The plant defence signal galactinol is used as a resource of high specificity by the pathogen *Agrobacterium fabrum*

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Running title: Galactinol and melibiose bound to MelB

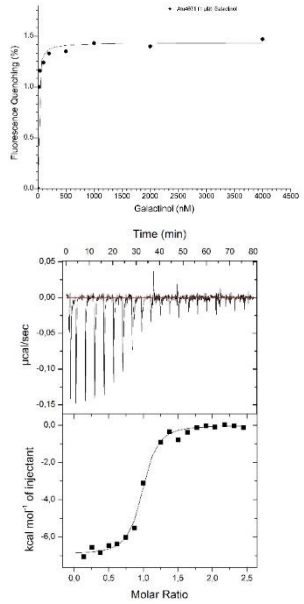
Supporting Information: Figs. S1-S2 and Tables S1-S2

Fig. S1. ITC and fluorescence K_D measurements of MelB towards RFOs and their derivatives. The top graphs show fluorescence monitoring of MelB upon titration with each ligand and fit (solid line) to a single binding model using Origin 7. Measures were done in triplicate. The lower graphs correspond to microcalorimetry experiments. The top panel shows heat differences upon injection of ligand and low panel shows integrated heats of injection with the best fit (solid line) to a single binding model using Microcal ORIGIN.

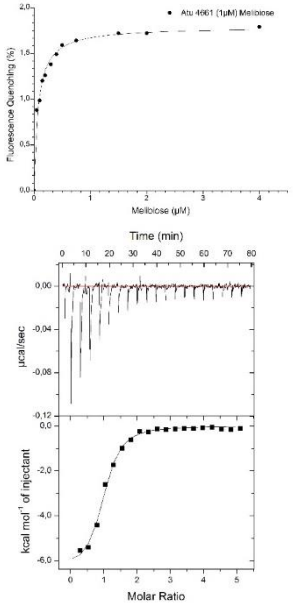
Fig. S2. DSC thermograms of MelB

Figure S1

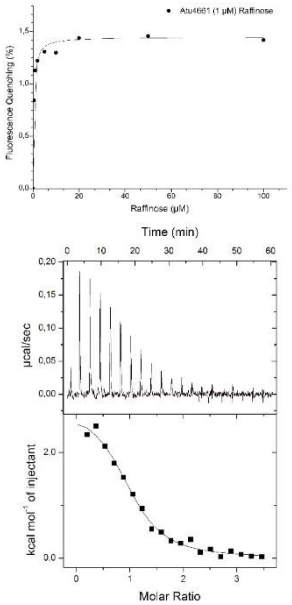
MelB-galactinol



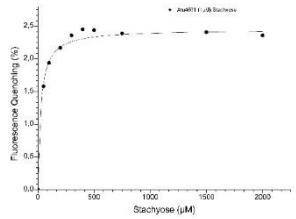
MelB-melibiose



MelB-raffinose



MelB-stachyose



Not determined by ITC

MelB-galactose

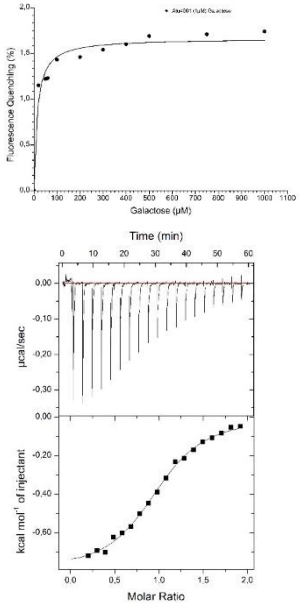
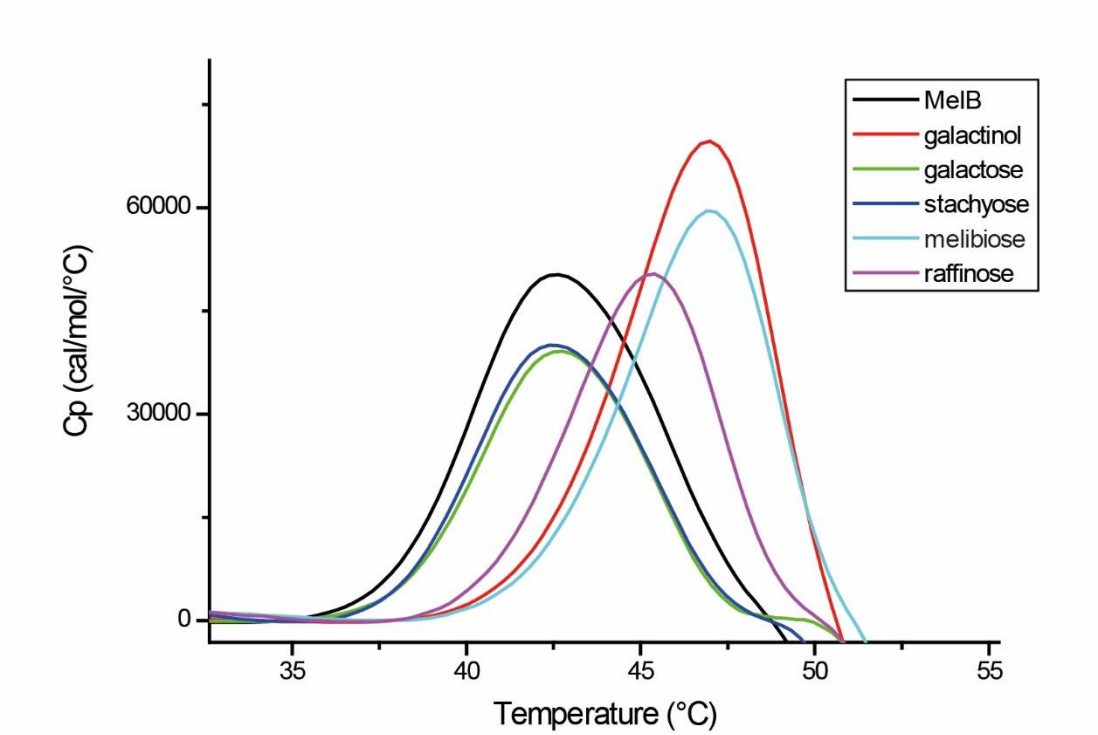


Figure S2



	Tm (°C)	**ΔH (Kcal/mol/°C)**
MelB	43.5	323
+ galactinol	46.5	362
+ galactose	43.3	225
+ stachyose	43.2	236
+ melibiose	46.6	314
+ raffinose	45.1	265

Table S1. Strains and plasmids used in this study

Strains	Relevant genotype and description	Reference or source
Escherichia coli		
JM109	*endA1 glnV44 thi-1 relA1 gyrA96 recA1 mcrB+ Δ(lac-proAB) e14- [F' traD36 proAB+ lacIq lacZΔM15] hsdR17(rK-mK+)*	NEB catalog
BL21	F$^-$ *ompT gal dcm lon hsdS*$_B$($r_B^- m_B^-$) [*malB*$^+$]$_{K\text{-}12}$(λ^S)	NEB catalog
Stellar	*F–, endA1, supE44, thi-1, recA1, relA1, gyrA96, phoA, Φ80d lacZΔ M15, Δ (lacZYA - argF) U169, Δ (mrr - hsdRMS - mcrBC), ΔmcrA, λ–*	Takara Clontech
Agrobacterium fabrum		
C58	Wild-type	CFBP 1903
C58Δ*melB (atu4661)*	C58 deleted of *melB* (*atu4661)* gene	This study
C58 pTi*atu6148*:Km	C58 disrupted for *atu6148* gene ; Km^R	(1)
Plasmids		
pJQ200SK	Suicide vector; P15A sacB; Gm^R	(2)
pOT1e	Promoter-probe vector based on pBBR1MCS-5 replicon; contains promoterless eGFP and MCS between two transcriptional terminators; Gm^R	(3)
pOT1eM	pOT1e derivative harboring *Ptac-m-cherry* inserted in ClaI-SalI site	(4)
pET-9(a)	*E. coli* expression vector (N-term T7-tag fusion), Km^R	Novagen
pOT1e-P*mel*	Upstream region *atu4660-atu4665* inserted in ClaI-SalI site in pOT1e; Gm^R	This study
pOT1eM-P*mel*	Upstream region *atu4660-atu4665* inserted in SpeI site in pOT1eM; Gm^R	This study
pET-9(a)-*melB*	*melB* inserted in NdeI-BamHI site in pET-29(a) (N-term T7-tag fusion), Km^R	This study

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4. Meyer, T., Renoud, S., Vigouroux, A., Miomandre, A., Gaillard, V., Kerzaon, I., Prigent-Combaret, C., Comte, G., Moréra, S., Vial, L., and Lavire, C. (2018) Regulation of hydroxycinnamic acid degradation drives *Agrobacterium fabrum* lifestyles. *Mol. Plant-Microbe Interact. MPMI*. 10.1094/MPMI-10-17-0236-R

Table S2. Primers used in this study

Primer Target	Primer name	Primer sequence (5'-3')	Reference
Transcriptional fusion			
mel promoter insertion into pOT1e	P*mel*F	ATCGATACCACATAATGGCGGTTCTC	This study
	P*mel*R	CGAGATTGTGCTGGCTGATA	
mel promoter insertion into pOT1eM	P*mel*F'	CGGGGGATCCACTAGACCACATAATGGCGGTTCTC	This study
	P*mel*R'	TTCTTCCTCCACTAGCGAGATTGTGCTGGCTGATA	
Deletion of melB			
melB flanking upstream region	Up*melB*F	GAATTCCTGCAGCCCCAACCACCAGACATGGTTC	This study
	Up*melB*R	GGATATGCTTCAGTTTTGCATCGTCATTCCTCCC	
melB flanking downstream region	Dw*melB*F	AACTGAAGCATATCCGGGA	This study
	Dw*melB*R	ACTAGTGGATCCCCCTCTCGCCACTCAGCATGT	