Expression of a Hydroxycinnamoyl-CoA Shikimate/Quinate Hydroxycinnamoyl Transferase 4 Gene from *Zoysia japonica* (*ZjHCT4*) Causes Excessive Elongation and Lignin Composition Changes in *Agrostis stolonifera*

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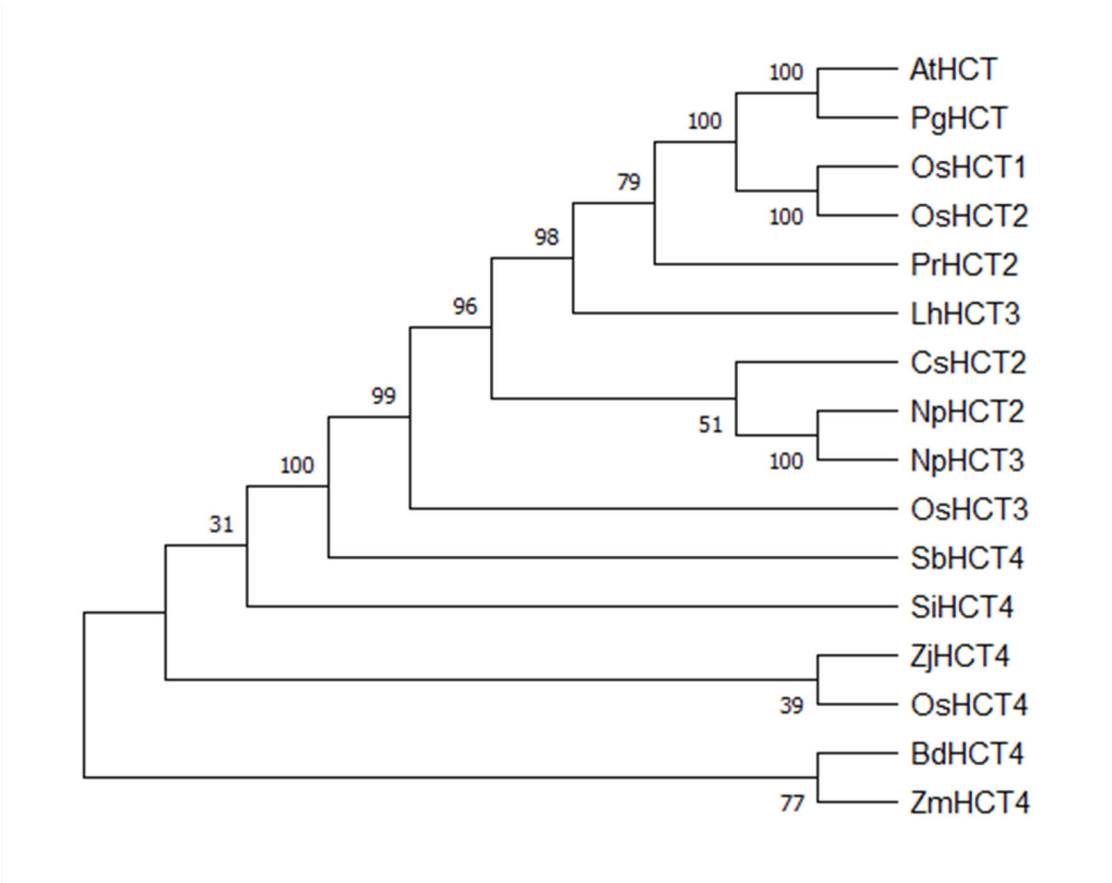
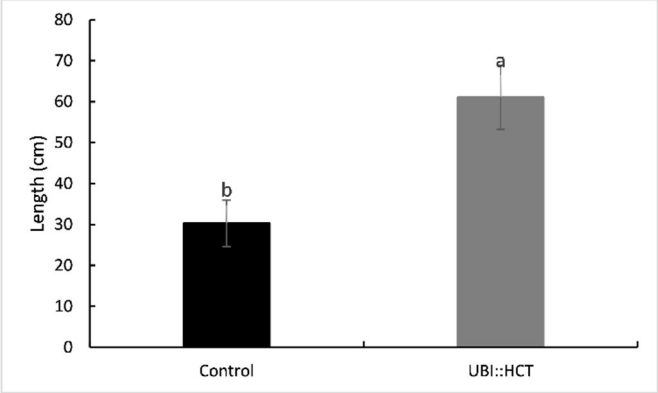
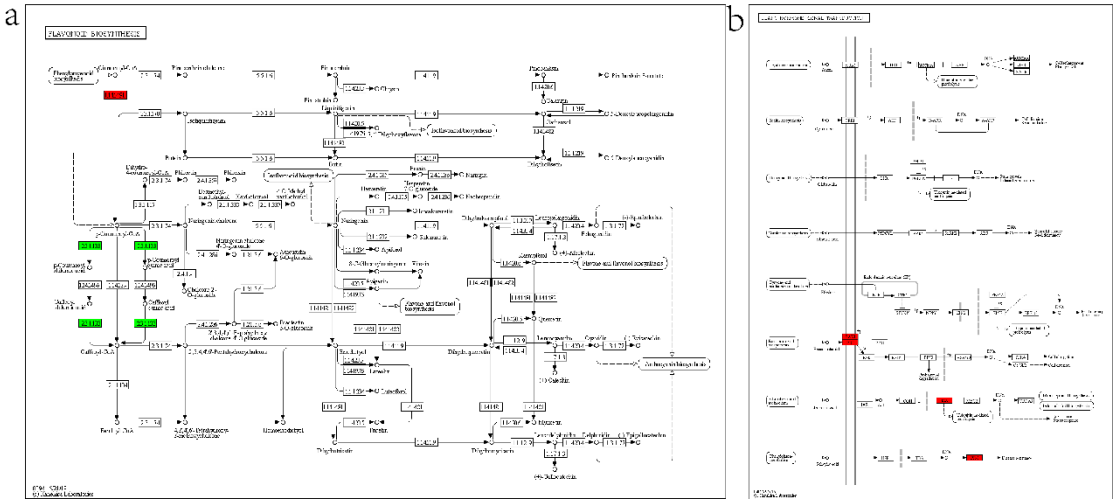


Supplementary Figure S1. Analysis of HCTs proteins. Protein sequence accession numbers are as follows: AT5G48930.1, AtHCT, *Arabidopsis thaliana*; AZS54120.1, PgHCT, *Punica granatum*; Q0JBZ8.1, OSHCT1, *Oryza sativa*; Q6K638.1, OsHCT2, *Oryza sativa*; ASM47232.1, PrHCT2, *Parasponia rugosa*; AKN80440.1, LhHCT3, *Lonicera hypoglauca*; KAF4871374.1, CsHCT2, *Colletotrichum siamense*; AXU39903.1, NpHCT2, *Narcissus papyraceus*; AXU39904.1, NpHCT3, *Narcissus papyraceus*; Q5SMM8.1, OsHCT3, *Oryza sativa* ; XP_002462397.1, SbHCT4, *Sorghum bicolor*; XP_004964652.1, SiHCT4, *Setaria italica*; Q5SMM6.1, OsHCT4, *Oryza sativa*; XP_010229918.1, BdHCT4, B*rachypodium distachyon*; PWZ06330.1 ZmHCT4, *Zea mays*.



Supplementary Figure S2. Length comparison of transgenic plants and control plants after 30 days of growth. Statistical significance of differences was assessed using the Student's t-test. Different letters above the columns indicate significant differences ($p < 0.05$, $n = 10$).



Supplementary Figure S3. KEGG pathways for DEGs in transgenic and control leaves. (a) DEGs mapped to flavonoid biosynthesis. EC:2.3.1.133, HCT; EC:1.14.14.91, trans-cinnamate 4-monooxygenase. (b) DEGs involved in plant hormone signal transduction

Supplementary Table S1. Protein sequence of HCT proteins.

Protein sequence
>ZjHCT4 MATVVTSVVVATAGAVWSNDAARRGYTTVYYRNGAADAVKDSSKAVAYAGRGVDTGRVVDCTGG AVVTARSDYVDDNVCMRDVATANCAVVTYRCGGVVGAMHHSVVDARGAAHTWASISRGDTAHA AVCDHSAARTTVYDHRYKVDVTASTYASAIITMTKGVSAKARCAGASTRSVVAVWCVCRARAATRY SMIDMRTRDAGYGNAVIRTSVSATVGVVANVGYAARRAAATTGDYTRSVDYGVDAMNRSGISRAH RAISWVGMSYKADGWGAAMGAIMYYSGVYVMNAAGKDGDAVSSMRKVADASVA
>SiHCT4 MAVVEVLTSEVAVPAEETPAGAIWLSNLDLAARRGYTPTVYFFRSNGEPGFFAAEVVKESLARALVAFY PLAGRLGVDATTGRVQIDCNGEGAVFVTARSGRYALDDLMSEFAPCREMRDLFVPPTPPPNPPCPLL FVQVTRLRCGSVVLGQAMHHSACDARGAAHFFETWASIARGDAAAAPVPPCFDHGLLAARPERAV TYDHPEYMPEPEPVDAAAASEYASAIITMTKAQVAALRARCPGASTFRAVVALVWRCACRARSLPHD AETRLYSMIDMRARLDPPLPPGYFGNAVVRTSVSATAAEVVSSPVGHVARRALAATSQGGDYARSLV DYLEGVDAMNLPRSGISRAHLRAISWVGMSLYKADFGWGAPAFMGPALMYYSGFVYVMNAAGKD GDLALVLSLEPESMPEFRKVFAEELARLDVV
>BdHCT4 MAKVEVLATELVVPAGETPGGSIWLSNLDLAARRGYTPTVYFYRPKNHGAVDPEEAFFAAGAVKCSL AKALVAFYPLAGRLGLDDAAGRLQIDCTGEGAVLVTARSDHYALDELMSEFVPCGEMRDLFVPATPA PNPPCALLLAQVTRLRCGGVVLGLALHHSVVDARSAAHFVETWASISRGNHQDAPLVPPCFDHRLL DARPYPARAVLYDHPEYKPEPAPVDVTPVSASTYASAIITVSKKQVAALRARCAGASTFRAVVALVWQ CACRARALAPGAETRLYSMIDMRPRLAPPLPQGYFGNAVVRTSAVVTVDEVVSSPVAYGARRARAA TSQGDDYARSLVDYLETVDTMNLPRSGISRAHLRAISWMGMSLSDADFGWGAPAFMGPALMYYSG FVYVMNAPGKDGAVALALSLEPDSMPEFKKTFADELARLEVEV
>ZmHCT4 MAMVELLSTELVVPAEETPAGAVWLSNLDLAARRGYTPTVYFYRTNGKPEFFETDAVKDSLARALVSF YPLAGRLGLDAATGRVQIDCTGEGAVFVTARWEQYALDELVGEFVPCDEMRALLVPATPAPNPPCPL LFAQVTRLRCGGVVLGLALHHSVVDARSAAHFVETWASIARGGAGAGAGTPLPPCFDHRLLNARPP GARAVAYDHPEYKAEAPAAADGGAGGGAAAGYASAIVTLSKAQVAALKARCAGASTFRAVVALVW QCACRARALPGDAETRLFSMVDMRARLAPPLPPGYFGNAVVRTSALATAGEVTGNPVGYAARRALA ATSPGDDYARSLVDYLEGVDAMNLPRSGISRAHLRAISWMGMSLHDSDFGWGAPVFMGPALMYYS GFVYVMQAPGKEGAVALALSLEPESMPEFRKVFAQELARLQAI
>SbHCT4 MAVEVVTSELVAPSETTPRRALWLSNLDLAARNGYTPTVYFFRRRCPQPQDGSGGDDDDRAPQQPS PDFFSADVLRAALAAALVQFYPFAGRLRAGRDDDGRAEIDCNAAGALFVVARSAAALEDFDGFAPSK AMNDTFVPKYDSTAGPDAPLLLLQVTFFRCGGVTLGTAMHPFVIDGRSAFHFIRTWASIARGDTAAA AVPPSLDRTPLRARPLPTVLFDHTYEYGGRTSRPPTTGGNNKAAEYASAILRVTGAQAAALRARAGAV STFRALVAHVWRCACAARALAPDAESRLYTMVDMRARLSPPLPDAFFGNAVARTSVSAVVGDLLAN PLGFGARRLRAATGHGDEYARSLLDYLETADLAALPRGGLAGTDLRVISWLGMPSYDADFGWGEPAL LAPALMYYPGFVYLLNCPGNGKGGGVAVAVALEPERMERFKELFFEELAALE
>AtHCT MKINIRDSTMVRPATETPITNLWNSNVDLVIPRFHTPSVYFYRPTGASNFFDPQVMKEALSKALVPFYP MAGRLKRDDDGRIEIDCNGAGVLFVVADTPSVIDDFGDFAPTLNLRQLIPEVDHSAGIHSFPLLVLQV TFFKCGGASLGVGMQHHAADGFSGLHFINTWSDMARGLDLTIPPFIDRTLLRARDPPQPAFHHVEY QPAPSMKIPLDPSKSGPENTTVSIFKLTRDQLVALKAKSKEDGNTVSYSSYEMLAGHVWRSVGKARGL PNDQETKLYIATDGRSRLRPQLPPGYFGNVIFTATPLAVAGDLLSKPTWYAAGQIHDFLVRMDDNYL RSALDYLEMQPDLSALVRGAHTYKCPNLGITSWVRLPIYDADFGWGRPIFMGPGGIPYEGLSFVLPSP TNDGSLSVAIALQSEHMKLFEKFLFEI

>PgHCT
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>OsHCT1
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>OsHCT2
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>PrHCT2
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RAIPNSQETRLHFAANGRNLRMKPRPQPGYFGNAVFVATSAVVASDLQYKPLWYAASRIRETLVRM
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>LhHCT3
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PPVLMGHTDALEEIKKETTIVMLKLDKKDIEKLKNKANNGENNYPLCYSRYEVVAGHMWRCACKARR
HASQQLTWLYFPMDVRNRIEPPLPQFYFGNAVYRAAATSTSGELISKPLSYASSKIREAKAKATDEYLR
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>LhHCT3
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HASQQLTWLYFPMDVRNRIEPPLPQFYFGNAVYRAAATSTSGELISKPLSYASSKIREAKAKATDEYLR
SSMMFLKNLPDVSQCRNFHSPIPEAFYGNPNIDINNSWGRQPSDGLDFGWGEEIYGGTAAHGSEGK
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>CsHCT2
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FPRSKINQLKANASRTEPNTWISSYDAIMALCWRCVSRARQSSVTGDTATTLMFAVNGRGRLKPPLS
KHYVGNVATLSWSELTFDEVVAPGAFPRLAALVRAANVEVDNDVYRSVVEWVAGIPDKRRVAFNM
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>NpHCT2
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>OsHCT3
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QVTFFRCGGVCLGAAIHHTAADGLAALDFVNTWAAIARDVAGDGEAAAAAVQRPWLDRTLLRARS
PPAVRFDHAEYSRRRGGGSKLPFDSAILPMSKNQLNALKGAGAGAGKRLSTFTAVVAHVWRCACKA
RGLAVAGTEAATRLYMTADARTRLHPPLPRGYLGNAIFRASAVSKVSDIVAAGPLGAVAEKVSAATAR
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>OsHCT4
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AARPARAVLYDHPEYKPEPAPPARAATASTYASAIITLTKQQVGALRAACAGASTFRAVVALVWQCA
CRARALPPEAETRLHSMIDTRQRLSPPLPPGYFGNAVIRTSTAATAGEVVSSPVGHAARRARAATSQG
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MNAPGKDGAVALALSLEPESMPEFRKVFADEVARLA

>NpHCT3
MVAIEVVKSELVFPSEETPKHRLWISNTDIMFATKLPTPMIYVYHPNGDPDFFNVEMLKAGLSKALVPF
YPLAGRLAKDRDGRLEVDCGRQGVLVVVARSDSGIDDFGEFLPSPELCRMLTPSIPSEDPDILFMTQVT
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LPACAATTTGPSATSILPLTKSQLALLTTNNKISTFCAISAHVWKCVSIARGLARDQAVQLKFVVNVRR
RMKPPLPDDYFGNAVCRMVVVSTADDVLSNPIEVVGNKIRDEIRKVDEEYVRSFIDHFEMSPTKGAKP
PASSAKFSLTPTMADFAMASWIGMPIYDADFGWGKPEIVRIAAITDGQYAYIMEIPKGGGVLVLVSLEE
ENMERFKEGFYEGLNMHGTIRTD

Supplementary Table S2. Primers used in the study.

Primer name	Primer sequence (5'–3')
ZjHCT-qPCR-F	GAGATGCGGGACCTGTTCGT
ZjHCT-qPCR-R	TACAGCACCGTTGGCGTGG
ZjHCT-F	ATGGCTTCAAGGACGACAGTTA
ZjHCT-R	GAGAAGGAAAGCAAGCCAGAA
35SYFP-ZjHCT-F	GGGGACTCTTGACCATGGTAATGGCGACGGTGGAGGTGTT
335YFP-ZjHCT-R	ACACGCGTACTAGTCAGATCGCCACCTCCAGGCTCGCCA
UBI-ZjHCT-F	GTGTTACTTCTGCAGAGGATGGCGACGGTGGAGGTGTT
UBI-ZjHCT-R	TAATCCAGATCTACCATAGGCTACGCCACCTCCAGGCTC
ZjACT-F	GGTCCTCTTCCAGCCATCCTTC
ZjACT-R	GTGCAAGGGCAGTGATCTCCTTG
AsACT-F	GAGGTCCTTCCTGATATCCA
AsACT-R	CCTTTTCCAGCCATCTTTCA
ASCSE-RT-F1	CCACTCTGGCAGACCAAT
ASCSE-RT-R1	TAGTCCATCCCGAACACG
ASCAD5-RT-F1	TCAGGTATCGCTTCGTCATC
ASCAD5-RT-R1	CATCCATCTCCAGCACAGTTT
ASCAD6-RT-F1	TCATTCCCGCTTATCTTCG
ASCAD6-RT-R1	AGCCTGGACCCATTCCCT
ASCAD8B-RT-F1	AAGCAAACCAAGAAGGCAGAT
ASCAD8B-RT-R1	TGTCAGGACCGAAAGGAGC
ASCCR2-RT-F1	TTCTTCGGATCACATGGGTC
ASCCR2-RT-R1	TCGGTAAACCGTGTTCTTTCT
ASCCR1C-RT-F1	AAAGAAGAGCGGTAAAGGTG
ASCCR1C-RT-R1	AAGCTAGTACGCAAGACTGGTA
ASPAL-RT-F1	ACCAGGGAAAGCACATCG
ASPPAL-RT-R1	TTGGCATCGGCATAGCAG
AS4CL5-RT-F1	GGGTTATGGGATGACAGAGG$
AS4CL5-RT-R1	CCGGTCCACGATGAAGAT$
AS4CLL7-RT-F1	GGGATGTCATTTACCCTGCTA$
AS4CLL7-RT-R1	TGTTCTCCGTATGCCTTGC$

AS4CLL4-RT-F1	CTTTGCTTAGTGGGTTGTTG$
AS4CLL4-RT-R1	CTGCTGCTTATTGTGATGGT
ASPOD1-RT-F1	TCACCGAATCTCAAATACCA
ASP0D1-RT-R1	CAAGTGCTCGTCATGTCTCA
ASCOMT-RT-F1	GGTTTCCCGTTGAAGCAC
ASCOMT-RT-R1	GCACGAGCACCTTTGACT
ASCCOAOMT1-RT-F1	CAAGAACGGGAAGAGCAG
ASCCOAOMT1-RT-R1	CAGGGAAGTGACAGCAAA
ASCCOAOMT2-RT-F1	CTCGTAGTATTCCCGGTTCA
ASCCOAOMT2-RT-R1	CTTCTCCGTCCTGCTCAA
ASCSE-RT-F1	CCACTCTGGCAGACCAAT
ASCSE-RT-R1	TAGTCCATCCCGAACACG

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