



Research article

Ensifer adhaerens strain OV14 seed application enhances *Triticum aestivum* L. and *Brassica napus* L. development

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ABSTRACT

Given the challenges imposed by climate change and societal challenges, the European Union established ambitious goals as part of its Farm to Fork (F2F) strategy. Focussed on accelerating the transition to systems of sustainable food production, processing and consumption, a key element of F2F is to reduce the use of fertilisers by at least 20% and plant protection products by up to 50% by 2030. In recent years, a substantial body of research has highlighted the potential impact of microbial-based applications to support crop production practices through both biotic/abiotic stresses via maintaining or even improving yields and reducing reliance on intensive chemical inputs. Here, we have characterised the ability of a new soil-borne free-living bacterium strain *Ensifer adhaerens* OV14 (EaOV14) to significantly enhance crop vigour index by up to 50% for monocot (wheat, *Triticum aestivum* L., $p < 0.0001$) and by up to 40% for dicot (oilseed rape, *Brassica napus* L., $p < 0.0001$) species under *in-vitro* conditions ($n = 360$ seedlings/treatment). The beneficial effect was further studied under controlled glasshouse growing conditions ($n = 60$ plants/treatment) where EaOV14 induced significantly increased seed yield of spring oilseed rape compared to the controls ($p < 0.0001$). Moreover, using bespoke rhizoboxes, enhanced root architecture (density, roots orientation, roots thickness etc.) was observed for spring oilseed rape and winter wheat, with the median number of roots 55% and 33% higher for oilseed rape and wheat respectively, following EaOV14 seed treatment compared to the control. In addition, EaOV14 treatment increased root tip formation and root volume, suggesting the formation of a more robust root system architecture post-seed treatment. However, like other microbial formulations, the trade-offs associated with field translation, such as loss or limited functionality due to inoculum formulation or environmental distress, need further investigation. Moreover, the delivery method requires further optimisation to identify the optimal inoculum formulation that will maximise the expected beneficial impact on yield under field growing conditions.

1. Introduction

The ambition of the EU's Green Deal and Farm to Fork (F2F) strategies in agriculture is to reduce the use and risk of synthetic agents

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for crop production by 20% for nutrients and up to 50% for plant protection products by 2030. Coupled with the increasing impacts of volatile weather patterns linked to climate change [1], it is clear that significant advancements are required to deliver integrated strategies that support crop resilience. This is especially the case with crop production, where plants must adapt to multiple biotic and abiotic stresses [2,3]. From the perspective of abiotic stress, European climate predictions suggest that more extreme events such as heatwaves, drought, and heavy precipitation can be expected [4,5], even within a single growing season [1]. Indeed, the weather events in the past decade suggest that the timeline for these events to occur has been accelerated beyond what models predict [1,6]. In the 21st century, the increase in daytime temperatures due to greenhouse gas emissions and atmospheric change has negatively influenced global wheat and maize production [7]. Moreover, since 2000, an increase of CO₂ levels in the atmosphere correlates with a rise in atmospheric ozone, which is responsible for approximately a 10% loss in wheat and soybean yield and up to 5% loss in rice and maize production, with severe impacts in Asian countries [7,8].

Plant biostimulants represent a promising solution to increase the resilience of crop production systems and overcome the current agricultural sector's limitations in the face of future challenges. It is well known that microorganisms are omnipresent on all organisms and surfaces, contributing to an ecosystem's well-being [9]. In plants, it has been shown that many rhizosphere bacteria positively influence nutrient availability [10]. Plant growth-promoting bacteria influence plant development via direct (facilitating nutrient acquisition) and indirect (biocontrol) mechanisms of action [11]. Direct mechanisms include phosphate/potassium solubilisation, nitrogen fixation, phytohormone synthesis (indolyl-3-acetic acid and cytokinin), and ACC deaminase production. Indirect mechanisms are represented by activating the induced systemic resistance (ISR) in the presence of pathogens and releasing in the environment inhibitory substances such as allelochemicals or lytic enzymes [11–15].

Ensifer represents a genus of Gram-negative α -proteobacteria with budding multiplication and a specific predatory characteristic for other bacteria. *Ensifer* and *Sinorhizobium* are taxonomic terms describing the same genus, with *Ensifer* first described by Casida in 1982 and *Sinorhizobium* by Chen, Yan and Li in 1988 [16]. When supplemented with the pCambia5105 plasmid, *Ensifer adhaerens* OV14 strain has been shown to successfully transform potato [17], rice [18], oilseed rape [19], and cassava [20]. Furthermore, several studies on alternative strains of *E. adhaerens* have already described the species' capability to promote plant growth via nutrient mineralisation [21], neonicotinoid insecticide (thiamethoxam) degradation [22], and indeed act as a biocontrol agent [23]. A previous study has indicated that *E. adhaerens* OV14 possesses genetic networks to indicate a possible ability to improve plant growth and enhance stress resilience (salicylic acid metabolism and quorum sensing communication system via *N*-acyl homoserine lactone metabolism) [24]. However, tangible evidence to support this hypothesis is lacking. Recently, through comparative genomic analyses, it has been established that species from the *Ensifer* genus are separated phylogenetically into two clades: symbionts and non-symbionts, with *E. adhaerens* OV14 located in the non-symbiont group [25]. In this context, the beneficial effect on improving plant growth that *E. adhaerens* OV14 might possess falls under the latest biostimulants definition stating that the microorganism must "stimulate natural processes to benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independently of its nutrient content" [26].

The goal of this study was to test the hypothesis that the wild-type *E. adhaerens* OV14 has indeed plant growth-promoting activity and could be utilised as a biostimulant for economically significant monocot (*Triticum aestivum* L.) and dicot (*Brassica napus* L.) species. For an in-depth understanding of the effect that *E. adhaerens* OV14 induces on plant development, *in-vitro* experiments looked at the early stages of development with a focus on seed germination and seedlings' vigour index. The beneficial effect was further confirmed under controlled glasshouse conditions with *in-vivo* seed-to-seed experiments for oilseed rape variety Ability and real time root system development observation using bespoke rhizoboxes.

2. Materials and methods

2.1. Bacteria growing conditions

E. adhaerens OV14 (hereafter EaOV14) was grown on TTY (Teagasc Tryptone Yeast: 10% tryptone, 5% yeast extract; after autoclaving, add 20% 1 M CaCl₂ to prevent aggregates formation, [19]) agar media supplemented with kanamycin 100 μ g/mL and incubated at 28 °C for 72 h for cultures development. Bacteria suspensions were obtained by inoculating single colonies on TTY broth media followed by incubation at 28 °C shaking at 220RPM (revolutions per minute) for 24 h or until an optical density at 600 nm wavelength (OD₆₀₀) of 0.8 was reached, the equivalent of 2×10^9 CFU/mL. The concentration of bacteria liquid culture was assessed using a 96 wells plate spectrophotometer (BioTek, Gen5 software).

2.2. Crop species selection

Two conventional varieties of oilseed rape (OSR) were selected, one spring OSR (Ability) and one winter OSR (Anastasia). The seeds were harvested in 2019 and recorded a post-harvest germination rate of >94%. Two varieties of winter wheat were selected (vars. Lilli and Rockefeller). Seeds were harvested in 2020, and the germination rate the following spring was 57% for Lilli and 61% for Rockefeller. The varieties were selected based on their prominence on the Irish recommended list from the year of study (2019 for OSR and 2020 for winter wheat).

2.3. Seed coating formulation

When working with biostimulants, the inoculum formulation is essential for a suitable seed delivery [27,28]. For oilseed rape,

conditions were adapted as per Lally et al. (2017) keeping the concentration of bacteria in the inoculum to 100% and exposure time to 10 min. In brief, EaOV14 liquid cultures at 0.8 OD₆₀₀ were centrifuged for 15 min at 3700 RPM, and the supernatant was discarded. The seed coating solution consisted of precipitated bacteria resuspended in 92.5% sterilised double distilled H₂O (H₂O), 7% Ringer solution ¼ strength, and 0.5% Glycerol. Sterilised double distilled H₂O and coating solution free of bacteria (CSC) were included as controls for all experiments. All solutions are sterilised by autoclaving prior to use. The unavailability of a comparable protocol for winter wheat led to the testing of a series of bacteria concentrations and exposure times. A concentration of 60% bacteria in the inoculum solution and 4 h of exposure were selected for the main experiments (Supplementary Fig. 1).

2.4. *In-vitro* plant growth-promoting activity

The *in-vitro* experiments were run in aseptic conditions where the coated seeds were placed on filter paper soaked with sterilised double distilled H₂O to saturation, the plates sealed with parafilm tape and left to germinate in the dark at 24 °C for seven days. Every two days, germination was assessed, and on day seven post-germination, seedlings were placed on a black background and a clear image captured, from which root and shoot length measurements were calculated in ImageJ™ software.

The seedling vigour index was calculated using the formula:

$$\text{Vigour index} = (\text{Shoot length} + \text{Root length}) \times \text{Germination \%}$$

The experimental design consisted of four biological replicates (four individual bacteria cultures started from independent starter cultures) of 30 seeds per treatment, and the overall experiment was repeated three times ($n = 360$ seeds/treatment). The treatments were water control (H₂O), coating solution free of bacteria (CSC) and coating solution with EaOV14.

2.5. Root architecture phenotyping using rhizoboxes

Bespoke rhizoboxes were built to study real-time root development. The rhizoboxes were filled with John Innes no.2 compost, and two coated seeds were sown left of centre for control and on the right for bacteria treated seed. Rhizoboxes were placed at a 45° angle in the glasshouse with the acrylic sheet facing down. Root morphology was quantified via images taken and analysed using Rhizo-Vision Explorer software v2.0.3 [29] which recorded 38 root morphology parameters. The data was analysed in RStudio v3.5.3, where a principal component analysis (PCA) identified clusters for parameters with different values between the treatments. A selection of parameters was then plotted on graphs with the “ggplot2” and “cowplot” packages to visualise the treatment effect.

2.6. Glasshouse experiments

Seeds of OSR variety Ability were coated following the same protocol as for the *in-vitro* experiments and sown in 3L pots filled with John Innes no.3 and MiracleGro Multi-Purpose compost in a 1:1 ratio. The pots were placed in a controlled environment glasshouse at 16 h-day/8 h-night conditions with 20 °C/16 °C temperatures. Plants were watered at two-day intervals or less if required. Pots were laid out in a completely randomised design, and within each experiment, the design consisted of four biological replicates (four starting bacteria cultures) of 15 plants per treatment (H₂O, CSC and EaOV14). The experiment was repeated twice ($n = 120$ plants/treatment).

Data were collected at four (growth stage (GS) 14) and nine (GS19) leaf stages for the stem height from the soil to the tip of the newest fully unfolded leaf. At mid flowering stage (GS65), the stem diameter was recorded using a digital calliper, and the number of inflorescences was counted. At the harvest stage (GS97), the pods were collected and stored in a dry cold room in paper bags before being weighed, threshed and seed collected in paper bags. The final seed analysis was completed with an OPTO-Agri analyzer (OPTO machines, France) to quantify seed biometrics: size, weight, and thousand seed weight.

2.7. Statistical analysis

Overall data analysis for the *in-vitro* and glasshouse experiments was implemented in RStudio v3.5.3 using “ggpubr”, “ggplot2”, “tidyverse”, “rstatix”, and “cowplot” packages [30]. A linear model was built using vigour index values as fixed effects and treatment, species, and variety as random effects. Normality assumptions were checked by analysing the QQ plots of the residuals. The data failed the Shapiro-Wilk test for normal distribution and Levene’s test for homogeneity of variance. As data transformation did not help to meet the assumptions, the treatment effects on plant development were analysed for each variety using the non-parametric Kruskal-Wallis test, followed by a multiple pairwise comparison using the Wilcoxon test. The resulting p-values from the Kruskal-Wallis test were adjusted with the Bonferroni correction.

3. Results

3.1. *In-vitro* plant growth-promoting activity of EaOV14

When applied as a seed treatment, EaOV14 promoted seedling development under *in-vitro* conditions for mono and dicot species. While the germination rate of oilseed rape (*Brassica napus* L.) was insignificant between treatments, the germination rate for variety Anastasia following EaOV14 seed treatment on Day 1 of incubation was 73% ± 8.38 versus 47% ± 19.57 (CSC [coating solution

control]) and $59\% \pm 13.85$ (H₂O) ($p = 0.15$) (Fig. 1). Winter wheat (*Triticum aestivum* L.) variety Lilli showed a 10% increase in germination rate across treatments, with germination rate on Day 7 at $82\% \pm 8.53$ (EaOV14) compared to $75\% \pm 11.78$ (CSC) and $70\% \pm 6.75$ (H₂O) ($p = 0.085$). Wheat var. Rockefeller showed insignificant changes in the germination rate in the presence of EaOV14 ($77\% \pm 4.04$) compared to the H₂O control $73\% \pm 6.33$, but a significantly higher germination rate compared to the CSC control $66\% \pm 5.40$ ($p = 0.031$) on Day 7 post germination (Supplementary Fig. 2).

Following EaOV14 treatment, the vigour index of oilseed rape seedlings increased by up to 40% (Fig. 2) over recorded control treatments. The oilseed rape var. Anastasia showed a median vigour index of 1420 ± 444.07 , significantly higher ($p < 0.0001$) compared to the controls (H₂O 1190 ± 438.70 and CSC 1209 ± 412.79). For the spring oilseed rape var. Ability EaOV14 treated seedlings' median value was 727.16 ± 268.79 ; significantly higher compared to the median of CSC 442.04 ± 216.58 and H₂O 463.16 ± 253.77 ($p < 0.0001$). Similarly, for the winter wheat variety Rockefeller, the EaOV14 seed treatment increased seedling vigour by up to 50% (median vigour index of 1410.33 ± 329.46) compared to the controls ($p < 0.0001$) (Fig. 2). The second winter wheat variety Lilly showed no significant improvement in seedling development compared to the H₂O control but recorded a significant increase compared to the coating solution control (CSC) ($p < 0.0001$) (Fig. 2).

3.2. Root phenotyping

Differential root system architecture (RSA) was observed for oilseed rape variety Ability plants when seeds were pre-treated with EaOV14 compared to the coating solution control (Fig. 3A). The skeletonized image in RhizoVision Explorer software showed a dense root biomass in both top and deep soil levels within the rhizobox (Fig. 3B). Visually, the RSA was shallow and steep in the topsoil, dense in medium soil, and steep at the greater depth for EaOV14 treatment (Fig. 3A and B). Winter wheat showed a higher density of roots in the topsoil when seeds were coated with EaOV14 (Fig. 3C). However, the two treatments showed similar effects on the depth of the roots. Nonetheless, the skeletonized image showed that the EaOV14-treated plants present enlarged roots at more depth (Fig. 3D).

Principal component analysis of the 38 parameters from the image analysis with the RhizoVision Explorer software revealed clusters including the median number of roots, number of root tips, root volume, the average degree of root orientation, width-to-depth ratio, shallow angle frequency, medium angle frequency, and steep angle frequency. The most significant impact of the EaOV14 treatment was observed on the median number of roots, the number of root tips, and root angle orientation. The median number of roots was 55% and 33% higher for oilseed rape and wheat, respectively, in the presence of EaOV14 compared to the control (Fig. 3E). The EaOV14 treatment induced a 2.5-fold increase in the number of roots for oilseed rape and a 1.5-fold increase for wheat compared to the control (Fig. 3F). An increased number of root tips suggests a higher number of root hairs that could benefit crops in drought conditions by increasing the water-holding capacity [31]. Regarding volume, a higher volume of roots, but also higher variability was recorded for the EaOV14 treatment compared to the control for both species (Fig. 3G).

The low average degree of root orientation and steep angle frequency indicate a tendency for deeper roots for both species in the presence of EaOV14 compared to the control (Fig. 3H and I). For both species, but especially for oilseed rape, the shallow angle frequency is higher, indicating a wider coverage of the topsoil (Fig. 3J). The medium angle frequency remained unchanged for oilseed

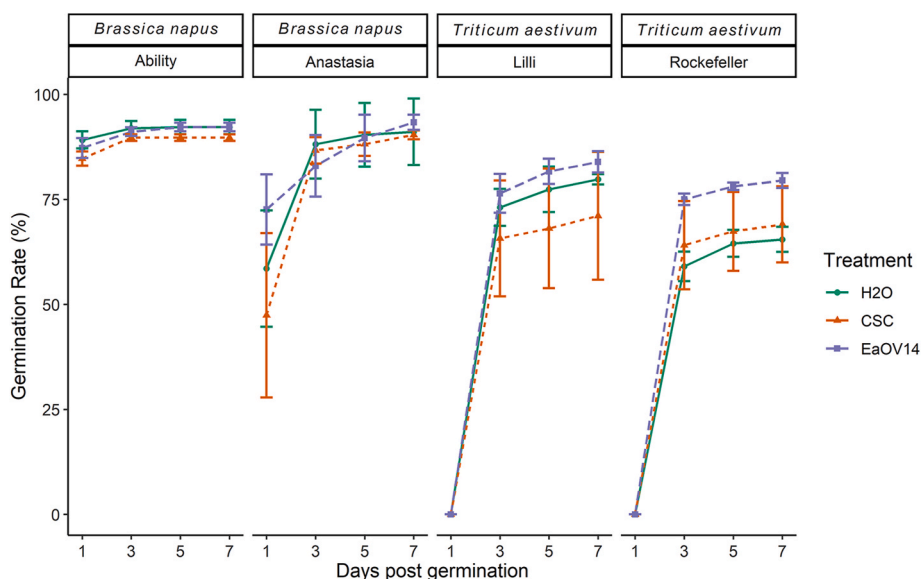


Fig. 1. The effect of EaOV14 treatment (EaOV14, square, purple, long-dashed line) on the germination rate of *Brassica napus* varieties Ability and Anastasia and *Triticum aestivum* varieties Lilli and Rockefeller over seven days compared to the H₂O control (H₂O, circle, green, straight line) and coating solution control (CSC, triangle, orange, short-dashed line). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

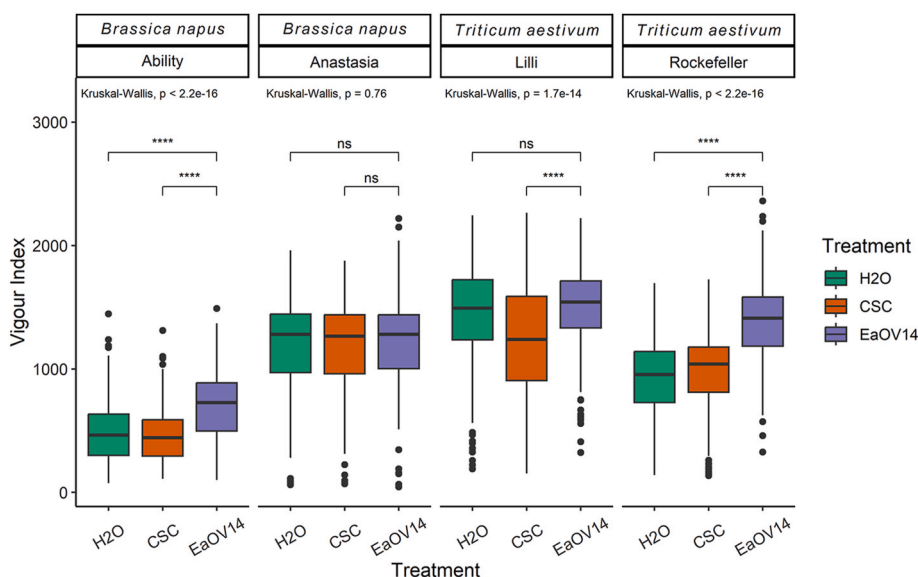


Fig. 2. The effect of EoOV14, water, and coating solution control seed treatment on seedling development of *Brassica napus* varieties Ability and Anastasia and *Triticum aestivum* varieties Lilli and Rockefeller represented as vigour index.

rape; however, it showed a slight increase for wheat (Fig. 3K). The width-to-depth ratio was lower for both species in the presence of EoOV14 compared to the control, suggesting a tendency to promote deeper root systems (Fig. 3L).

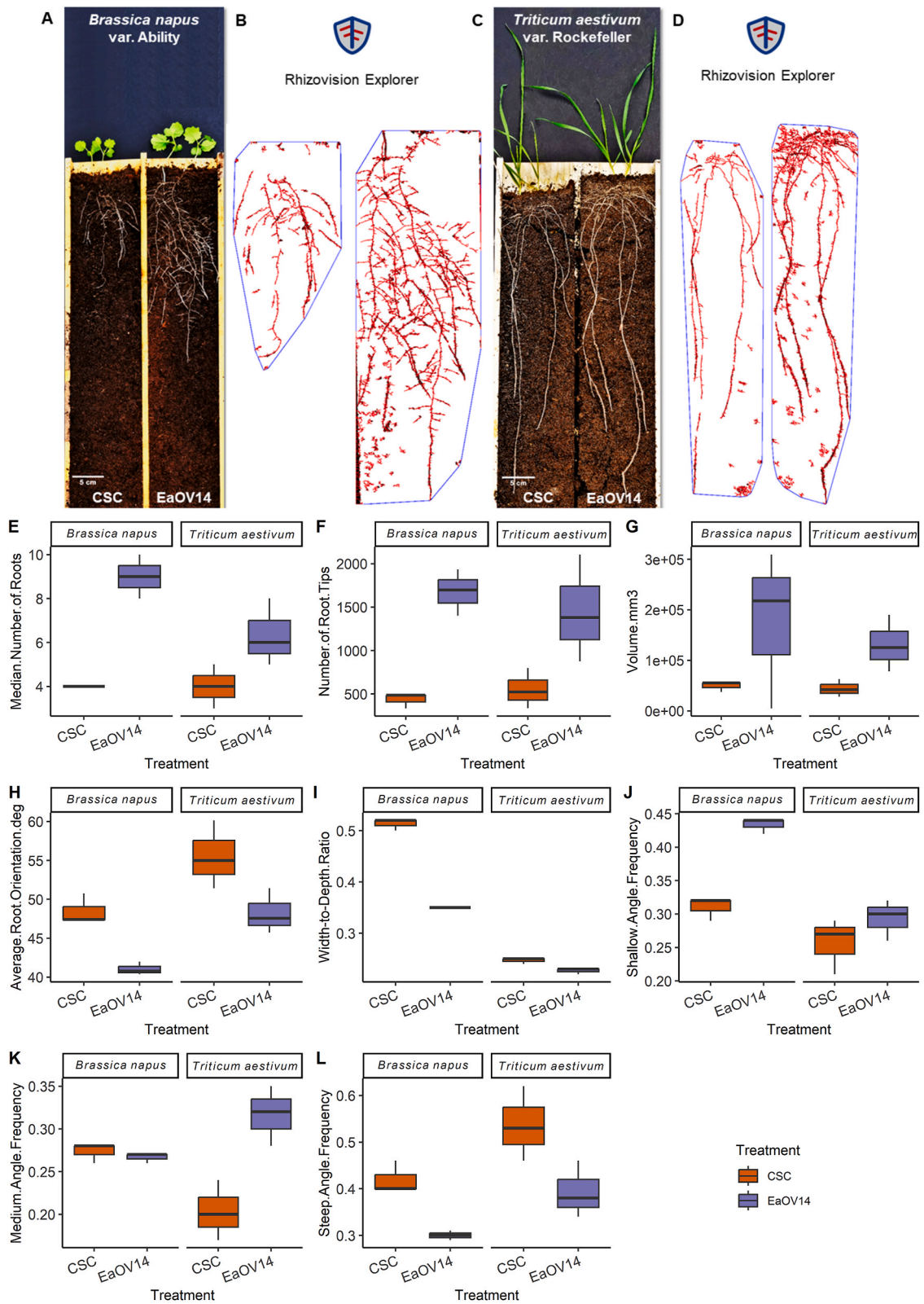
3.3. Glasshouse plant growth-promoting activity of EoOV14

Under glasshouse conditions, the EoOV14 seed treatment showed a positive effect on oilseed rape variety Ability. At the early stages of development (GS14), the height of the plants showed no difference between treatments (EaOV14 15.1 ± 2.8 cm, H₂O 14.7 ± 2.46 cm, CSC 14.4 ± 2.96 cm, $p = 0.21$) (Fig. 4A). At the nine-leaf stage (GS19), a significant difference between EaOV14 (24.3 ± 4.08 cm) and H₂O (22.7 ± 4.33 cm) control was observed ($p < 0.01$, Fig. 4B). At the flowering stage (GS65), EaOV14-treated plants showed a significantly higher number of inflorescences (EaOV14 16.1 ± 3.9 , H₂O 14.5 ± 4.04 , CSC 14.6 ± 3.62 , $p < 0.001$) and stem diameter (EaOV14 18.4 ± 2.88 mm, H₂O 17.3 ± 2.65 mm, CSC 17.5 ± 2.41 mm, $p < 0.01$) compared to the controls (Fig. 4C and D). Finally, at the harvest stage (GS97), significant differences were recorded between the treatments. An insignificant difference was recorded between EaOV14 (229 ± 60.4) and the controls (H₂O 229 ± 59.6 , CSC 210 ± 59.4) treatments in the number of pods per plant ($p = 0.1$; Fig. 4E). The pods' weight per plant was significantly higher for EaOV14-treated plants (25.0 ± 5.41) compared to the controls (H₂O 23.1 ± 5.67 g, CSC 22.5 ± 6.75 g, $p < 0.01$; Fig. 4F). Moreover, the seed weight per plant was significantly higher for EaOV14 treatment (10.8 ± 2.9 g) compared to the controls (H₂O 9.47 ± 3.16 g, $p < 0.001$; CSC 8.95 ± 3.55 g, $p < 0.0001$; Fig. 4G). Nonetheless, an insignificant effect on the thousand seed weight was observed between treatments (EaOV14 3.92 ± 0.74 g, H₂O 4.03 ± 0.75 g, CSC 4.11 ± 0.78 g, $p = 0.27$) (Fig. 4H).

4. Discussion

In the agricultural sector, a new 'green revolution' is required to sustain the continuously growing population [32] and counter the socio-economic challenges the world is facing [33,34]. Identifying and validating a low-input, cost-effective alternative that can maintain or even improve plant yield while enhancing stress resilience is essential [35–38]. An important step towards sustainable crop production was the observation that naturally soil-borne organisms positively influence plant development, entering the plant nutrient cycle and actively enriching nutrient availability, representing a continuous source of nutrients for the developing plant [39–45].

Previous research has looked at the biostimulant potential of *E. adhaerens* under stressful environmental conditions, where it was able to remediate heavy metal-contaminated soil [46] and biodegrade thiamethoxan, a neonicotinoid insecticide [22]. Neonicotinoid insecticides are the most widely seed-applied insecticides and have proven to be harmful to wild bees [47] and in response, the EU limited the use of some compounds from this class, thiamethoxan included [48]. Furthermore, the strain *E. adhaerens* SZMC 25856 isolated from soil improved tomato seedlings' development under *in-vitro* conditions and induced high tolerance to salinity, drought, and heavy metals [49]. There is still a lack of knowledge though on the ability of *E. adhaerens* to extend its beneficial impact into important agricultural crop cultivars. In this regard, the goal of this work was to determine the growth-enhancing potential of the soil-borne bacterium strain *E. adhaerens* OV14 and identify if applied as a seed treatment, whether EaOV14 has the potential to enhance the development of an important mono and dicot crop species.



(caption on next page)

Fig. 3. The effect of EaOV14 on root system architecture as observed for oilseed rape variety Ability on rhizoboxes (A) and skeletonized image (B), and wheat variety Rockefeller on rhizoboxes (C) and skeletonized image (D). EaOV14 impact was recorded for the median number of roots (E), number of root tips (F), root volume (G), average root orientation (H), width-to-depth ratio (I), shallow angle frequency (J), medium angle frequency (K), and steep angle frequency (L).

The findings of this work showed that EaOV14 had a minimal effect on seed germination yet induced a strong positive effect on the seedling vigour index. EaOV14 showed a positive response for both spring and winter OSR varieties studied and displayed varietal dependency in the case of the wheat varieties examined. Genotype dependency for the efficiency of microbial biostimulants was previously observed for wheat [50] and rice [51]. Previous studies on *Pseudomonas* sp. strains [52] or consortium of *Pseudomonas* sp. and other bacteria strains (*Pseudomonas* sp. strain B14, *Sphingobacterium* sp. strain B16, and *Microbacterium* sp. strain B19) [53] isolated from oilseed rape rhizosphere showed enhanced plant biomass under glasshouse-controlled conditions. However, it was observed that the biostimulant effect of rhizobacteria on oilseed rape seed yield under glasshouse conditions is insufficiently studied for optimal growing conditions. This work shows that, under glasshouse conditions, oilseed rape variety Ability plants pre-treated with EaOV14 present higher seed yield compared to the controls (H₂O and CSC), even though the number of pods and thousand seed weight (TSW) were similar across treatments (Fig. 4F–H). These observations suggest that applying EaOV14 as a seed treatment could contribute in part to an integrated nutrient management strategy in support of sustainable agricultural practises.

The glasshouse experiments showed that EaOV14 significantly enhanced the root architecture of the oilseed rape variety Ability and wheat variety Rockefeller. These changes in the root system architecture are similar to the findings of Sakhivel et al. [54] that evaluated the impact of *Bacillus altitudinis* FD48 on rice growth. This study observed that seedlings inoculated with FD48 presented higher number of roots, increased lateral root formation and overall root thickness as response to FD48 modulating the expression of genes in the auxin metabolism pathway (*IAA1*, *IAA4*, *IAA11*, *IAA13*). Other studies also highlighted the positive impact of plant growth-promoting bacteria on root development through modulating hormone production [55–58] and have been linked to drought stress alleviation capabilities [59].

Strong and healthy root systems are indicators of thriving plants [60]. Biologically, roots are divided into two classes: shallow and deep roots. The shallow roots inhabit the topsoil, where they absorb immobile nutrients such as potassium and phosphorous. The deeper roots absorb nutrients available at more depth or the mobile water-soluble nutrients that travel with the water, such as nitrates [61]. In this context, studying how biostimulants modulate root system architecture becomes essential to fully understand the extent of their potential benefit on plant development [62]. The evolution of technology, together with the rise of computer sciences, has led to significant improvements in the root phenotyping field with high-throughput facilities now available in Europe [63]. Moreover, the accessibility to deeply understand dynamics of root architecture has also been improved by the release of free software sources (e.g. RhizoVision Explorer) [29]. Nonetheless, for this work, the difficulty in sourcing accessible phenotyping tools drove the design and building of low-cost rhizoboxes that allowed the study of the changes EaOV14 induces on root systems in real time.

‘Steep, cheap, and deep’ represents the ideal root structure for resilient crops in the current agricultural challenges as it requires minimum energy costs to access nutrients at different levels in the soil [64]. Shallow root angles (steep) and branching represent cost-effective metabolic solutions to facilitate the uptake of topsoil nutrients such as potassium and phosphorous [64–66]. Nonetheless, crops with deeper roots have increased access to nutrients and water, especially when they are available at depth, in scarce conditions such as drought [65]. This work, under controlled conditions, clearly highlights that pre-treatment of seed with *E. adhaerens* OV14 stimulates oilseed rape and wheat to develop root systems following the ‘steep, cheap, and deep’ pattern indicating the stress resilience enhancing potential of *E. adhaerens* OV14.

The primary challenges associated with the use of beneficial soil bacteria include selecting the right inoculation method, ensuring adequate shelf life, and successfully translating laboratory findings to field applications [67]. Studying microbial biostimulants requires particular attention to optimising the inoculation method. Several inoculation methods have been assessed in the last decade: soil application, foliar application, and seed application. The latter represents the most accessible method to ensure uniform and persistent microbial coverage [27, 28, 68, 69]. Moreover, the plant gets access to the beneficial microbe at the early stages of development, while the bacteria gain a head start in the competition for root colonisation [67]. In this work, seed coating was selected as the inoculation method and optimised for wheat and oilseed rape. For oilseed rape, the concentration selected was adapted after Lally et al. (2017) to 100% of bacteria at 0.8 OD₆₀₀ with 10 min of exposure to the inoculum, while for wheat, 4-h exposure to a concentration of 60% of bacteria inoculum showed the highest beneficial impact on seedlings development. This unique approach highlights the importance of optimising delivery methods even for single bacteria strains. With the delivery method selected for this study, the results suggest significantly increased seed yield for oilseed rape plants treated with EaOV14 when grown in controlled environmental conditions (10.8 ± 2.9 g of seed per plant compared to 9.47 ± 3.16 g of seed per plant for H₂O and 8.95 ± 3.55 g of seed per plant for CSC control treatments). Nonetheless, further optimisation would be required to ensure successful field application, especially for winter crops where bacteria might lose efficiency after the vegetative stage during the winter months.

When translated from lab to field, the lack of consistency represents the bottleneck of microbial plant biostimulants. Although the beneficial effect is highly successful under lab and glasshouse conditions, even when non-sterilised compost is used, the effects are often lost under field growing conditions [67]. This happens primarily because of the high variability and competition of the field environment alongside the soil’s physical properties and reduced shelf life of the bacteria. Considering that *E. adhaerens* OV14 is a gram-negative bacterium, its viability on the seed could be compromised by drying processes [70] and shifts in temperature [71]. In this work, the inoculum formulation included 7% ¼ Ringer solution, an osmolarity regulator aiming to protect EaOV14 against desiccation [52]. This approach allowed EaOV14 to induce a higher vigour index of oilseed rape seedlings compared to the control,

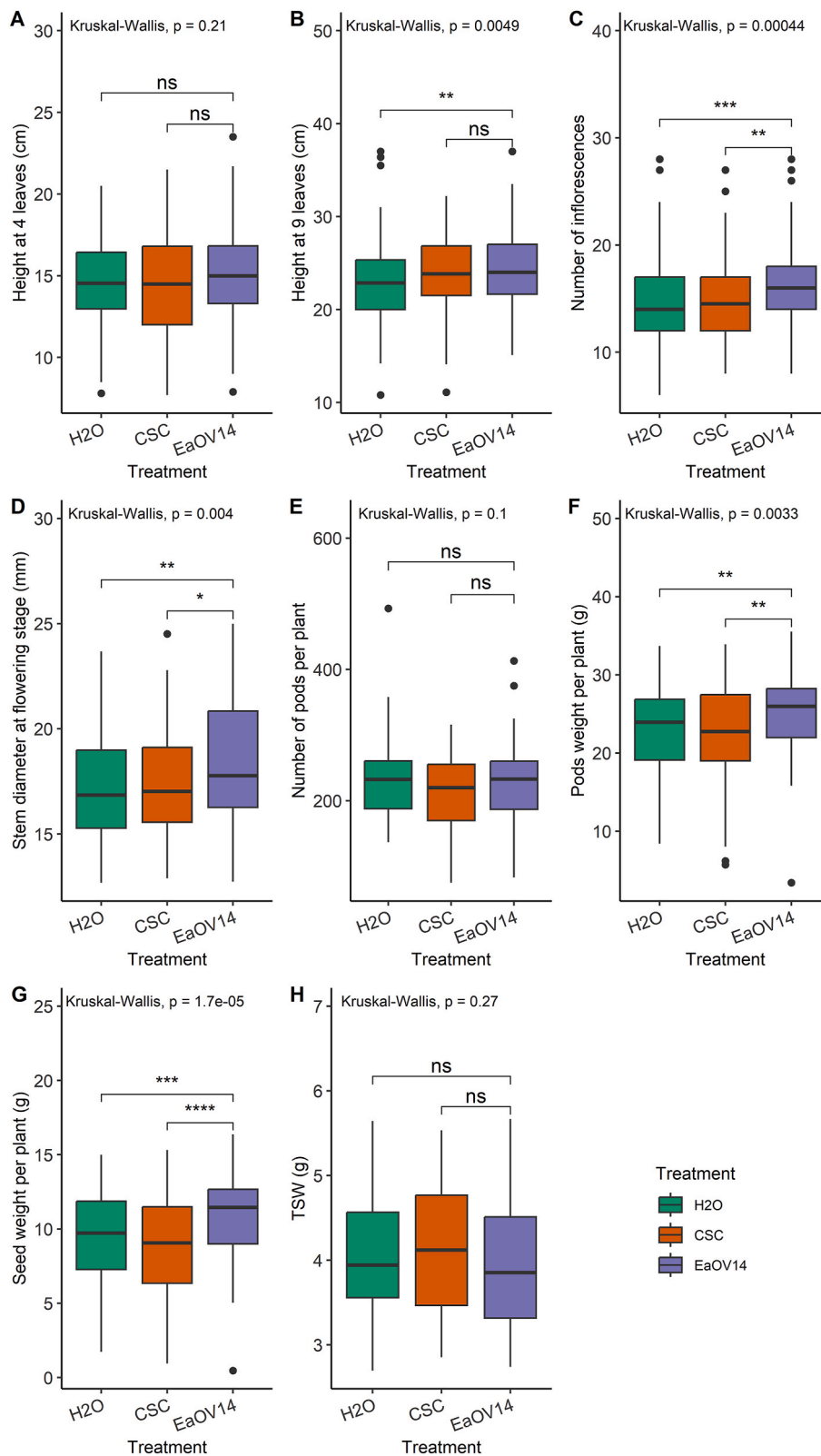


Fig. 4. The influence of EaoV14 seed treatment on oilseed rape var. Ability under glasshouse growing conditions. Differences between treatments were recorded at (A) height at four leaves stage, (B) height at nine leaves stage, (C) number of inflorescences, (D) stem diameter at the flowering stage, (E) number of pods per plant, (F) pods weight per plant, (G) seed weight per plant, and (H) thousand seed weight (TSW).

even after the coated seeds were stored for 14 days at 4 °C (data not shown). Some of the limitations that EaOV14 could face under field conditions could be overcome if a consortium including EaOV14 with other beneficial microorganisms were considered, their synergistic activities inducing beneficial effects on the target crop while maintaining cell viability under field conditions. Recently, Chaparro-Rodríguez et al. [72] showed that hydrogel encapsulation of several gram-negative plant growth-promoting bacteria allowed viability of up to 10⁷ CFU/g capsules for up to three months of storage at 18 °C. Baliyan et al. [73] demonstrated that sugarcane straw ash conserves the viability of the consortium *Ensifer adhaerens* MSN12 and *Bacillus cereus* MEN8 for up to 12 months at ambient temperature. Taken together, it is clear that further work is required to elucidate the optimum approach that maximises the potential impact of *E. adhaerens* OV14 and its role as a biostimulant.

5. Conclusions

It is clear from the literature that integrated approaches are required to support sustainable crop productivity goals [74,75]. In this regard, the goal of this study was to determine the growth-enhancing potential of the soil-borne bacterium strain *E. adhaerens* OV14. The results show that when applied as a seed treatment, EaOV14 has the potential to enhance the development of both an important mono and dicot species. These findings indicate that EaOV14 represents a suitable candidate with the potential to contribute to reducing reliance on chemical fertilisers in line with the very ambitious F2F European goals.

In conclusion, EaOV14 had a minimal impact on seed germination but exhibited a significantly positive effect on the seedling vigour index. EaOV14 showed a positive response for both spring and winter OSR varieties studied and displayed varietal dependency in the case of the wheat varieties examined. This observation highlights the need to test the efficiency of microbial biostimulants on the varieties listed on the annual recommended list and select the optimal species for the varieties recommended for cultivation.

Based on the glasshouse studies, EaOV14 significantly enhanced the root architecture of the oilseed rape variety Ability and wheat variety Rockefeller. Moreover, oilseed rape variety Ability recorded a significant increase in seed yield in response to EaOV14 treatment compared to the controls. Considering that insignificant changes between EaOV14 and controls were observed for the pod number and thousand seed weight (TSW) one can conclude that an increased seed yield is a response to EaOV14 treatment on the pod filling process.

This work highlights the biostimulant potential of EaOV14, with the positive response on plant development recorded through both aerial and root development. While the results are limited to controlled optimal growing conditions (*in-vitro* and glasshouse), the first stage of proof-of-concept is now confirmed. This will serve as a benchmark for follow on studies that seek to investigate the field performance of *E. adhaerens* OV14 as a plant biostimulant.

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Data availability

Data and scripts will be made available on request.

CRediT authorship contribution statement

Elena Grosu: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. **Dheeraj Singh Rathore:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Guiomar Garcia Cabellos:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Anne-Marie Enright:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization. **Ewen Mullins:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e27142>.

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