Materials and Methods

Bacterial strains, plasmids and culture conditions. The bacterial strains and plasmids used in this study are detailed in Table S1. *Rhizobium* strains were grown at 28°C on either tryptone yeast extract (TY) (1), or Universal Minimal Salts (UMS) which is modified from previously described AMS (2) as follows; EDTA Na_2 1μM, $CoCl_2.6H_2O$ 4 μM, $CaCl_2.2H_2O$ 510 μM and $FeSO_4.7H_2O$ 40 μM. Antibiotics were used at the following concentrations (μg ml^{-1}): streptomycin, 500 (Rlv3841); chloramphenicol 10 (ATCC14479); kanamycin, 20 (*E. coli*); tetracycline 10 (*E. coli*) or 2 (LMB134 and LMB136); spectinomycin 50; carbenicillin 50, gentamicin 10, neomycin 80 .

Mutation of *matA, matB and matC*. Primers p1149/p1150 and p1152/p1153 (all primers listed in Table S2) were used to amplify internal gene fragments from *matA* (RL0990) and *matC* (RL0992) respectively from Rlv3841. The products were cloned into pK19mob, using HindIII sites incorporated into the primers forming pRU2027 and pRU2028 respectively. Plasmids pRU2027 and pRU2028 were conjugated into strain Rlv3841 and single cross-over insertions selected as previously described (3). Primers p1151 (*matA*) and p1154 (*matC*) were used with pK19A and pK19B to confirm the mutations. Primers pr0068/pr0069 and pr0071/pr0072 were used to amplify *matA* and *matC* respectively from ATCC14479. The products were cloned into pJET1.2/Blunt (Fermentas Inc) producing pLMB37 and pLMB38. Ω::Tc cassettes were cloned into unique StuI sites in pLMB37 and pLMB38, yielding pLMB39 and pLMB40. pLMB39 and pLMB40 were digested with SacI and XhoI/XbaI respectively and cloned into pJQ200SK, yielding pLMB47 and pLMB41. These plasmids were conjugated into ATCC14479 and cells plated on UMA (Universal Minimal Agar) supplemented with sucrose (10%) and tetracycline to select for gene replacement. Mutations in *matA* and *matC* were confirmed by PCR amplification using primers pr0070/pOTfarforward and pr0073/ pOTfarforward respectively. Primers pr1352/1353 and pr1354/1355 were used to amplify *matB* from Rlv3841 and ATCC14479. PCR products were cloned into pJET1.2/Blunt as described earlier resulting in plasmids pLMB638/pLMB639. Plasmid pLMB638 was digested with BmgBI to remove 244bp of *matB* of ATCC14479 and this was ligated with a SmaI digested ΩSp cassette, resulting in plasmid pLMB645. The BglII fragment from pLMB645 containing the ΩSp was cloned into the BamHI site of pJQ200SK, resulting in pLMB647. Plasmid pLMB639 was digested with AfeI to remove 386bp of *matB* of Rlv3841 and this was ligated to a SmaI digested ΩSp cassette, resulting in the plasmid

pLMB646. The PstI/XbaI fragment from pLMB646 was cloned into pJQ200SK, resulting in pLMB648. The plasmids pLMB647 and pLMB648 were conjugated into ATCC4479 and Rlv3841 and then plated onto UMA supplemented with sucrose (10%) and spectinomycin to select for gene replacement. Mutations in *matB* were confirmed by PCR amplification using primers pr1357/pOTfarforward and pr1356/ pOTfarforward respectively.

Complementation of ATCC14479 malonate mutants. Primers pr1313/pr1314, pr1313 /pr1315 and pr1316/pr1317 were used to PCR amplify *matA, matAB* and *matC* respectively from ATCC14479. PCR products were digested with XbaI/BamHI and cloned into the promoterless broad host range plasmid pRU1097 (4), yielding pLMB605 (*matA*), pLMB610 (*matAB*) and pLMB606 (*matC*) respectively. Another series of complementing clones containing *matA* and *matABC* were made in pBBR1MCS5 which has a p*lac* promoter vector to drive expression of the genes. Primers pr1329/pr1330, pr1328/pr1330, pr1317/pr1360 were used to amplify *matC* , *matABC, matRABC* respectively. PCR products were BamHI/XbaI digested and cloned into pBBR1MCS5, resulting in pLMB628 (*matABC*), pLMB629 (*matC*) and pLMB654 (*matRABC)*.

Complementation of *matC* using p*Tau* vector. Primers pr1358/1359 containing flanking NdeI/SacI sites were used to amplify *matC* from ATCC14479 and this was digested and cloned into pLMB509 yielding pLMB653. Plasmid pLMB509 contains a regulatable taurine promoter (5) allowing controlled expression of *matC*.

Plant growth and acetylene reduction. Pea seeds (*Pisum sativum* cv Avola) were surface sterilised and grown for 3-4 weeks in 1L vermiculate pots . White and red clovers were grown in 250ml pots (4 plants/pot) for 4-5 weeks. After growth, acetylene reduction was determined for plants incubated in 95% air and 5% acetylene for 1 h in 250-ml Schott bottles for peas and 100-ml Schott bottles for clover (6).

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66 **TABLE S1. Bacterial strains and plasmids used in this study**

Strain or plasmid	Description	Source/Reference
R. leguminosarum bv. *viciae* 3841	St^r derivative of *R. leguminosarum* bv. *viciae* strain 300, wild type strain	(7)
R. leguminosarum bv. *trifolii* ATCC14479	Cm^r ,wild type strain	ATCC culture collection
***mat* mutants**		
RU4053	Rlv3841 *matA*::pk19mob	This study
RU4054	Rlv3841 *matC*::pk19mob	This study
LMB134	ATCC14479 *matA* :: ΩTc	This study
LMB136	ATCC14479 *matC ::* ΩTc	This study
LMB510	ATCC14479 *matB* :: ΩSp	This study
LMB557	Rlv3841 *matB* :: ΩSp	This study
Plasmids		
pK19 mob	pUC19 derivative *lacZα*, mob; Km^r	(8)
pJET1.2/Blunt	PCR product cloning vector; Ap^r	Fermentas Inc
pRU1097	Broad host range cloning vector ; Gm^r	(4)
pBBR1MCS5	Broad host range cloning vector; Gm^r	(9)
pLMB509	Taurine inducible vector	(5)
pHP45Ω	pBR322 derivatives with the Sp and Tc cassette; Ap^r Sp^r ; Ap^r Tc^r	(10)
pRK2013	ColEI replicon with RK2 *tra* genes, helper plasmid used for mobilizing plasmids; Km^r	(11)
pJQ200SK	mob^+ *orip*15A, *lacZ*α^+ *sacB*; suicide vector; Gm^r	(12)
pRU2027	p1149-p1150 PCR product of Rlv3841 *matA* (RU0990) cloned into pK19 via BD cloning; Km^r	This study
pRU2028	p1152-p1153 PCR product of Rlv3841 *matC* (RU0992) cloned into pK19 via BD cloning; Km^r	This study
pLMB37	pr0068-0069 PCR product in pJET/Blunt; Ap^r	This study
pLMB38	pr0071-0072 PCR product in pJET/Blunt; Ap^r	This study
pLMB39	pLMB37 with ligated ΩTc cassette into *matA* gene via StuI digestion to generate *matA* mutant; Ap^r Tc^r	This study
pLMB40	pLMB38 with ligated ΩTc cassette into *matC* gene via StuI digestion to generate *matC* mutant; Ap^r Tc^r	This study
pLMB47	SacI fragment from pLMB39 cloned into pJQ200SK; Gm^r Tc^r	This study
pLMB41	XhoI/XbaI fragment from pLMB40 cloned into	This study

	pJQ200SK; Gm^r Tc^r	
pLMB605	Xba/BamHI fragment from pLMB601 containing *matA* gene from ATCC14479 cloned into pRU1097; Gm^r	This study
pLMB606	Xba/BamHI fragment from pLMB603 containing *matC* gene from ATCC14479 cloned into pRU1097; Gm^r	This study
pLMB610	Xba/BamHI fragment from pLMB602 containing *matAB* gene from ATCC14479 cloned into pRU1097; Gm^r	This study
pLMB628	XbaI/BamHI fragment containing *matC* gene from ATCC14479 cloned into pBBR1MCS5; Gm^r	This study
pLMB638	pr1352-pr1353 PCR product containing *matB* from Rlv3841 cloned into pJET1.2 /Blunt; Ap^r	This study
pLMB639	pr1352-pr1353 PCR product containing *matB* from ATCC14479 cloned into pJET1.2 /Blunt; Ap^r	This study
pLMB645	pLMB638 with ΩSp cassette ligated into BmgBI site of *matB* to generate *matB* mutant in Rlv3841; Ap^r Sp^r	This study
pLMB646	pLMB639 with ΩSp cassette ligated into AfeI site of *matB* gene to generate *matB* mutant in ATCC14479; Ap^r Sp^r	This study
pLMB647	BglII fragment from pLMB645 cloned into pJQ200SK BamHI site; Gm^r Sp^r	This study
pLMB648	PstI/XbaI fragment from pLMB645 cloned into pJQ200SK; Gm^r Sp^r	This study
pLMB653	pTau::*matC* in pLMB509 ; Gm^r	This study
pLMB654	Xba/BamHI fragment containing *matRABC* gene from ATCC14479 was cloned into pRU1097; Gm^r	This study

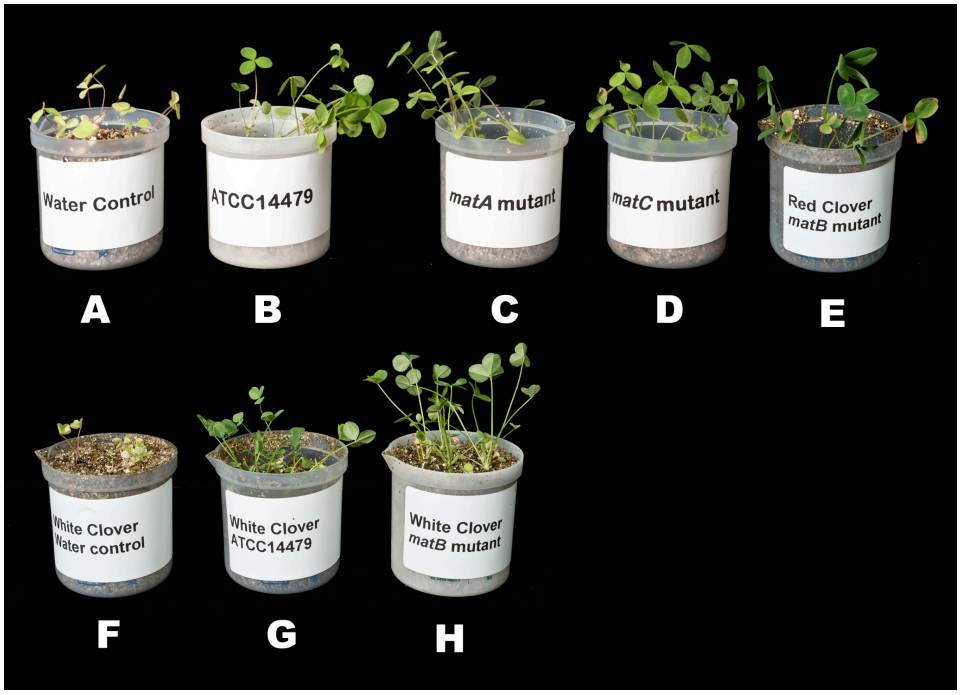
TABLE S2. Primers used in this study

Primer	Sequence
p1149	GACCATGATTACGCCAAGCTGACGCCGACTTCACGCATCTGTTC
p1150	GACCTGCAGGCATGCAAGCTCGGGCCGTTTCGGATAAGAACC
p1151	CCGACCCAACAGAAAATACC
p1152	GACCATGATTACGCCAAGCTCATCCACCCGGTCATGATGG
p1153	GACCTGCAGGCATGCAAGCTGATTGCTGCCTTCTGAGTCTTCG
p1154	TATACCAGGAGGTAAGGCGGCTGC
pr0068	GCCCTCCAGTTCCGCCATAA
pr0069	GGTCGAACTTCGACAGCAGG
pr0070	AAGATGGTTCGGTTCCCTA
pr0071	ACGGCATGACGGAAACCAAT
pr0072	ACCCGAATGGAATTGCGGTC
pr0073	ATCGCCAAGCCCCGCGGTGC
pr1313	AAATCTAGAGGGAGAGAACTCGTTGGAAA
pr1314	AAAGGATCCAGACAGGCGAGATAGAGGAT
pr1315	TTTGGATCCTATTTCGTTGGTTTTCATCC
pr1316	TTTTCTAGAATGAAAAGGCCATCGTCAGC
pr1317	TTTGGATCCTTTTCCACGTTTCCGTAATT
pr1328	AAAGGATCCGGGAGAGAACTCGTTGGAAA
pr1329	TTTGGATCCATGAAAAGGCCATCGTCAGC
pr1330	TTTTCTAGATTTTCCACGTTTCCGTAATT
pr1352	TTTCTGCAGACCAGGCGATTGAAAATTAC
pr1353	TTTTCTAGAGATCTGGTTGGTGATACCGC
pr1354	TCACGCTATCGCCCGTTCCA
pr1355	GATGCTGATCGGCGAGAAGC
pr1356	ACCGCCAAGCTCGTCCGGAT
pr1357	GCCTCTGCGCGGTCGAATTT
pr1358	TTTCATATGATGAAAAGGCCATCGTCAGC
pr1359	TTTGAGCTCTTTTCCACGTTTCCGTAATT
pr1360	AAATCTAGACATGCAAGGCAACTTCATTG
pK19A	ATCAGATCTTGATCCCCTGC
pK19B	GCACGAGGGAGCTTCCAGGG
pOTfarforward	GACCTTTTGAATGACCTTTA

FIG S1 Growth of *Rhizobium* strains on malonate.

Growth measured on UMS supplemented with NH_4Cl (10 mM) and carbon sources as stated. Growth is scored as shown below. A, RU4053 (*matA*) on 5mM Malonate (-); B, Rlv3841 growth on 5mM Malonate (+); C, Rlv3841 on 20mM Succinate (++); D, LMB134 (*matA*) with complementing plasmid pLMB628 (p*matABC)* on 5mM Malonate; E, LMB134 (*matA*) with complementing plasmid pLMB654 (*pmatRABC*) on 5mM Malonate

FIG S2 Clover plants inoculated with *R. leguminosarum bv trifolii* wild type and mutant strains.



Red clover plants were grown for 4 weeks (A-D) or 5 weeks (E). White clover plants (F-G) were grown for 5 weeks. A, red clover uninoculated; B, red clover plus ATCC14479 (wild type); C, red clover plus LMB134 (*matA*); D, red clover plus LMB136 (*matC*); E, red clover plus LMB510 (*matB*); F, white clover uninoculated; G, white clover plus ATCC14479 (wild type); H, white clover LMB510 (*matB*).

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