Regulation of root architecture by Pseudomonas oryzihabitans is mediated by strigolactones and redox processes.

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Abstract

Mechanisms that control of root system architecture are well characterised but little is known about how these processes respond to plant growth promoting rhizobacteria. We therefore studied how the presence of Pseudomonas oryzihabitans PGP01 altered wild type RSA and how these changes were modified in mutants that are defective antioxidant capacity (vtc2-1, vtc2-2, pad2-1, cad2-1 and rax1-1) or strigolactone (SL) synthesis (max3-9 and max4-1) or signalling (max2-3). The presence of P. oryzihabitans PGP01 decreased the length of primary and lateral roots but increased the number of lateral roots and lateral root density in the wild type roots but not in the SL mutants. The presence of synthetic SL, GR24 in combination with P. oryzihabitans PGP01 significantly decreased the number and length of lateral roots in the WT, max3-9 and max4-1 but not max2-3 seedlings. Lateral root density was increased in all genotypes in the presence of bacterium, but this effect was less pronounced in the ascorbate deficient vtc2-1 and vtc2-2 roots and absent from glutathione –deficient (pad2-1, cad2-1 and rax1-1) seedlings. Taken together, these results demonstrate the importance of SL-mediated signalling in root responses to growth promoting rhizobacteria, as well roles of cellular redox controls in these processes.

Pseudomonas oryzihabitans,

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Summary statement

The presence of the plant growth promoting rhizobacterium,*Pseudomonas oryzihabitans* PGP01 alters root system architecture in *Arabidopsis thaliana* in a strigolactone-and glutathione-dependent manner.

Introduction.

Root system architecture (RSA), which is the term used to describe the organization of the primary and lateral roots (and any accessory roots and root hairs on a microscale), is a key determinant of nutrient- and water-use efficiency in plants. Root architecture is defined by the distribution and size of the primary root, adventitious roots, as well as the arrangement of secondary and tertiary roots (Shekhar *et al.* 2019). The first root structure that appears upon germination is the radicle, from which the primary root develops. Taproots are characterized by the dominance of the primary roots after germination and from which secondary lateral roots develop. This class of root system, also known as allorhizic, is found in the model

plant*Arabidopsis thaliana* . In contrast, fibrous or homorhizic root systems are found in monocotyledonous species, where post-embryonic secondary roots that develop adventitious roots, dominate root system architecture after germination. The molecular control of root branching that facilitates the elaboration of root system architecture is essential for the adaptation of the plant to the nutrients and microbiome of the local soil environment. While the regulation of RSA by phytohormones and nutrients is relatively well characterised, much less information is available on how soil microorganisms shape the root system. *A. thaliana* is frequently used as model for studies on root system architecture (Kellermeier *et al.* 2014) largely because of the simplicity of the root system, and the broad range of molecular tools that have been developed for this species (Shekhar*et al.* 2019). However, the genetic networks that control RSA in response to the microbiome remain largely unclear.

The successful detection and uptake of nutrients from the soil depends on the capacity of post-embryonic organogenesis and the plasticity of the primary root (PR). Lateral roots (LR) are formed from xylem pools pericycle (XPP) founder cells on the PR. Eight distinctive stages of LR development have been described. In the early stages (I to IV) XPP cells dedifferentiate and proliferate within the endodermis to form the lateral root primordia (LRP), which continually divide and grow through the cellular layers from the endodermis, emerging only at the latest stages of development (Péret *et al.* 2009). Pre-branch sites in which cells acquire competence to develop LRs are marked by an auxin-dependent signal in the transition zone between proliferating and elongating cells of the PR meristem (Möller *et al.* 2017). Both the initiation and development of LRs are complex processes that are controlled by a range of molecular pathways. For example, reactive oxygen species (ROS) play a crucial role in LR formation (Manzano*et al.* 2014). Genetic manipulation of ROS levels increases the number of pre-branch sites and facilitates the emergence of LR primordia (Orman-Ligeza *et al.* 2016). Other redox-active compounds such as the glutathione (γ-glutamyl-cysteinyl-glycine; GSH), thioredoxins (TRX) and glutaredoxins (GRX) are important in the control of root development. Soil microorganisms, such as plant growth promoting rhizobacteria (PGPR) also modulate root development and RSA. For example, PGPR increase LR density in a salicylic acid (SA)-dependent manner and stimulate root hair (RH) elongation (Poitout *et al.*2017).

Many microorganisms inhabit the rhizosphere and establish communication with the plant roots. It has been estimated that 10^6–10^9 bacteria, and 10^5–10^6 fungi per gram of soil compete for carbon-based metabolites derived from the root (Chuberre*et al.* 2018). However, the effect of microorganisms on the root system depends on the species that establishes communication and interactions with the root. Some interactions are beneficial to the plants and others are harmful. Pathogenic soil microorganisms such as*Fusarium oxysporum* and *Ralstonia solanacearum* cause severe soil borne-diseases in important crop species (Haas & Défago 2005). Soil-borne diseases can be controlled by the microbiome status of the soil in what is known as disease-suppressive soil effects, which largely rely on competition between species of microorganisms for the uptake of plant nutrients from the rhizosphere (Schlatter *et al.* 2017). Conversely, PGPR, which are comprised of different orders of bacterial species, can establish mutualistic interactions with the plants in the rhizosphere, which positively affects the capacity of the root to explore the soil and uptake nutrients (Glick 2012). The mechanisms by which PGPR induce positive changes in the root system remain poorly characterised, but they are considered to rely on either the modification of nutrients sources in the rhizosphere or by direct or indirect effects on phytohormones (Poitout *et al.* 2017). Bacteria such as *Rhizobium* spp. secrete nitrogenases that improve the fixation of nitrogen in anaerobic soils, as well as releasing organic acids to increase plant uptake of phosphorus (Yanni *et al.* 2001). They also induce changes in RSA by altering the levels of ethylene through the synthesis of rhizobitoxine, which inhibits the activity of 1-aminocyclopropane-1-carboxylate synthase and thus decreases the synthesis of ethylene (Yuhashi *et al.* 2000). Some PGPR species such as *Pseudomonas aeruginosa* , *Klebsiella* spp.,*Rhizobium* spp., *Mesorhizobium* spp., also have the capacity to secrete phytohormones that alter RSA, such as the auxin indole-3-acetic acid (IAA), which is a major player in the control of the root system (Ahemad & Kibret 2014).

Low molecular weight antioxidants, such as ascorbate (ASC) and GSH are involved in the regulation of signalling processes, which affect plant growth and development (Kocsy *et al.* 2013). The characterisation of *A. thaliana* mutants that have low levels of GSH and ASC has impacted these antioxidants in the control of

root development. While the GSH-deficient *root meristemless 1* cannot generate a root system, other weak mutants in the *GSH1* gene that accumulate 20-30% wild-type GSH are able to produce roots. For example, the phytoalexin-deficient2 (*pad2-1*), the cadmium-sensitive (*cad2-1*) and *rax1-1* show little impairment of the root system (Cobbett *et al.* 1998; Parisy *et al.* 2007; Schnaubelt *et al.* 2013). The ASC-deficient vitamin C2 (*vtc2*) mutants show altered growth that is linked to changes in the expression of genes encoding proteins involved in phytohormone pathways, particularly auxin, cytokinins, abscisic acid, brassinosterioids, ethylene and salicylic acid (Caviglia *et al.*2018). The *vtc1* mutants contain between 30-60% of the ascorbate present in wild-type plants and it showed enhanced sensitivity to drought stress (Niu *et al.* 2013). The *Gossypium hirsutum*(Gh)VTC1 gene was reported to be a positive regulator of root cell elongation in *A. thaliana* mutants, a process that was under the control of ethylene (Song *et al.* 2019). However, it is not known how ROS and antioxidants such as GSH and ASC influence root responses to PGPR. In this study, the responses of RSA to *P. oryzihabitans*strain PGP01 were compared in the wild type and mutants that are deficient in either strigolactone (SL) synthesis (*More Axillary Growth* {*max*}*3-9* and *max4-1*) or SL signalling (*max2-3*) to test the hypothesis that SL-dependent pathways are important in plant responses to PGPR. SLs play a key role in RSA through the inhibition of branching (Kapulnik *et al.* 2011; Rasmussen *et al.* 2012). They interact with other phytohormone, particularly auxins, to control root morphology (Agusti *et al.* 2011; Ruyter-Spira *et al.*2011; Jong *et al.* 2014). They are also important signalling molecules in plant-microbe interactions attracting arbuscular mycorrhizal fungi or favouring the nodulation between bacteria*Rhizobium* spp. with legumes (López-Ráez *et al.* 2017). Secondly, we compared the root responses of the wild type *A. thaliana* , the ASC-deficient *vtc2-1* and *vtc2-2* mutants and the GSH-deficient *pad2-1, cad2-1 and rax1-1* mutants to*Pseudomonas oryzihabitans* strain PGP01. These studies show that plant responses to PGPR are either decreased or absent from the SL mutants and the ASC-deficient and GSH-deficient mutants relative to the wild type.

Material and methods.

Growth on plates.

Seeds of the *A. thaliana* Columbia-0 (Col-0) wild-type (WT), the SL-deficient mutants (*max2-3* , *max3-9* and *max4-1*), the ASC-deficient (*vtc2-1* and *vtc2-2*) mutants and the GSH-deficient (*pad2-1, cad2-1 and rax1-1*) mutants were surface sterilized for 5 min in 50% bleach, followed by three 5-min-washes with sterilized water. Sterile seeds were then cultured in 10 cm square petri dishes containing half-strength Murashige and Skoog medium ($\frac{1}{2}$ MS, pH 5.7), supplemented with 0.01% myo-inositol, 0.05% MES, 1% sucrose and 1% plant agar. Plates were stored at 4^{o}C in a dark room between 2-4 days to synchronise germination, and then plated vertically in a plant growth cabinet with a 16-h photoperiod and 22^{o}C of temperature during 6 days. In order to test the combined effect of the synthetic SL, GR24, and bacteria, 6-day-old Col-0, *max2-3* , *max3-9* and*max4-1* seedlings were transferred to new plates containing the same medium plus GR24 (2 µM) prior the inoculation with bacteria. For each experiment, at least three plates per treatment and genotype and 6 seeds per plate were used.

Inoculation of bacteria.

Pseudomonas oryzihabitans strain PGP01 was obtained from the IRTA Postharvest Plant Growth Promoter Microorganism (PGPM) Collection (Lleida, Catalonia, Spain). The bacterium were grown in nutrient yeast dextrose agar (NYDA: nutrient broth, 8 g/L; yeast extract, 5 g/L; dextrose, 10 g/L; and agar, 20 g/L) medium for 48 h. The application of the bacterium to Arabidopsis seedlings was conducted following Zamioudis et al. (2013) protocol with some modifications. Bacterial cells were collected from plates in 10 mM $MgSO_4$, and washed by centrifugation at 5000 g during 5 min. After resuspension in 10 mM $MgSO_4$, bacterium concentration was adjusted to 1x10^6 and 1x10^7 CFU/ml by measuring turbidity at 600 nm. Fifty µl of both bacterial inocula were applied at 5-cm of root tip of 6-days-old Arabidopsis Col-0 seedlings to test their effects in root architecture. For experiments involving mutant's seedlings, only 1x10^6 CFU/ml was used.

Root architecture.

After 7 days of co-culture with bacterium, pictures of control and bacteria-treated plates were taken, and

different parameters such as primary root length, number of visible lateral roots and length of lateral roots were measured using ImageJ software. Lateral root density was calculated by dividing the number of lateral roots by the primary root length for each root analysed.

Statistical analysis.

All the experiments were repeated at least three times. Data represent the mean ± standard error (SE) of the mean. Data from the experiments using Col-0 and bacteria were analysed by one-way ANOVA, and the analysis of data from SLs, ASC and GSH mutants experiments was performed by a two-way ANOVA. Statistical significance was judged at the level$P < 0.05$, and the Duncan's *post hoc* test was used for the means separation when the differences were significant using IBM SPSS statistics 25 program.

Results.

The presence of *P. oryzihabitans* PGP01 reduced the length of the WT Arabidopsis primary root by about 36 and 28%, at bacterial concentrations of $1x10^6$ and $1x10^7$CFU/ml respectively, after 7 days of co-culture (Figure 1A, E). Moreover, the seedlings had a significantly higher number of lateral roots at the lower ($1x10^6$ CFU/ml) but not the higher bacterial concentration (Figure 1B, E). The length of the visible lateral roots was not significantly changed in response to the presence of *P. oryzihabitans* PGP01 at either bacterial concentration (Figure 1C). As a consequence of the decreased primary root length and the higher number of lateral roots, lateral root density was increased in the presence of both concentrations ($1x10^6$ and $1x10^7$ CFU/ml) of *P. oryzihabitans* PGP01, the effect being greatest at the $1x10^6$ CFU/ml concentration (100% increase compared to the WT) (Figure 1D). Based on these results, we choose to use the $1x10^6$ CFU/ml concentration in all the following experiments.

Root responses in strigolactone (SL) deficient mutants.

In the absence of bacteria, the *max2-3* , *max3-9* and*max4-1* seedlings had a shorter primary root than the WT seedlings, with decreases of 32, 18 and 42%, respectively (Figure 2A, E). However, the number of lateral roots was not greatly changed in the mutants compared to the WT seedlings (Figure 2B, E). Moreover, no significant differences were detected for the length of lateral roots in absence of bacteria in either the WT or *max2-3* , *max3-9* and*max4-1* seedlings (Figure 2C). Lateral root density was increased by 12, 12 and 25% in these lines respectively, relative to the wild type but these changes were not significant (Figure 2D). While the presence of *P. oryzihabitans* PGP01 decreased the primary root length in the WT, with a higher number of lateral roots and a greater lateral root density after 7 days of co-culture (Figure 2), these bacterium-induced changes were largely absent from the SLs mutants. Only*max3-9* showed a significant 20% reduction in primary root length after the presence of *P. oryzihabitans* PGP01 (Figure 2A).

After 7 days growth in media supplemented with the SL analogue GR24 in combination with *P. oryzihabitans* PGP01, a significant decrease in primary root length was observed in the mutant seedlings relative to the WT (Figure 3A). The presence of GR24 in combination with *P. oryzihabitans* PGP01 significantly decreased the number of lateral roots in the WT, *max3-9* and *max4-1* seedlings but not*max2-3* seedlings (Figure 3B). While the presence of GR24 and*P. oryzihabitans* PGP01 had no consistent effect on the length of lateral roots in the WT and *max2-3* seedlings, the lateral roots were significantly shorter in *max3-9* and *max4-1* seedlings that the WT under these conditions (Figure 3C). Similarly, the lateral root densities of the *max3-9* and *max4-1* seedlings were lower than the other genotypes under these conditions (Figure 3D).

Ascorbate-deficient mutants.

The primary roots were significantly shorter in the *vtc2-1* and*vtc2-2* mutants than the WT in the absence of bacteria (Figure 4A, E). However, the number of lateral roots, the length of lateral roots and lateral root density were similar in all genotypes (Figure 4B, C, D). The number of lateral roots was significantly increased in the wild type seedlings in the presence of *P. oryzihabitans* PGP01 but this effect was less pronounced in the ASC-deficient mutants (Figure 4B). The presence of *P. oryzihabitans* PGP01 significantly reduced the length of the lateral roots in the wild type and the ASC-deficient mutants (Figure 4C). Lateral

root density was increased in all genotypes in the presence of bacteria, but this effect was less pronounced in the*vtc2-1* and *vtc2-2* seedlings than the WT (Figure 4D).

*Glutathione-deficient mutants.*There were no differences in the length of the primary roots in the GSH deficient mutants (*cad2-1* , *pad2-1* and *rax1-1*) and the WT in the absence of the bacterium (Figure 5A, E). However, the length of the primary roots of all genotypes was significantly decreased in the presence of *P. oryzihabitans* PGP01 (Figure 5A). The *cad2-1* , *pad2-1* and *rax1-1* mutants had fewer visible lateral roots than the WT seedlings in the absence of bacterium (Figure 5B, E). While the presence of *P. oryzihabitans* PGP01 led to a significant increase in the number of lateral roots in the WT seedlings, no significant bacterium-induced changes were observed for the GSH deficient mutants (Figure 5B). The length of lateral roots was significantly decreased in the WT seedlings in the presence of *P. oryzihabitans* PGP01 but not in the GSH-deficient mutants (Figure 5C). Only the *pad2-1* mutants had a lower lateral root density than the WT in the absence of*P. oryzihabitans* PGP01 (Figure 5D). Lateral root density was significantly increased in all the genotypes in the presence of bacteria. However, while, this increase was about 83% in the WT compared to seedlings grown in the absence of bacteria, the bacteria-induced effects on this parameter were less pronounced in the GSH-deficient mutants (Figure 5D).

Discussion.

SLs regulate many important processes in plants including shoot branching, root growth, secondary growth and tolerance to cold and drought stresses (Foo & Reid 2013; Quain *et al.* 2014; Jong*et al.* 2014; Cooper *et al.* 2018). They are also important regulators of the *Rhizobium* -legume symbiosis and other rhizosphere plant–microbe interactions, such as the symbiosis with arbuscular mycorrhizal fungi, particularly when nutrients such as phosphorus and nitrogen are limiting (Aliche *et al.* 2020). The synthetic SL analogue GR24 stimulating swarming motility of the alfalfa symbiont *Sinorhizobium meliloti* (Pelaez-Vico *et al.,*2020). The data presented here show that SL are crucial for the appropriate responses of roots to the plant growth promoting rhizobacterium *P. oryzihabitans* PGP01. The presence of this bacterium resulted in a strong response of the WT *A. thaliana*root system, leading to a decrease in primary root length and an increased lateral root density, changes that were absent from the SL mutants.

Previous studies have shown that the inoculation of nectarine (*Prunus persica* cv. Nectarina) embryos with *P. oryzihabitans* PGP01 induced root modifications that promote plant growth under greenhouse conditions (Cantabella *et al.* 2020). The effects of bacteria belonging to *Pseudomonas* genus have been previously studied in *A. thaliana* . For example, a strain of*P. fluorescens* was able to promote the growth of *A. thaliana* seedlings and increased the number of lateral roots (Zamioudis*et al.* 2013). A similar effect was observed by Ortiz-Castro*et al.* (2020) using *P. fluorescens* and *P. putida* , although the influence of *P. oryzihabitans* in *A. thaliana*root system has not been previously studied. The data presented here clearly implicate SL in *A. thaliana* root responses to *P. oryzihabitans* PGP01. The PGPR *P. simiae* WCS417r was found to stimulate lateral root formation in *A. thaliana* , a response that was partly regulated by the production of volatile organic compounds by the bacterium. Although nearly all Arabidopsis accessions examined were found to respond positively to WCS417r, there was a large variation between accessions in terms of the extra number of lateral roots formed, and the effect on primary root length (Wintermans *et al.* 2016). Cyclodipeptides produced by *P. putida* and *P. fluorescens* cultures were shown to stimulate lateral root and root hair formation through the induction of auxin responses (Ortiz-Castro*et al.* 2020). Since auxins and SLs closely interact in the regulation of RSA, it is root responses to *P. oryzihabitans* are absent from mutants that are defective in either SL synthesis or signalling. The changes in the root systems of the SL mutants relative to wild type *A. thaliana* seedlings in the absence of bacteria are consistent with previous reports (Ruyter-Spira *et al.* 2011). Moreover, the application of GR24 supressed the lateral root primordia in the WT decreasing to the number of lateral roots and lateral root density, as reported previously (Ruyter-Spira *et al.* 2011). In this study, roots of all genotypes growing in the presence of GR24 together with *P. oryzihabitans* PGP01 show a decrease in primary root length but there was an increase in lateral root density only in the wild type and not any of the lines that are defective in SL synthesis and signalling. These data are surprising because it would be predicted that GR24 would recover the WT phenotype in the *max3-9*and *max4-1* seedlings.

We have previously shown a link between SL and GSH signalling that was dependent on MAX2 in *A. thaliana* (Marquez-Garcia *et al.*2014). Redox processes are also important in the control of root growth and development (Kocsy *et al.* 2013) but little attention has been paid thus far to how ROS-processing molecules such as ASC and GSH contribute to plant responses to PGPR. The effects of *P. oryzihabitans* PGP01 on lateral root density were less pronounced in the*vtc2-1* and *vtc2-2* mutants than the wild type. Moreover, the number of lateral roots was significantly increased but the length of the lateral roots was decreased in the wild type seedlings in the presence of *P. oryzihabitans* PGP01 but not in the *cad2-1* ,*pad2-1* and *rax1-1* mutants. Taken together, these results suggest that an appropriate cellular redox balance is required to promote appropriate root responses to the bacterium. This result is perhaps not surprising given that mutants lacking glutathione peroxidases (GPX) have an altered root phenotype (Passaia *et al.*2014) and glutaredoxins such as GRXS8 are considered to be major regulators of RSA (Ehrary *et al.* 2020). Moreover, GSH is required for indole butyric acid (IBA) conversion to indole acetic acid (IAA), suggesting an important role for GSH-dependent regulation of the auxin pathway in root development (Trujillo-Hernandez *et al.* 2020).

Taken together, these data show that SL and GSH are key components in the *A. thaliana* root response to *P. oryzihabitans* PGP01 to*A. thaliana* . They confirm that the pathways of signalling by SL, auxin and GSH are interlinked in the regulation of root architecture in response to rhizosphere signals. It is tempting to suggest therefore that a bacterium-induced oxidative burst in the plasma membrane of root cells is a primary signal of recognition by the plant that redox changes thereafter trigger phytohormone pathways that mediate appropriate responses in root architecture.

Author contributions

Daniel Cantabella, Christine H. Foyer, Ramon Dolcet and Neus Teixidó developed the project; Daniel Cantabella, Christine H. Foyer and Barbara Karpinska designed and performed experiments; Daniel Cantabella and Barbara Karpinska measured root parameters; Daniel Cantabella and Christine H. Foyer analysed data; Daniel Cantabella and Christine H. Foyer wrote the article, and all other authors read and contributed to previous versions and approved the final version.

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FIGURE LEGENDS

Figure 1.- Effect of the application of two different doses of*P. oryzihabitans* PGP01 (1x10^6 and 1x10^7 CFU/ml) on primary root length (A), number of lateral roots (B), length of lateral roots (C) and lateral root density (D) of *A. thaliana* Col-0. 6-days-old seedlings were inoculated with this bacterium at 5 cm of the root tip, and the measurements of the different parameters were measured 7 days after the inoculation. In order to support the results, representative pictures of the seedlings (E) are shown. Data shown mean ± standard error (SE) of the mean of three independent biological replications. In each graph, different letters indicate significant differences ($P < 0.05$; analysis of variance and Duncan's *post hoc* test).

Figure 2.- Effect of the application of *P. oryzihabitans*PGP01 on primary root length (A), number of lateral roots (B), length of lateral roots (C) and lateral root density (D) of mutants that are defective in SLs (*max2-3*, *max3-9* and *max4-1*).6-days-old seedlings were inoculated with this bacterium at 5 cm of the root tip, and the measurements of the different parameters were measured 7 days after the inoculation. In order to support the results, representative pictures of the seedlings (E) are shown. Data shown mean ± standard error (SE) of the mean of three independent biological replications. In each graph, different letters indicate significant differences ($P < 0.05$; analysis of variance and Duncan's *post hoc* test).

Φιγυρε 3.- Εφφεςτ οφ τηε αππλιςατιον οφ τηε σψντηετις ΣΛς ΓΡ24 2 μΜ ανδ τηε βαςτεριυμ *Π. ορψζιηαβιτανς* ΠΓΠ01 ον πριμαρψ ροοτ λενγτη (Α), νυμβερ οφ λατεραλ ροοτς (Β), λενγτη οφ λατεραλ ροοτς (˚) ανδ λατεραλ ροοτ δενσιτψ (Δ) οφ μυταντς τηατ αρε δεφεςτιε ιν ΣΛς (*μαξ2-3*, *μαξ3-9* ανδ *μαξ4-1*). 6-days-old seedlings were inoculated with this bacterium at 5 cm of the root tip, and the measurements of the different parameters were measured 7 days after the inoculation. In order to support the results, representative pictures of the seedlings (E) are shown. Data shown mean ± standard error (SE) of the mean of three independent biological replications. In each graph, different letters within the same treatment (Control, GR24 2 μM and GR24 2 μM + PGP01) indicate significant differences ($P < 0.05$; analysis of variance and Duncan's *post hoc* test).

Figure 4.- Effect of the application of *P. oryzihabitans*PGP01 on primary root length (A), number of lateral roots (B), length of lateral roots (C) and lateral root density (D) of mutants that are defective in reduced ASC (*vtc2-1* and *vtc2-2*). 6-days-old seedlings were inoculated with this bacterium at 5 cm of the root tip, and the measurements of the different parameters were measured 7 days after the inoculation. In order to support the results, representative pictures of the seedlings (E) are shown. Data shown mean ± standard error (SE) of the mean of three independent biological replications. In each graph, different letters indicate significant differences ($P< 0.05$; analysis of variance and Duncan's *post hoc*test).

Figure 5.- Effect of the application of *P. oryzihabitans*PGP01 on primary root length (A), number of lateral roots (B), length of lateral roots (C) and lateral root density (D) of mutants that are defective in reduced glutathione (*cad2-1, pad2-1 and rax1-1*).6-days-old seedlings were inoculated with this bacterium at 5 cm of the root tip, and the measurements of the different parameters were measured 7 days after the inoculation. In order to support the results, representative pictures of the seedlings (E) are shown. Data shown mean ± standard error (SE) of the mean of three independent biological replications. In each graph, different letters indicate significant differences ($P < 0.05$; analysis of variance and Duncan's *post hoc* test).