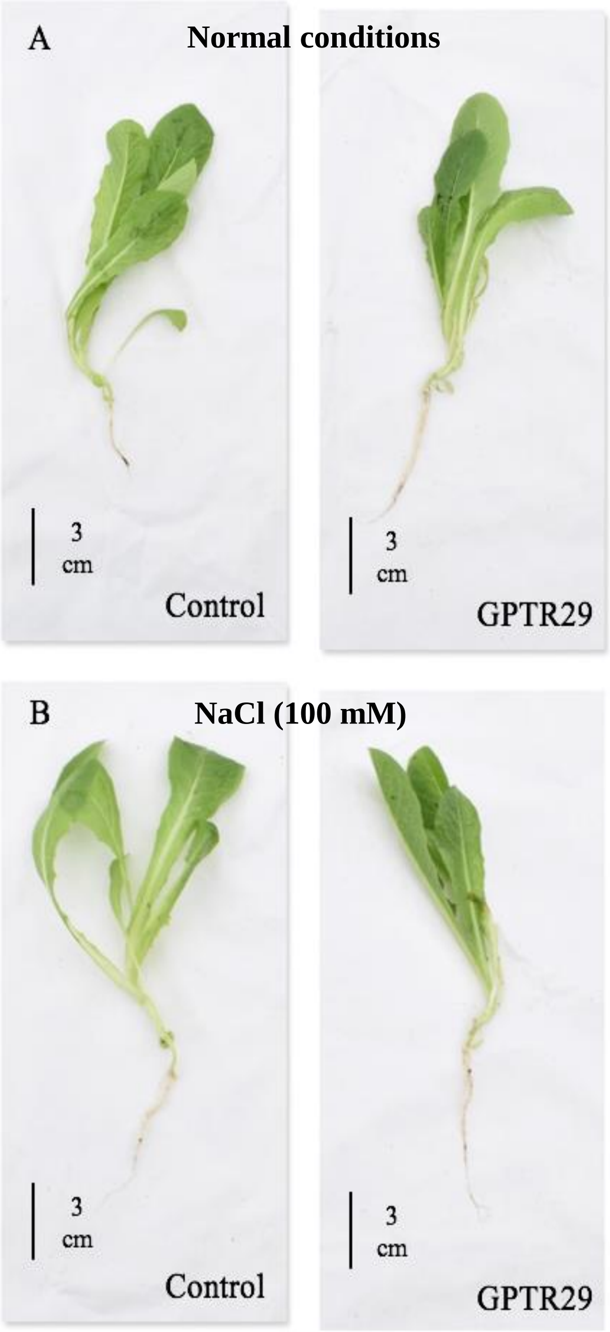
Greenhouse assay under normal conditions and salt stress



Comparison between plants inoculated with GPTR29 and uninoculated plants, under normal (A) and salinity (B) conditions.



Parameters analyzed 20 days post inoculation (DPI)

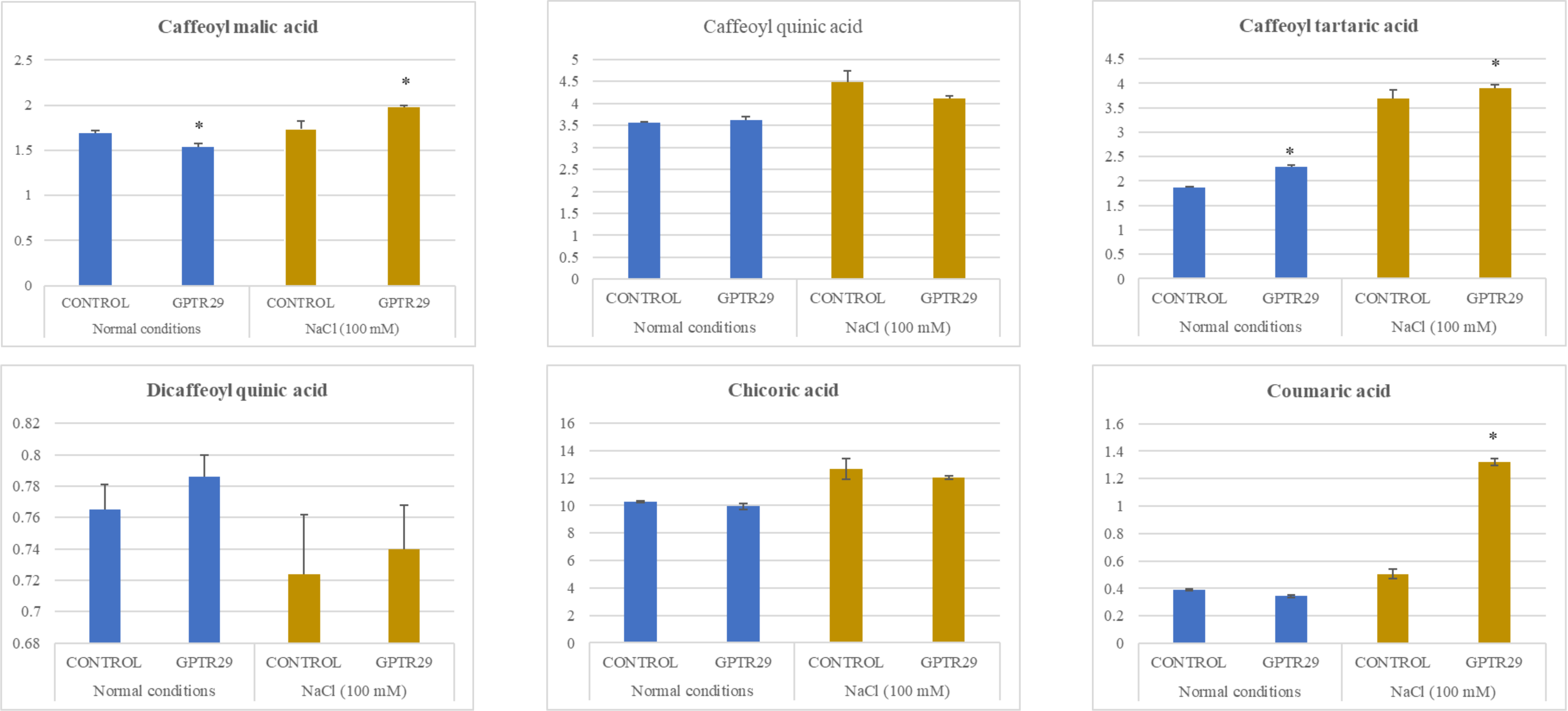


Lettuce growth 20 DPI in greenhouse

Nutritional analysis of *Lactuca sativa* L. leaves

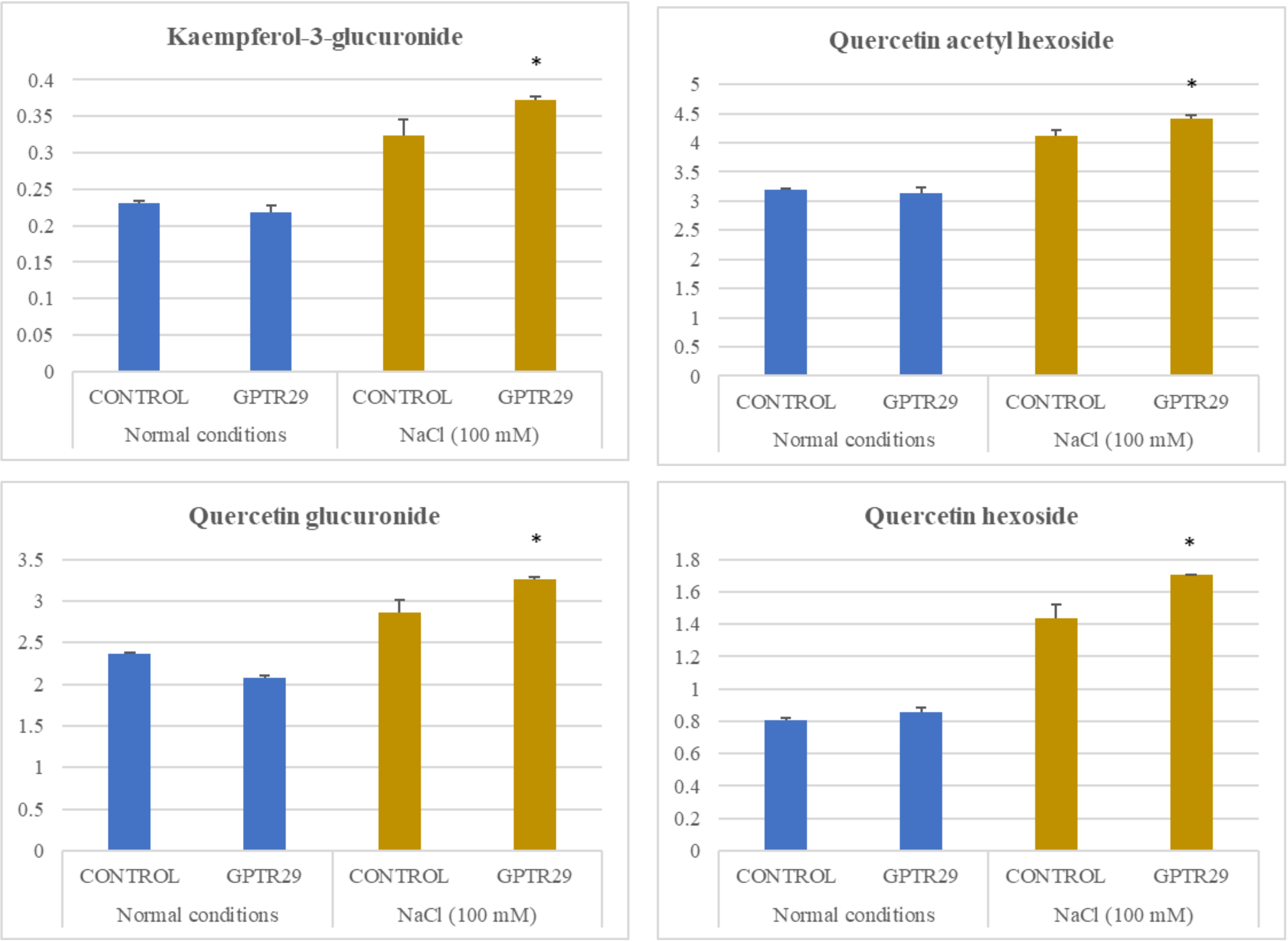
Phenolic acid content (g kg^{-1}) of control lettuce plants and plants inoculated with GPTR29, under greenhouse conditions

Results and Discussion



Nutritional analysis of *Lactuca sativa* L. leaves

Flavonoid content (g kg^{-1}) of control lettuce plants and plants inoculated with GPTR29, under greenhouse conditions



CONCLUSIONS

1. ***Rhizobium* sp. GPTR29 can interact with *Lactuca sativa* L. root system**, by colonizing it successfully.

2. **GPTR29** strain significantly promotes the development of lettuce under greenhouse conditions, **by increasing the values of various parameters of agroeconomic interest for this crop**, such as number of leaves or weight of the aerial part. This makes it possible to consider this strain ideal for use as biofertilizer in promoting plant growth and relieving salt stress.

3. The results obtained in phenolic compounds analysis of *Lactuca sativa* L. leaves allow to determine the important role of *Rhizobium* sp. GPTR29 in the improvement of this horticultural species nutritional value.

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