EcfE, a master regulator of pea root attachment and colonization of *Rhizobium leguminosarum* bv *viciae* 3841

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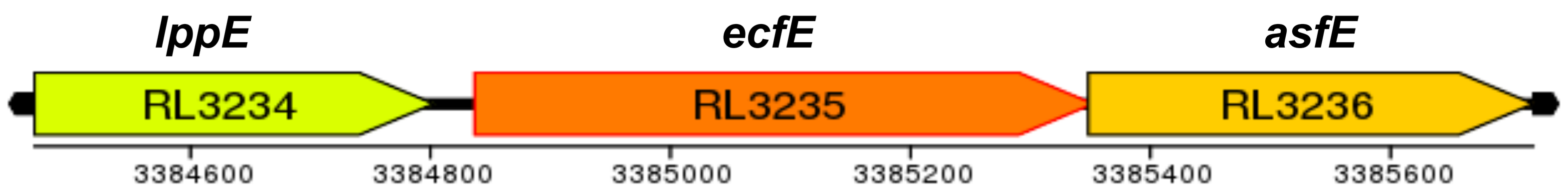
Background

- Legumes tightly regulate attachment and colonization by very specific time-dependent signals and the ability to perceive and respond to such signals by rhizobia is a requirement for successful colonization (1).
- Rhizobia respond to host signals by activating a subset of genes, directed by extracytoplasmic function sigma factors (ECF). We found the *lppE, ecfE, asfE* operon to be up-regulated in the rhizosphere (Panel A)
- Root attachment and colonization requires an orchestrated transcription of a number of genes encoding polysaccharides (exo-, lipopolysaccharides and glucomannan), extracellular proteins (rhicadesin and rhizobium adhering proteins), celluose fibrils and secretion systems (2).
- We used Lux and fluorescence reporter marked bacteria to follow attachment to roots and colonization.

A. *lppE* operon



lppE **– lipoprotein**

ecfE **– extracytoplasmic sigma factor E**

asfE **– anti sigma factor E**

Comparative rhizosphere transcriptomics (3)

Growth conditions	fold up Vs Glucose - lab culture		
	RL3234 *lppE*	RL3235 *ecfE*	RL3236 *asfE*
pea rhizosphere	118	3	2
alfalfa rhizosphere	29	3	2
sugar beet rhizosphere	29	2	2
lab culture (+ Phenylalanine)	7	8	6

Results

1. Spatial induction of lppE promoter on pea root elongation zone (REZ)

Lux promoter fusions were used to study the spatial activation of *lppE* operon. Fig 1, shows the colonization of Rlv3841 marked with constitutive promoter demonstrating rhizobial root colonization. Fig 2, shows a very specific spatial induction of *lppE* promoter on the root elongation zone (REZ). (Below) Induction was also observed over time (Figs 3 - 5). The lux reporter, growth of pea plant and NightOWL lux imaging was performed as described in Pini *et al*., 2017.

Fig 1: constitutive

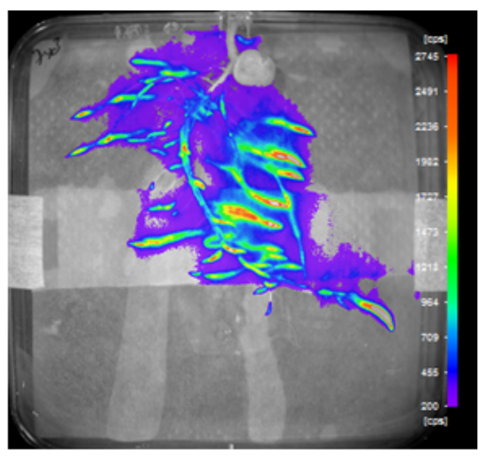
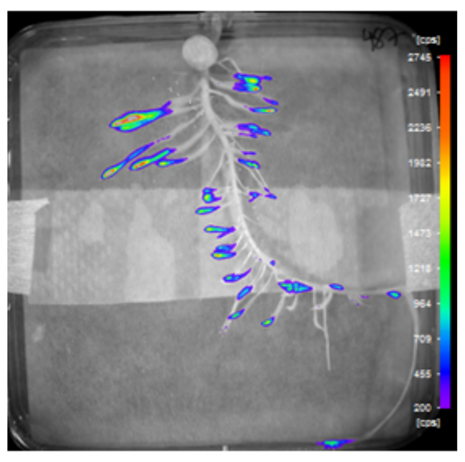
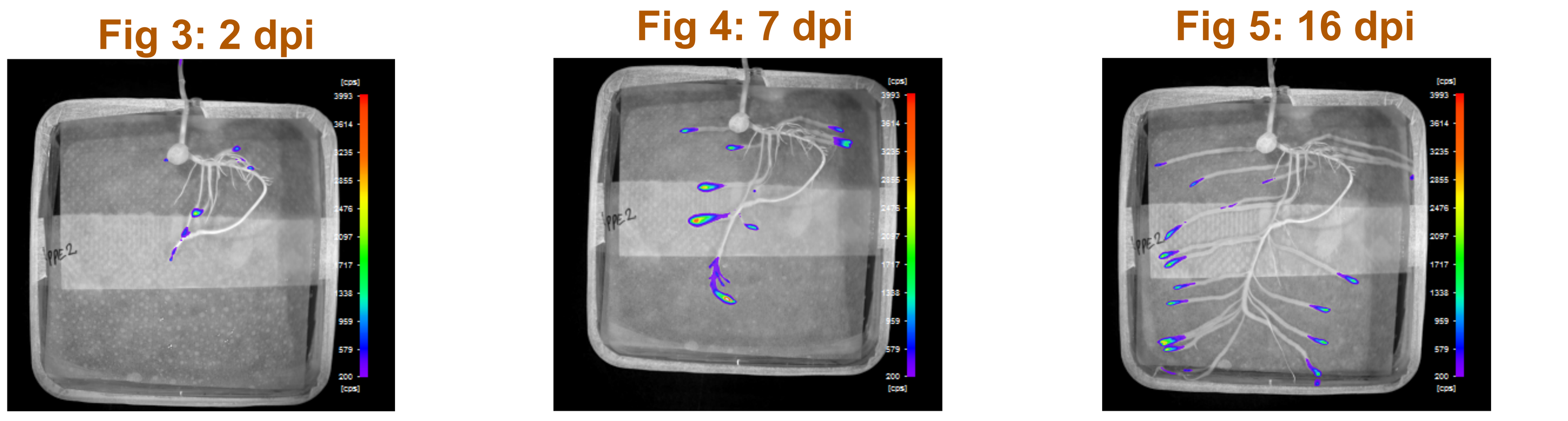


Fig2: *lppE*

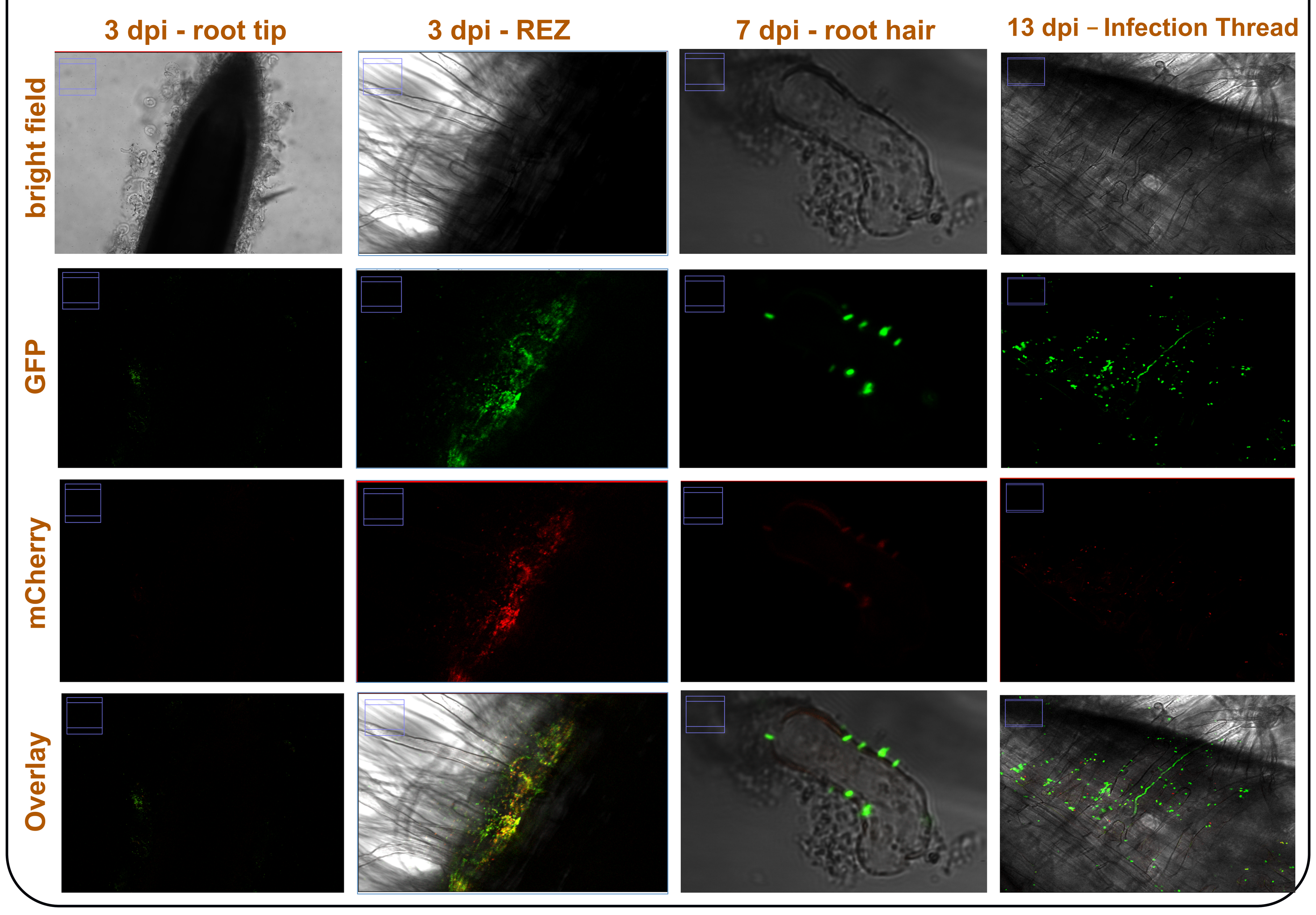


Induction of lppE promoter on pea root elongation zone (REZ) over time

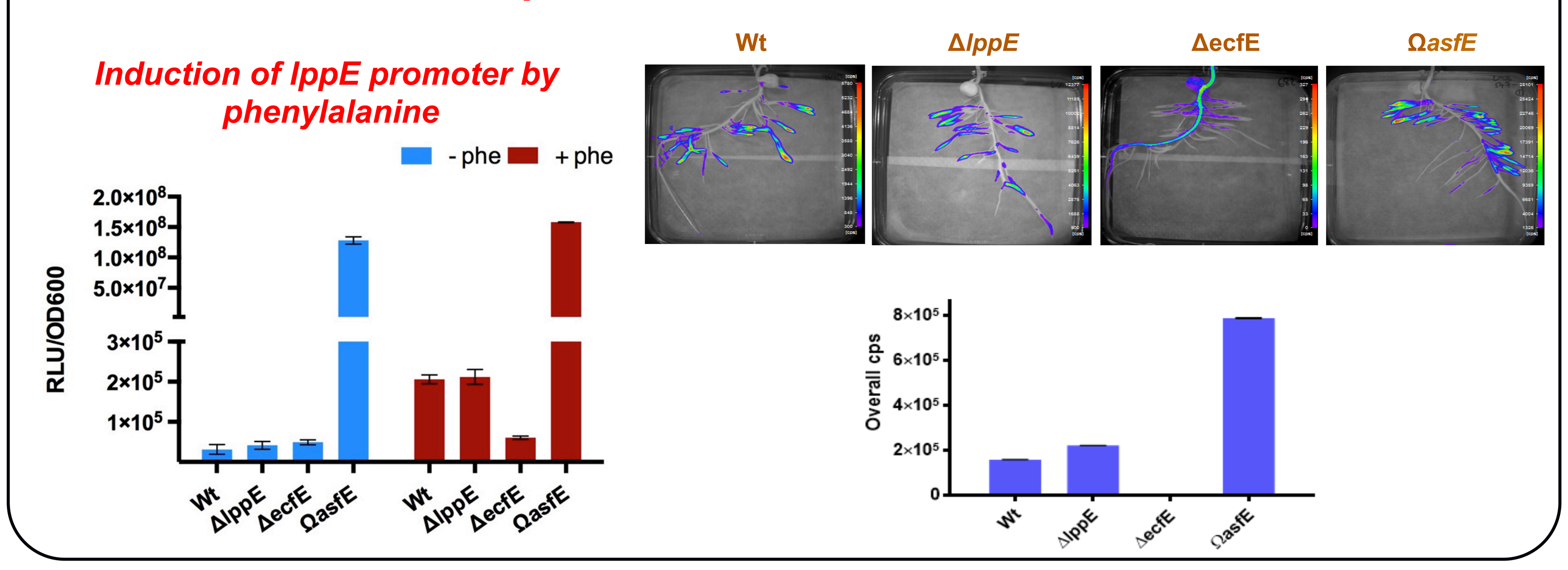


Confocal microscopy of rhizobial colonization of pea roots (dual reporter)

To view the spatial induction on a higher resolution, a dual (fluorescence) reporter was constructed. Dual reporter has a constitutive promoter (pTac) driving GFP and plppE promoter driving mCherry. Confocal images taken at different days post inoculation (dpi) time points demonstrates that *lppE* operon is switched on only in the root elongation zone (REZ) and switches off as the rhizobia progress to the next stage in the symbiosis

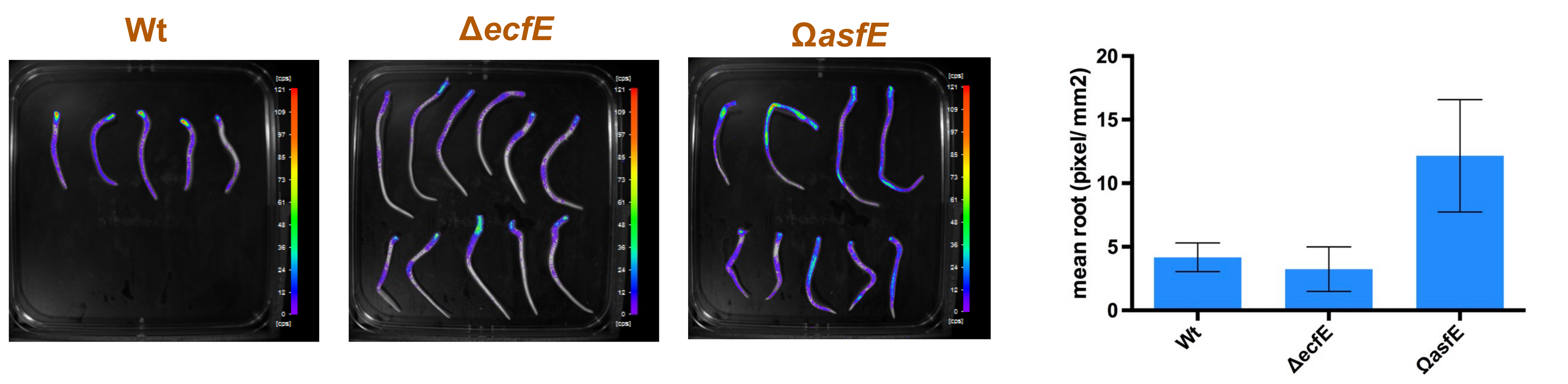


2. EcfE, activates lppE operon in response to phenylalanine or plant- derived metabolite



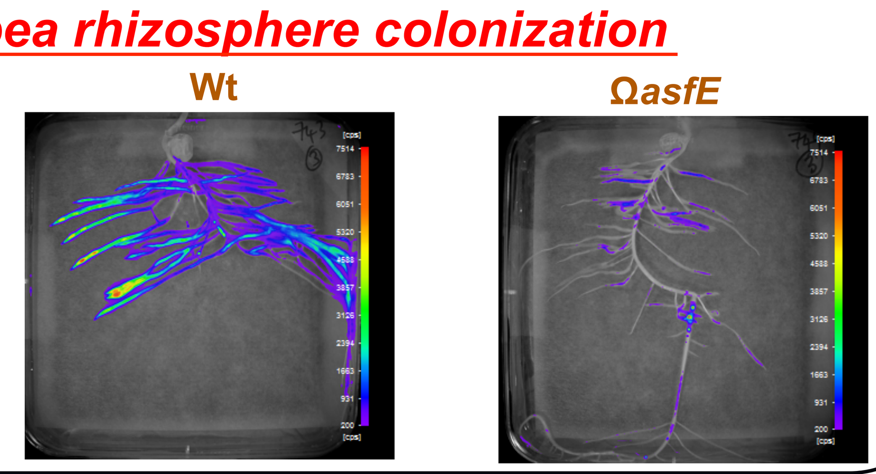
3. ΔecfE mutant is attenuated in attachment and shows spatial bias

Pea root attachment assay (1 hr) were performed with Wt, *ecfE and asfE* mutants harboring constitutive lux reporters. The data showed that, Δ*ecfE* mutant is slightly compromised in overall attachment but showed a strong reduction in attachment to the REZ. However, an Ω*asfE* mutant showed higher attachment compared to Wt.

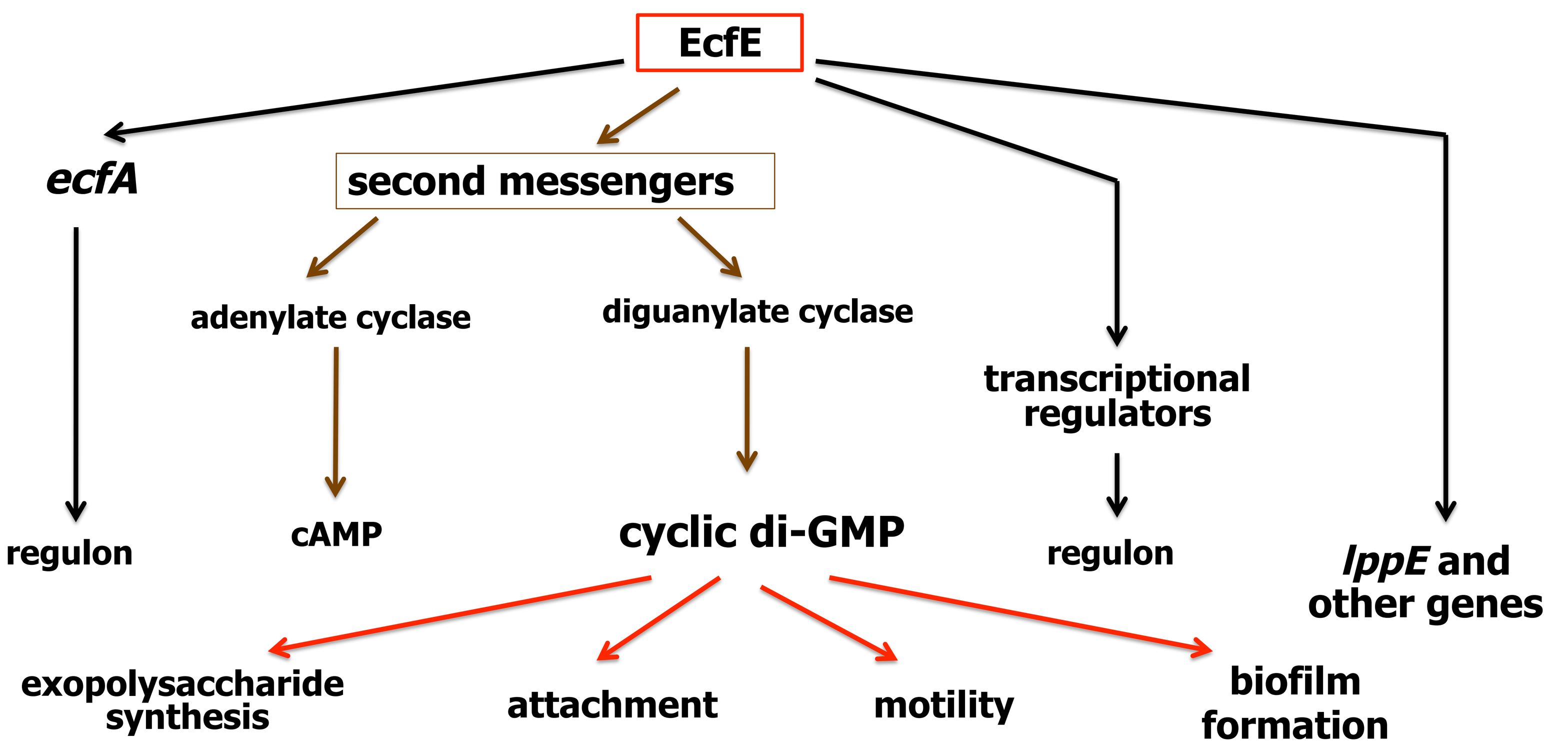


4. ΩasfE mutant compromised for pea rhizosphere colonization

Pea root colonization assay (7d pea seedling with 7 dpi) were performed with Wt, *ecfE and asfE* mutants harboring constitutive lux reporters. The data showed that, Ω*asfE* mutant is highly compromised in root colonization compared to wildtype. However, an Δ*ecfE* mutant colonized as Wt.

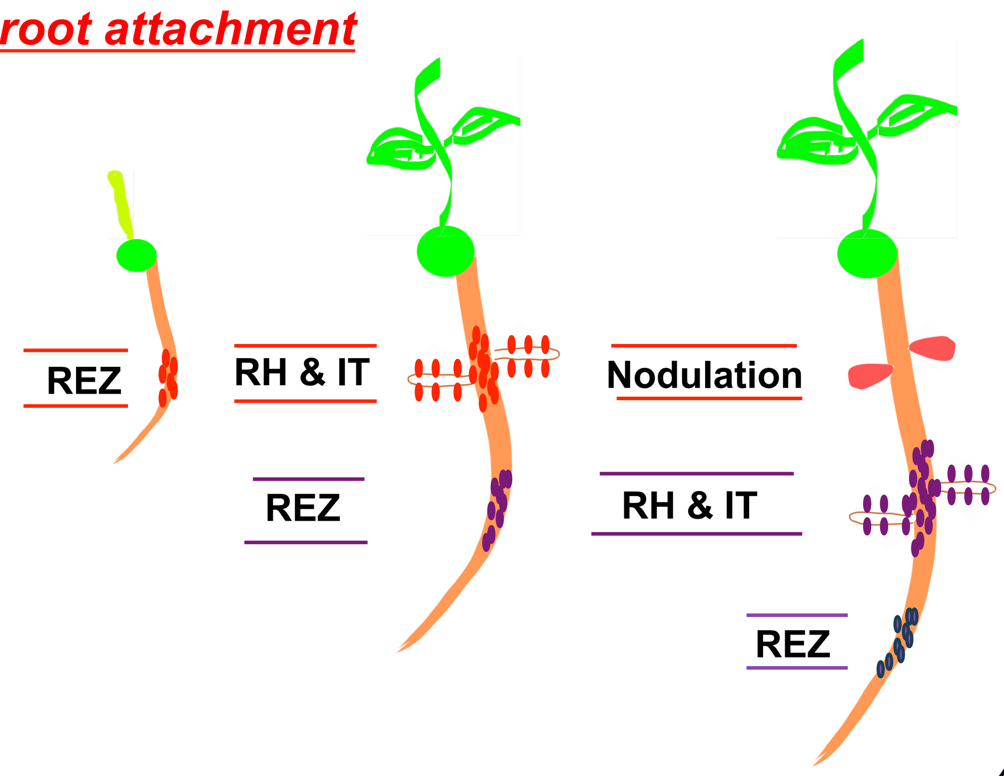


5. EcfE, a master regulator of attachment on pea roots



6. Model of EcfE mediated primary root attachment

Based on this data, we hypothesis that *lppE* operon mediates primary attachment to the REZ of a pea root. When rhizobia encounters REZ, a very specific signal from the REZ activates EcfE regulon, which aid rhizobial attachment to REZ. As the root grows, the REZ matures and becomes the part of matured root, which stops the secretion of metabolite. Thus attached rhizobia, spread and colonize root and root hairs, to enter into infection threads and initiate nodulation.



Summary

- EcfE is the master switch controlling rhizobial pea root attachment, in response to a pea root elongation zone derived signal.
- Temporal switching off of EcfE is critical, to enable rhizobia to spread and colonize, rather than just attaching to roots.

References

1. G. Oldroyd *et al*., (2011) *Annual Review of Genetics* 45:119-144.
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3. V.K. Ramachandran *et al*., (2011) *Genome Biology* 2011, **12**:R106.
4. Pini et al., (2017) Plant Physiology