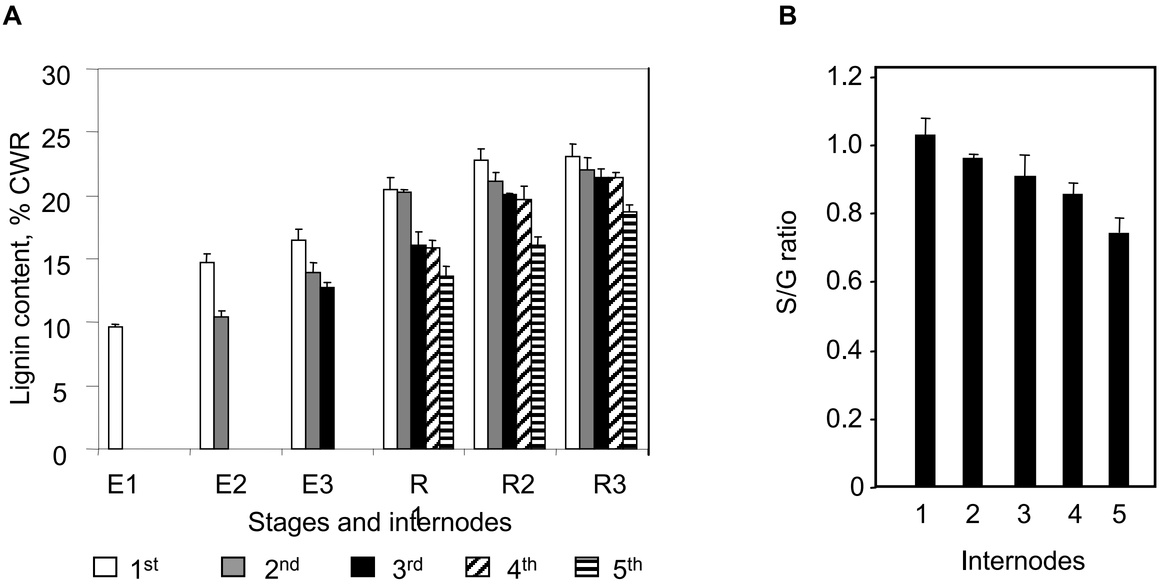
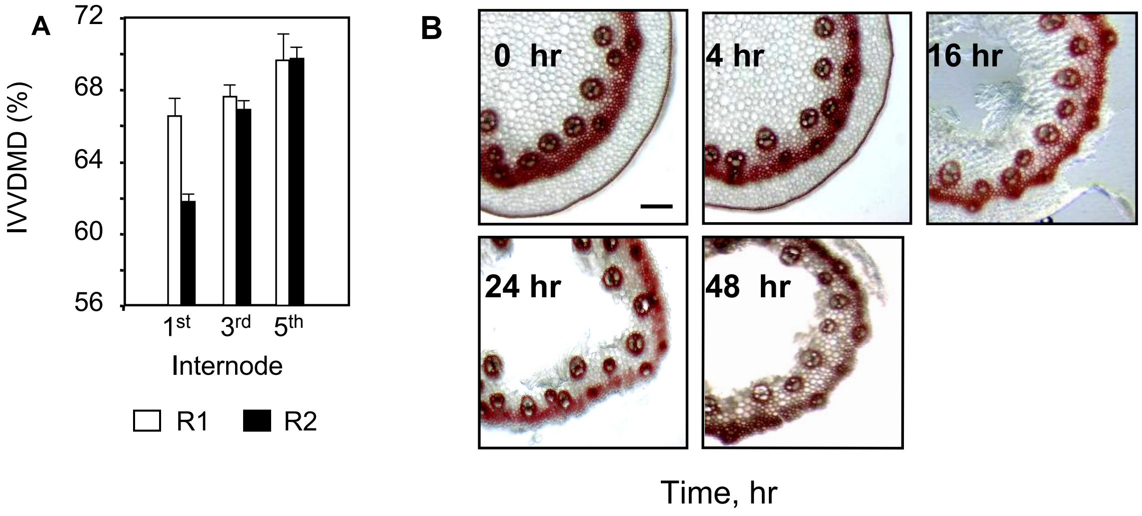


Supplementary Figure 1. Quantification of lignin content and composition in perennial ryegrass stems

(A) Acetyl bromide soluble lignin content in developing stems. The error bars are based on two biological and three technical replicates. Developmental stages as for Figure 1. Internodes were analysed separately and at each stage, results from the basal internode are shown on the left-hand column and those from the apical internode in the right-hand column. Results from the remaining internodes are shown in the other columns. CWR: cell wall residues.

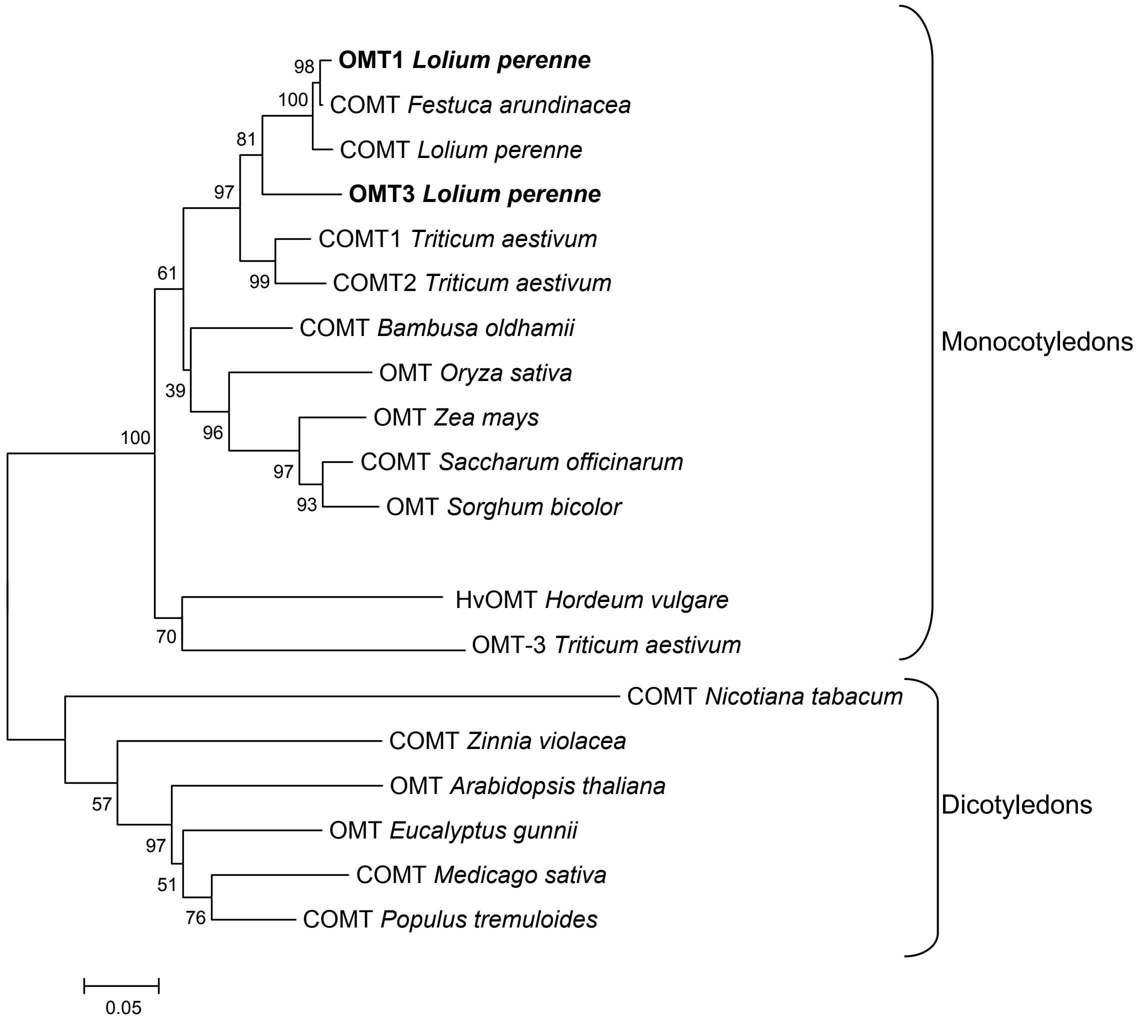
(B) Syringyl: Guiaicyl (S/G) monolignol ratio measured in different internodes of stems at the R3 stage. Internodes were numbered from the base to the apex of the stem.



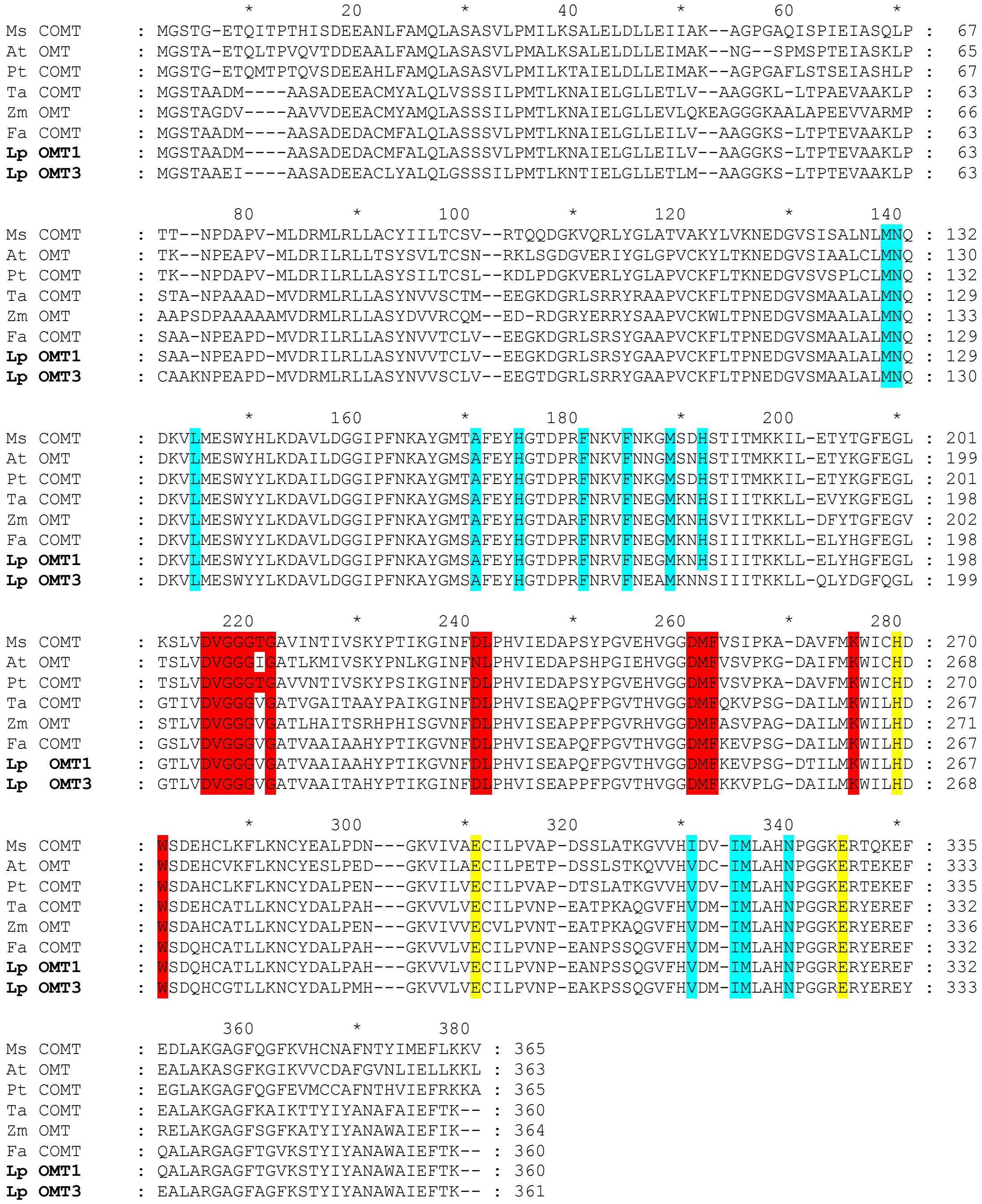
Supplementary Figure 2. Assessment of the digestibility of perennial ryegrass stems

(A) Estimation of *in vivo* dry matter digestibility (IVVDMD) of 1st, 3rd and 5th internodes of stems at the R1 and R2 stages, measured using near-infrared spectroscopy (NIRS). IVVDMD is a regression of the pepsin-cellulose digestibility compared to in vivo digestibility of known standards of similar class. white bars: R1 stage, black bars: R2 stage.

(B) Mäule staining of sections taken from the 1st internode of stems at the E3 stage incubated *in vitro* with bovine rumenal fluid over a 48 h time course. Each micrograph is representative of at least 4 stems from different plants. Bars = 160 µm for all sections



Supplementary Figure 3. Phylogenetic tree showing the relationships between the deduced amino acid sequences of plant caffeic acid O-methyltransferases. The Swissprot accession numbers are as follows: AAD10253, AAK68907.1, AAC18623, AAD10255, ABP63535.1, AAP23942.1, ABS18316.1, ABB90678.1, AAB03364.1, CAA13175.1, AAL57301.1, BAC54275.1, ABP93669.1, AAL91506.1, AAA86718.1, AAB96879.1, CAA52814.1, AAB46623.1 and AAB61731.1.



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At OMT        : MGSTA-ETQLTPVQVTDDEAALFAMQLASASVLPMALKSALELDLLEIMAK--NG--SPMSPTEIASKLP :  65
Pt COMT       : MGSTG-ETQMTPTQVSDEEAHLFAMQLASASVLPMILKTAIELDLLEIMAK--AGPGAFLSTSEIASHLP :  67
Ta COMT       : MGSTAADM----AASADEEACMYALQLVSSSILPMTLKNAIELGLLETLV--AAGGKL-LTPAEVAAKLP :  63
Zm OMT        : MGSTAGDV----AAVVDEEACMYAMQLASSSILPMTLKNAIELGLLEVLQKEAGGGKAALAPEEVVARMP :  66
Fa COMT       : MGSTAADM----AASADEDACMFALQLASSSVLPMTLKNAIELGLLEILV--AAGGKS-LTPTEVAAKLP :  63
Lp OMT1       : MGSTAADM----AASADEDACMFALQLASSSVLPMTLKNAIELGLLEILV--AAGGKS-LTPTEVAAKLP :  63
Lp OMT3       : MGSTAAEI----AASADEEACLYALQLGSSSILPMTLKNTIELGLLETLM--AAGGKS-LTPTEVAAKLP :  63

                        80         *       100         *       120         *       140
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At OMT        : TK--NPEAPV-MLDRILRLLTSYSVLTCSN--RKLSGDGVERIYGLGPVCKYLTKNEDGVSIAALCLMNQ : 130
Pt COMT       : TK--NPDAPV-MLDRILRLLASYSILTCSL--KDLPDGKVERLYGLAPVCKFLTKNEDGVSVSPLCLMNQ : 132
Ta COMT       : STA-NPAAAD-MVDRMLRLLASYNVVSCTM--EEGKDGRLSRRYRAAPVCKFLTPNEDGVSMAALALMNQ : 129
Zm OMT        : AAPSDPAAAAAMVDRMLRLLASYDVVRCQM--ED-RDGRYERRYSAAPVCKWLTPNEDGVSMAALALMNQ : 133
Fa COMT       : SAA-NPEAPD-MVDRILRLLASYNVVTCLV--EEGKDGRLSRSYGAAPVCKFLTPNEDGVSMAALALMNQ : 129
Lp OMT1       : SAA-NPEAPD-MVDRILRLLASYNVVTCLV--EEGKDGRLSRSYGAAPVCKFLTPNEDGVSMAALALMNQ : 129
Lp OMT3       : CAAKNPEAPD-MVDRMLRLLASYNVVSCLV--EEGTDGRLSRRYGAAPVCKFLTPNEDGVSMAALALMNQ : 130

                         *       160         *       180         *       200         *
Ms COMT       : DKVLMESWYHLKDAVLDGGIPFNKAYGMTAFEYHGTDPRFNKVFNKGMSDHSTITMKKIL-ETYTGFEGL : 201
At OMT        : DKVLMESWYHLKDAILDGGIPFNKAYGMSAFEYHGTDPRFNKVFNNGMSNHSTITMKKIL-ETYKGFEGL : 199
Pt COMT       : DKVLMESWYYLKDAILDGGIPFNKAYGMTAFEYHGTDPRFNKVFNKGMSDHSTITMKKIL-ETYKGFEGL : 201
Ta COMT       : DKVLMESWYYLKDAVLDGGIPFNKAYGMSAFEYHGTDPRFNRVFNEGMKNHSIIITKKLL-EVYKGFEGL : 198
Zm OMT        : DKVLMESWYYLKDAVLDGGIPFNKAYGMTAFEYHGTDARFNRVFNEGMKNHSVIITKKLL-DFYTGFEGV : 202
Fa COMT       : DKVLMESWYYLKDAVLDGGIPFNKAYGMSAFEYHGTDPRFNRVFNEGMKNHSIIITKKLL-ELYHGFEGL : 198
Lp OMT1       : DKVLMESWYYLKDAVLDGGIPFNKAYGMSAFEYHGTDPRFNRVFNEGMKNHSIIITKKLL-ELYHGFEGL : 198
Lp OMT3       : DKVLMESWYYLKDAVLDGGIPFNKAYGMSAFEYHGTDPRFNRVFNEAMKNNSIIITKKLL-QLYDGFQGL : 199

                       220         *       240         *       260         *       280
Ms COMT       : KSLVDVGGGTGAVINTIVSKYPTIKGINFDLPHVIEDAPSYPGVEHVGGDMFVSIPKA-DAVFMKWICHD : 270
At OMT        : TSLVDVGGGIGATLKMIVSKYPNLKGINFNLPHVIEDAPSHPGIEHVGGDMFVSVPKG-DAIFMKWICHD : 268
Pt COMT       : TSLVDVGGGTGAVVNTIVSKYPSIKGINFDLPHVIEDAPSYPGVEHVGGDMFVSVPKA-DAVFMKWICHD : 270
Ta COMT       : GTIVDVGGGVGATVGAITAAYPAIKGINFDLPHVISEAQPFPGVTHVGGDMFQKVPSG-DAILMKWILHD : 267
Zm OMT        : STLVDVGGGVGATLHAITSRHPHISGVNFDLPHVISEAPPFPGVRHVGGDMFASVPAG-DAILMKWILHD : 271
Fa COMT       : GSLVDVGGGVGATVAAIAAHYPTIKGVNFDLPHVISEAPQFPGVTHVGGDMFKEVPSG-DAILMKWILHD : 267
Lp  OMT1      : GTLVDVGGGVGATVAAIAAHYPTIKGVNFDLPHVISEAPQFPGVTHVGGDMFKEVPSG-DTILMKWILHD : 267
Lp  OMT3      : GTLVDVGGGVGATVAAITAHYPTIKGINFDLPHVISEAPPFPGVTHVGGDMFKKVPLG-DAILMKWILHD : 268

                         *       300         *       320         *       340         *
Ms COMT       : WSDEHCLKFLKNCYEALPDN---GKVIVAECILPVAP-DSSLATKGVVHIDV-IMLAHNPGGKERTQKEF : 335
At OMT        : WSDEHCVKFLKNCYESLPED---GKVILAECILPETP-DSSLSTKQVVHVDC-IMLAHNPGGKERTEKEF : 333
Pt COMT       : WSDAHCLKFLKNCYDALPEN---GKVILVECILPVAP-DTSLATKGVVHVDV-IMLAHNPGGKERTEKEF : 335
Ta COMT       : WSDEHCATLLKNCYDALPAH---GKVVLVECILPVNP-EATPKAQGVFHVDM-IMLAHNPGGRERYEREF : 332
Zm OMT        : WSDAHCATLLKNCYDALPEN---GKVIVVECVLPVNT-EATPKAQGVFHVDM-IMLAHNPGGKERYEREF : 336
Fa COMT       : WSDQHCATLLKNCYDALPAH---GKVVLVECILPVNP-EANPSSQGVFHVDM-IMLAHNPGGRERYEREF : 332
Lp OMT1       : WSDQHCATLLKNCYDALPAH---GKVVLVECILPVNP-EANPSSQGVFHVDM-IMLAHNPGGRERYEREF : 332
Lp OMT3       : WSDQHCGTLLKNCYDALPMH---GKVVLVECILPVNP-EAKPSSQGVFHVDM-IMLAHNPGGRERYEREY : 333

                       360         *       380
Ms COMT       : EDLAKGAGFQGFKVHCNAFNTYIMEFLKKV : 365
At OMT        : EALAKASGFKGIKVVCDAFGVNLIELLKKL : 363
Pt COMT       : EGLAKGAGFQGFEVMCCAFNTHVIEFRKKA : 365
Ta COMT       : EALAKGAGFKAIKTTYIYANAFAIEFTK-- : 360
Zm OMT        : RELAKGAGFSGFKATYIYANAWAIEFIK-- : 364
Fa COMT       : QALARGAGFTGVKSTYIYANAWAIEFTK-- : 360
Lp OMT1       : QALARGAGFTGVKSTYIYANAWAIEFTK-- : 360
Lp OMT3       : EALARGAGFAGFKSTYIYANAWAIEFTK-- : 361
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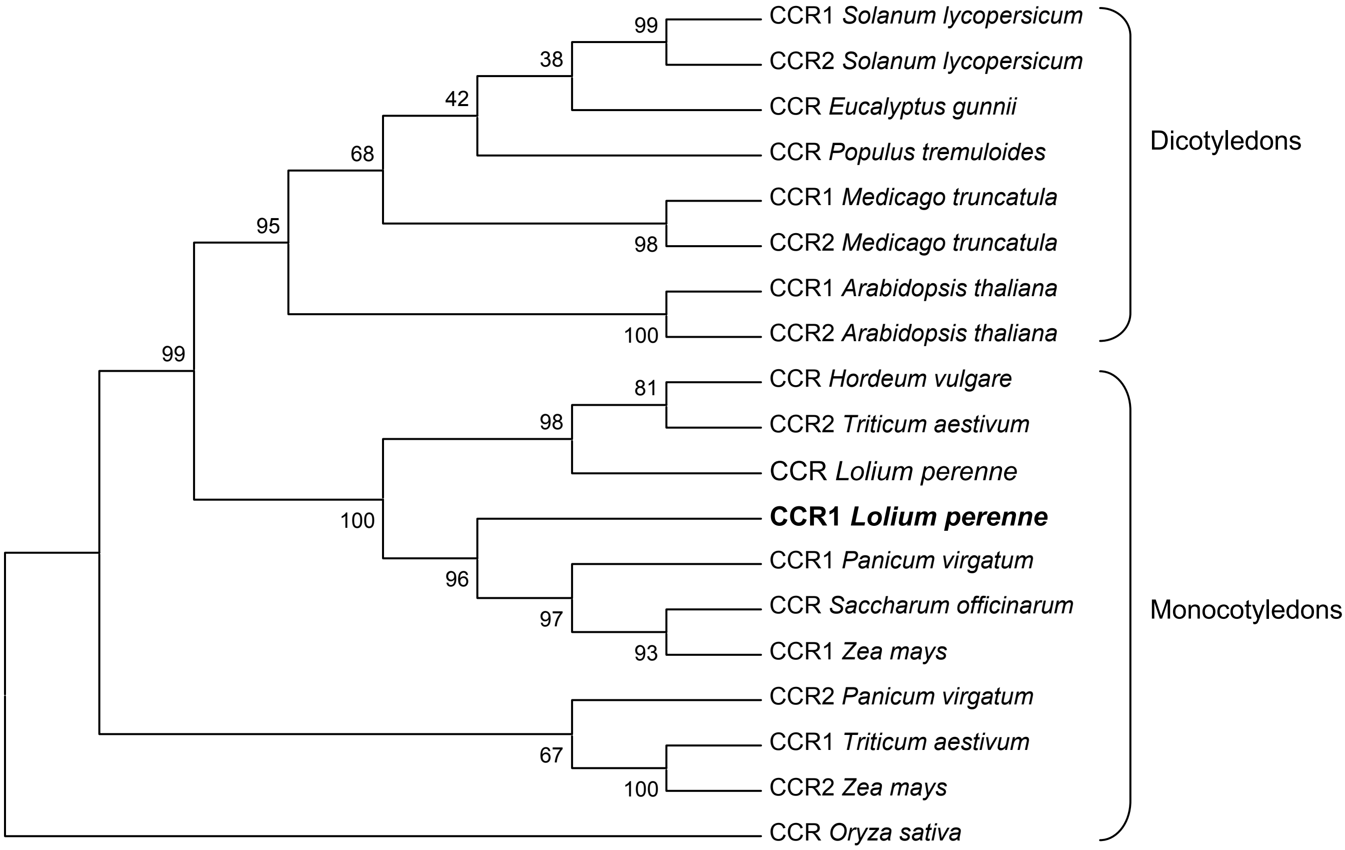
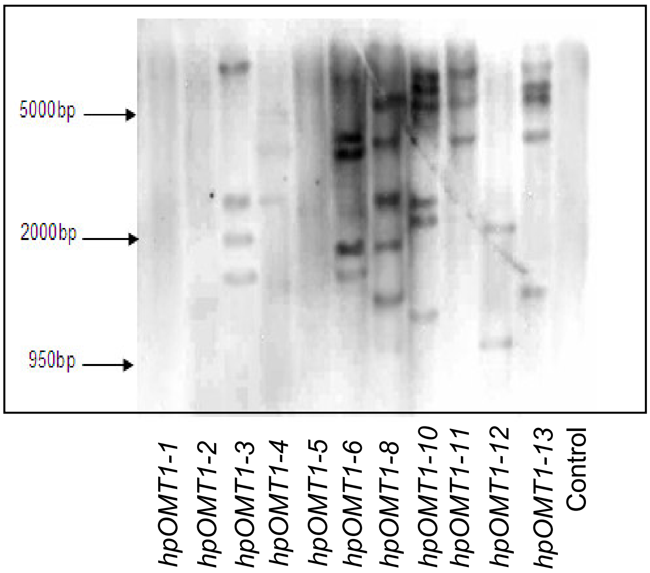
Supplementary Figure 4. Multiple sequence alignment of caffeic acid O-methyltransferases.from perennial ryegrass and other plant species.

Residues involved in SAM binding, catalytic activity and substrate specificity are highlighted in blue, red and yellow, according to structural analysis of Zubieta et al. (2002). Lp: *Lolium perenne*, Ms: *Medicago sativa*, At: *Arabidopsis thaliana*, Pt: *Populus tremuloides*, Ta: *Triticum aestivum*, Zm: *Zea mays*, Fa: *Festuca arundinacea*. The accession numbers are as follows. Swissprot AAB46623.1, AAB96879.1, AAB61731.1, ABP63535.1, AAB03364.1, AAK68907.1, AF033538 AF033540

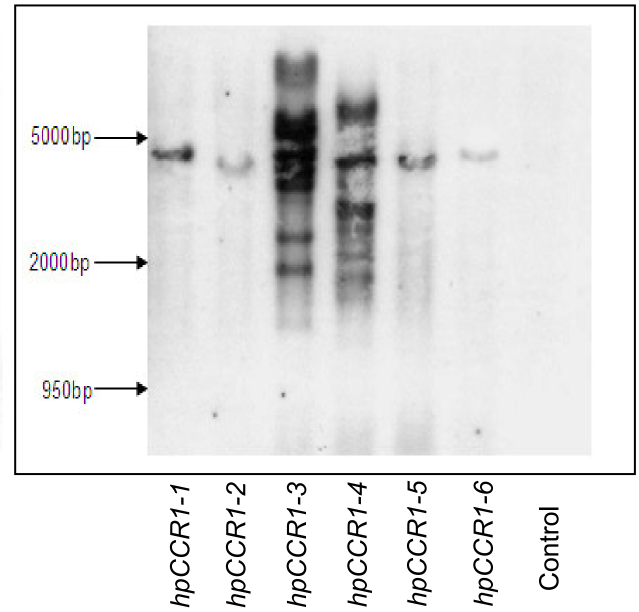


Supplementary Figure 5. Phylogenetic tree showing the relationships between the deduced amino acid sequences of plant cinnamoyl-CoA-reductases.

Genbank accession numbers are as follows: CAA13176.1, NP_001105488.1, ACZ74580.1, AAL47182.1, AAG09817.1, AAX08107.1, AAN71760.1, AAG46037.1, NP_178197.1, AAF43141.1, CAA56103.1, AAY41879.1, AAY41880.1, CAD21520.1, ACZ74585.1, NP_001105715.1, ABE01883.1, Mt CCR1 (TC 128336), Mt CCR2 (TC 132256),

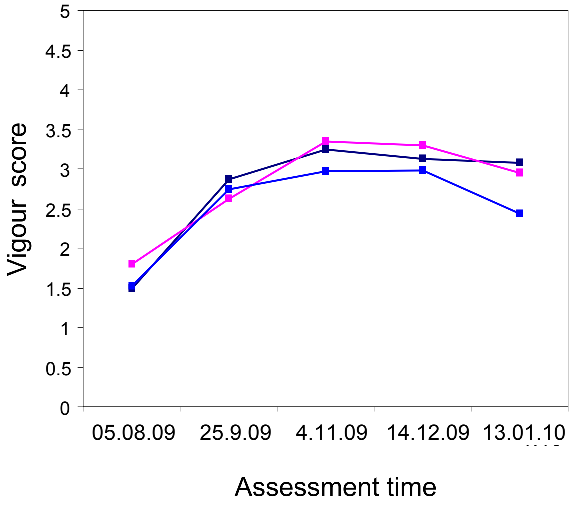


Supplementary Figure 6. Southern hybridization analysis of transgene copy number in perennial ryegrass plants containing an *hpOMT1* construct.
Genomic DNA was extracted from 11 transgenic lines and 1 wild type control plant, digested with *Eco*RI and hybridized to a probe that was complementary to the maize *Ubiquitin* promoter present in the transgene.

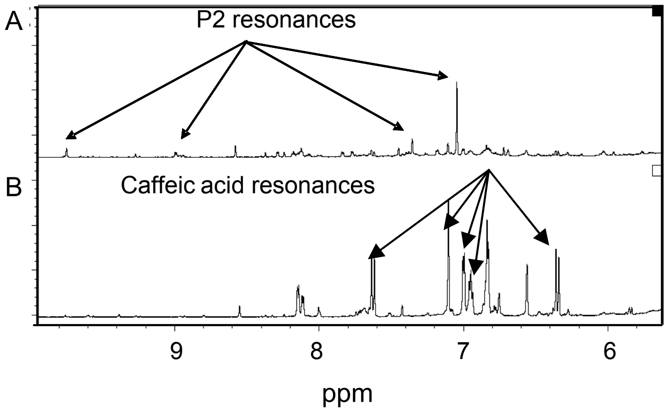


Supplementary Figure 7. Southern hybridization analysis of transgene copy number in perennial ryegrass plants containing an *hpCCR1* construct.

Genomic DNA was extracted from 6 transgenic lines and 1 wild type control plant, digested with *Eco*RI and hybridized with a probe that was complementary to the maize *Ubiquitin* promoter present in the transgene cassette



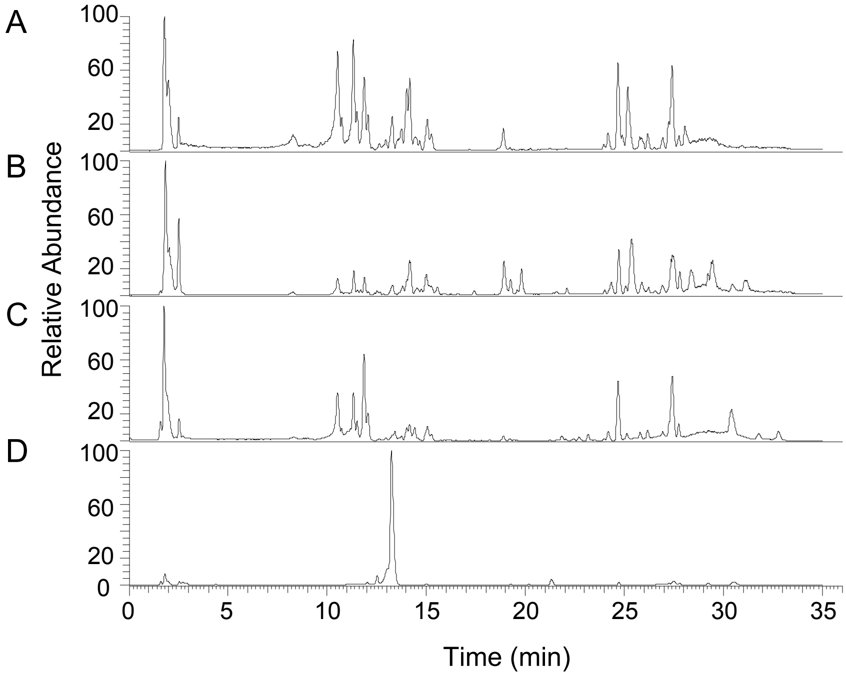
Supplementary Figure 8. Phenotypic analysis of the plants grown under field conditions.
Transgenic *hpCCR1-1* (blue line), *hpOMT1-3* (red line) and control plants (black line) were assessed for vigour growth (scores 1-5) under field condition from August 2009 till January 2010.



Supplementary Figure 9. NMR analysis of aqueous soluble metabolites from *hpCCR1-1* and control plants. Expansion of the aromatic region of the ^{1}H NMR spectrum (acquired in MeOD for maximum phenolic solubility).

A. Control plants

B. *hpCCR1-1* plants



Supplementary Figure 10. LCMS chromatograms of a *hpCCR1-1* mutant compared to control in both positive and negative acquisition modes.

(A) *hpCCR1-1* ESI negative base peak chromatogram

(B) Control ESI negative base peak chromatogram

(C) *hpCCR1-1* ESI positive base peak chromatogram

(D) Control ESI positive base peak chromatogram

Supplementary Table 1 Homology between members of COMT proteins from perennial ryegrass and tall fescue

	Lp OMT1	**Lp OMT3**	**La COMT**
Lp OMT1		90%	98%
Lp OMT3	90%		90.3%

The Swissprot accession numbers are as follows:
Lp OMT1: AF033538
Lp OMT3: AF033540
La COMT: AAK68907

Supplementary Table 2 Homology between members of CCR proteins from monocots

	Lp CCR1	**Lp CCR**	**Ta CCR1**	**Hv CCR**	**So CCR**	**Zm CCR1**	**Pv CCR1**
Lp CCR1		75%	80%	80%	82%	85%	89%
Lp CCR	75%		77%	78%	77%	77%	79%

The Swissprot accession numbers are as follows:
Lp CCR1: AAL47182
Lp CCR: AAG09817.1
Ta CCR1: ABE01883.1
Hv CCR: AAN71760.1
So CCR: CAA13176.1
Zm CCR1: NP_001105488.1
Pv CCR1: ACZ74580

Supplementary Table 3 List of primers

Gene	**Primer sequence (5'-3')**	
OMT1 (qRT-PCR)	Forward	GGCCATCGAGTTCACCAAGTAG
	Reverse	GGCAGCAGCCAGCAAGAT
OMT3 (qRT-PCR)	Forward	TCTCTCGCTCATACTACCAAAAACA
	Reverse	ATCTCTGCCTGTGTCTGCTTCTC
CCR1 (qRT-PCR)	Forward	GATGTCGAACCAGAAGCTCCA
	Reverse	GCTAGGGTTTCCTTGTCCGC
His (qRT-PCR)	Forward	TGCTTGCCCTTCAGGAGGCT
	Reverse	ATCCTCCTGGCAAGCTGAATGA
GAPDH (qRT-PCR)	Forward	TGGTGCCAAGAAGGTCATCAT
	Reverse	GACCATCAACAGTCTTCTGG
Tub (qRT-PCR)	Forward	AGTTGCCCCCAGTGATGGTA
	Reverse	GCCACGATACACAAACAAGCA
CCR1 (*hpCCR1*)	Forward	CGGGATCCTTCAGGCCGGTGAGCC
	Reverse	CTAGCTTATTTGTAGAGATGGTGCTTAAGG
OMT1 (*hpOMT1*)	Forward	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGGCTCC
	Reverse	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTGGTGC
hph (probe)	Forward	CGCATAACAGCGGTCATTGACTGGAGC
	Reverse	GCTGGGGCGTCGGTTTCCACTATCGG
Ubi (probe)	Forward	CCAAGCGAAGCAGACGGCAC
	Reverse	GGACCGGAACGCCGATCTAG

Supplemental Methods. Phylogenetic analysis.
Plant sequences shown in Supplemental Data Set 1 and Supplemental Data Set 2 were aligned using ClastalW program. The phylogenetic tree was constructed using the neighbor-joining method in the MEGA4.0.2 package (Tamura et al. 2007)

Supplemental Reference.

Tamura, K., Dudley, J., Nei, M., and Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol **24,** 1596-1599.