

SmCAD 1 MGSLES - PRTAHGWAHDSGLI LSPYEFPLREVD PDNVEIKVLYCGI CHTDLHQ LKNDY G 59
PtCAD 1 MGSLES - EKTVTGYAARDS SGHLSPYTYNLRKK GPEDVI VKVI YCGI CHSDLV QMRNE MG 59
PotCAD 1 MGSLET - ERKIVGWAATDSTGH LA PYTYS LRDTGPE DVL I KVI SCGICH TD I HQI KND LG 59
AtCAD4 1 MGSVEA GEK KALGWAARDPSGV LSPY SYTLRS TGADDVYI KVI CCGICH TD I HQI KND LG 60
AtCAD5 1 MGIMEA - ERKTTGWAARDPSGLI LSPYTYTLRE TGPE DVI NI RI ICCGICH TD LHQ TKN DLG 59
NtCAD19 1 MGS LDV - EKSAI GWAARDPSGL LSPYTYTLRN TGPE DVI QV KVL YCGI CHSD LHQV KND LG 59
OscAD2 1 MGS LAA - EKTVT GWAARDA SGHL TPYNYTLR K TGPE DVI VVKVLYCGI CHTD I HQAKNHL LG 59
SoCAD 1 MGS LAS - ERKVV GWAARDA TGH LA PYTYTLRS TGPE DVI VVKVLYCGI CHTD I HQAKNHL LG 59
ZmCAD2 1 MGS LAS - ERKVV GWAARDA TGH LA PYTYTLRN TGPE DVI VVKVLYCGI CHTD I HQAKNHL LG 59
Bmr6 1 MGS LAS - ERKVV GWAARDA TGH LSPYTYTLRN TGPE DVI VVKVLYCGI CHTD I HQAKNHL LG 59
SbCAD4 1 - - MEQCKTAH GWAARDA SGHLSPY SFSARI QGDADV TI KVLFCGI CHTDLHQ VIKNE WG 57
MGSLE . GERK . . GWAARD . SGHLSPYTYTLR TGPE DVI . VVKVLYCGI CHTDI HQ . KN . LG

SmCAD 60 MSRYPMVA GHEVVG TVTKV GAGVERFKVGD CVGVG CIVGSC QDCNA CTGDM E QYC SKKR W 119
PtCAD 60 MSHYPMVPGHEVVG I VTEIGSEV KKFKVGEH VGVG CIVGSC RSCGN CNQSM E QYC SKRI W 119
PotCAD 60 MSHYPMVPGHEVVG E VEVGSDV TKFKAGDVVGVGVI VGSCKN CHPKS E LE QYCNKKI W 119
AtCAD4 61 MSNYPMPGHEVVG E VEVGSDV SKFTVGDV VGVV VGGCCG SCKP CS E LE QYCNKKI W 120
AtCAD5 60 MSNYPMPGHEVVG E VEVGSDV SKFTVGD I VGVG CLVGGC GGCS P CERDLE QYC PKKI W 119
NtCAD19 60 MSNYP L VPGHEVVG K VVEVGA DV SKFKVGD TVGVGLLVG SCRN CGPKREI E QYCNKKI W 119
OscAD2 60 ASKYPMVPGHEVVG E VEVGPEV TKYSAGDVVGVGVI VGCCRE CHPKCANVE QYCNKKI W 119
SoCAD 60 ASKYPMVPGHEVVG E VEVGPEV TKYGVGDV VGVGVI VGCCRE CKPKCANVE QYCNKKI W 119
ZmCAD2 60 ASKYPMVPGHEVVG E VEVGPEV AKYGVGDV VGVGVI VGCCRE CS PCKANVE QYCNKKI W 119
Bmr6 60 ASKYPMVPGHEVVG E VEVGPEV SKYGVGDV VGVGVI VGCCRE CS PCKANVE QYCNKKI W 119
SbCAD4 58 NAMYPV VPGHEVVG V T D VGHG VTKFKAGD TVGVGYFV DSCRT CES CSTGH ENYCPDLV L 117
MS . YPMVPGHEVVG E VEVG . V . KF VGDV VGVGVI VGCCR C PCK . EQYCNKKI W

SmCAD 120 TYN - - DVFPDGEPTQGGFADIMVVDQRFVV TIPEGLALDA AAPLLCAGI TVYSPMKHF GM 177
PtCAD 120 TYN - - DVNH DGTPTQGGFAS SMVVDQMFVVRIPEN LPLEQAAPLLCAGVTVF SPMKHF AM 177
PotCAD 120 SYN - - DVYTDGKPTQGGFAESMVVDQKFVVRIPDGMSP EQAAPLLCAGL TVYSP LKHFG L 177
AtCAD4 121 SYN - - DVYTDGKPTQGGFAD TMI VN QKFVVKIPEGM AVEQAAPLLCAGVTVYSP LSHFGL 178
AtCAD5 120 SYN - - DVY INQPTQGGFAKATV VH QKFVVKIPEGM AVEQAAPLLCAGVTVYSP LSHFGL 177
NtCAD19 120 NCN - - DVYTDGKPTQGGFAN SMVVDQNFVVKIPEGM AVEQAAPLLCAGI TVYSP FNHF GF 177
OscAD2 120 SYN - - DVYTDGRPTQGGFASAMVVDQKFVVKI PAAGLAPEQAAPLLCAGL TVYSP LKHFG L 177
SoCAD 120 SYN - - DVYTDGRPTQGGFAS TMVVDQKFV MKI PAAGLAPEQAAPLLCAGVTVYSP LKAFGL 177
ZmCAD2 120 SYN - - DVYTDGRPTQGGFAS TMVVDQKFVVKI PAAGLAPEQAAPLLCAGVTVYSP LKHFG L 177
Bmr6 120 SYN - - DVYTDGRPTQGGFAS TMVVDQKFVVKI PAAGLAPEQAAPLLCAGVTVYSP LKAFGL 177
SbCAD4 118 TSN GVDH HHHGATTKGGFSDV L VVS QDFVVRVPE SLP L DGAAPLLCAGVTVYSP MAQYAL 177
SYNGVDVYTDG . PTQGGFA . MVVDQKFVVKIPEGLAPEQAAPLLCAGVTVYSP LKHFG L

*

SmCAD 178 TEPGKKCGI LGLGGVGHMGVK FAKAFGLHVTVI STSR SKKEAREV LGADD FLVSDTPEQ 237
PtCAD 178 TEPGKKCGI LGLGGVGHMGVK IAKAFGLHVTVI SSSDKKKEEAMEV LGADAYLVSKD TEK 237
PotCAD 178 KQSGLRGGI LGLGGVGHMGVK IAKAMGHHVTVI SSSDKKRE EAMEHLGAD EYLVSSD VES 237
AtCAD4 179 MASGLKGGI LGLGGVGHMGVK IAKAMGHHVTVI SSSDKKKEEAEI EHLGADDYVVS SDPAE 238
AtCAD5 178 KQPGLRGGI LGLGGVGHMGVK IAKAMGHHVTVI SSSNKKRE EALQD LGADDYVIGSD QAK 237
NtCAD19 178 NQSGFRGGI LGLGGVGHMGVK IAKAMGHHVTVI SSSNKKRQEAL EHLGADDYLVSSD TDK 237
OscAD2 178 MSPGLRGGV LGLGGVGHMGVKV AKSMGHHVTVI SSSARKRGEAMD D LGADAYLVSSDAAA 237
SoCAD 178 TTPGLRGA I LGLGGVGHMGVKV AKAMGHHVTVI SSSSKKRAEAMDHLGADAYLVSSDAAA 237
ZmCAD2 178 TTPGLRGGI LGLGGVGHMGVKV AKAMGHHVTVI SSSSKKRAEAMDHLGADAYLVSSDAAA 237
Bmr6 178 TAPGLRGGI VGLGGVGHMGVKV AKAMGHHVTVI SSSSKKRAEAMDHLGADAYLVSDAAA 237
SbCAD4 178 NEPGKHLGVVGLGGLGHMAVK FAKAFGMTVTVI SSSPGKRDEALGR LGADAF LVSHDA AQ 237
PGLRGGI LGLGGVGHMGVK . AKAMGHHVTVI SSS KKR EAM . HLGADAYLVSSD . A

SmCAD 238 MAAAQSLDYI LDTIPAP HPLDMYLP L LGMNGKF ILLGVAPAPL QFVAPNI LLGRRM IAG 297
PtCAD 238 MMEAAESLDYI MDTIPVA HPLEPYLALLK TNGKLVMLGVVPEPL HFVTP LLI LGRRS IAG 297
PotCAD 238 MQKAAADQLDYI IDTVPV HPLEPYL SLLKLDGKLI LMGVIN TPL QFVSPMVMLGRKSI TG 297
AtCAD4 239 MQR LADSLDYI IDTVPV HPLDPYLA CLKLDGKLI LMGVIN TPL QFVTP LV I LGRKVI SG 298
AtCAD5 238 MELADSLDYI IDTVPV HHALEPYL SLLKLDGKLI LMGVIN NPL QFLTPL LMLGRKVI TG 297
NtCAD19 238 MQEAAADSLDYI IDTVPV GHPL ELYL SLLK I DGKLI L IGVIN TPL QFI SPMVMLGRKSI TG 297
OscAD2 238 MAAAAGDSLDYI IDTVPV HHPLEPYLALLKLDGKLI LMGVIN QPL SFISPMVMLGRKAI TG 297
SoCAD 238 MAAAADSLDYI IDTVPV HHPLEPYLALLKLDGK H VLLGVI GEPL SFVSPMVMLGRKAI TG 297
ZmCAD2 238 MGPAADSLDYI IDTVPV HHPLEPYLALLKLDGKLV L L GVI GEPL SFVSPMVMLGRKAI TG 297
Bmr6 238 MAAAADSLDYI IDTVPV HHPLEPYL SLLR LDGKH VLLGVI GEPL SFVSPMVMLGRKAI TG 297
SbCAD4 238 MKAAAATLDGI IDTVSA GHQIVP L LALLKPMGQMVVVGAP S TPL ELPAYA I I TGGRVAG 297
M AADSLDYI IDTVPV HPLEPYLALLKLDGKLI L . GVI PL FVSPMVMLGRK . ITG

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SmCAD 298 S F I G S M K E T Q E M L E F S A E K K I E C M I E T V T F D Y I N E A M K R L E K N D V R Y R F V I D V E K S N K S A 357
PtCAD 298 S F I G S M E E T Q E T L D F C A E K K V S S M I E V V G L D Y I N T A M K R L E K N D V R Y R F V V D V A A S K L D N 357
PotCAD 298 S F I G S M K E T E E M L E F C K E K G L A S M I E V I K M D Y I N T A F E R L E K N D V R Y R F V V D V A G S K L I P 357
AtCAD4 299 S F I G S I K E T E E V L A F C K E K G L T S T I E T V K I D E L N I A F E R L R K N D V R Y R F V V D V A G S N L V E 358
AtCAD5 298 S F I G S M K E T E E M L E F C K E K G L S S I I E V V K M D Y V N T A F E R L E K N D V R Y R F V V D V E G S N L D A 357
NtCAD19 298 S F I G S M K E T E E M L D F C K E K G V T S Q I E I V K M D Y I N T A M E R L E K N D V S Y R F V V D V A G S K L D Q 357
OsCAD2 298 S F I G S M A E T E E V L N F C V D K G L T S Q I E V V K M D Y V N Q A L E R L E R N D V R Y R F V V D V A G S N I D D 357
SoCAD 298 S F I G S I D E T A E V L Q F C V D K G L T S Q I E V V K M G Y V N E A L D R L E R N D V R Y R F V V D V A G S N V E E 357
ZmCAD2 298 S F I G S I D E T A E V L Q F C V D K G L T S Q I E V V K M G Y V N E A L E R L E R N D V R Y R F V V D V A G S N V E A 357
Bmr6 298 S F I G S I D E T A E V L Q F C V D K G L T S Q I E V V K M G Y V N E A L E R L E R N D V R Y R F V V D V A G S N V E E 357
SbCAD4 298 N G V G S V A D C Q A M L D F A G E H G V T A D I E V V Q M D Y V N T A I E R L E K N D V R Y R F V I D V A G S K M E E 357
S F I G S M E T E L F C E K G L T S I E V V K M D Y V N A E R L E K N D V R Y R F V V D V A G S N . .

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SmCAD 358 - - - - - 357
PtCAD 358 - - - - - 357
PotCAD 358 - - - - - 357
AtCAD4 359 E A A T T T N - - - 365
AtCAD5 358 - - - - - 357
NtCAD19 358 - - - - - 357
OsCAD2 358 A - - - D A P P A 363
SoCAD 358 V - - A A D A P S N 365
ZmCAD2 358 E A A A A D A A S N 367
Bmr6 358 D - - A A D A P S N 365
SbCAD4 358 T V A - - - - - 360
A A A A D A P S N

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Figure S1. Protein alignment of Bmr6, SbCAD4 and CAD2 sequences.

The amino acid sequences from *Arabidopsis* AtCAD4 (At3g19450) and AtCAD5 (At4g34230); aspen PotCAD (AAF43140); eucalyptus EgCAD2 (CAA46585); loblolly pine PtCAD (CAA86073); maize ZmCAD2 (BM1 locus) (NM_001112184); rice OsCAD2 (Os02g0187800); *Selaginella moellendorffii* SmCAD (estExt_fgenes2_pg.C_390191); sorghum Bmr6 (Sb04g005950), SbCAD4 (Sb02g024190.1); sugarcane SoCAD (CAA13177); and tobacco NtCAD19 (CAA44217) were aligned using ClustalW. * below the alignment denotes the stop codon found in *bmr6*. The NADPH binding domain is underlined.

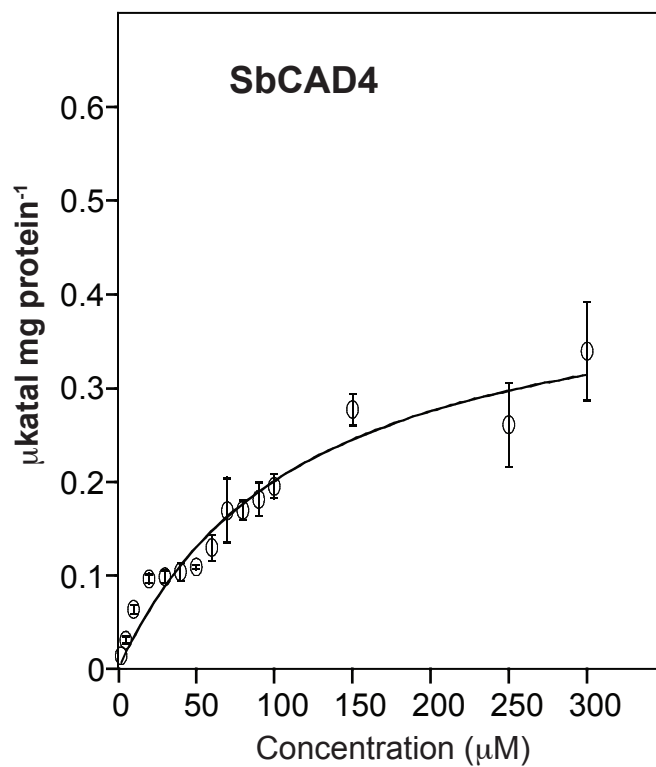
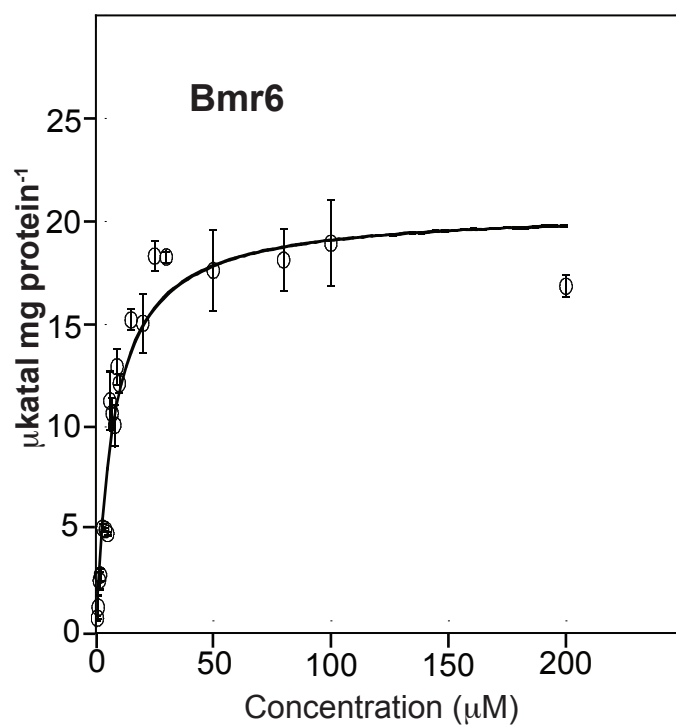


Figure S2. Bmr6 and SbCAD4 enzyme velocities for coniferyl alcohol

The enzymatic velocities for Bmr6 and SbCAD4 were measured over a range of concentrations of coniferyl alcohol in order to determine the enzyme kinetic parameters (Table I; see Materials and methods). (N=4).

| Name | forward (5'-3') | reverse (5'-3') |
|-------------------|--------------------------------------|-------------------------------------|
| <i>cDNA</i> | ggagaatggggagcctggcgt | ccgtcagttgctcggcgcatc |
| <i>Genomic</i> | | |
| segment 1 | ggagaatggggagcctggcgt | cctgccagtacgacaagctac |
| segment 2 | ggagtagctgtcgtactggcagg | ctagggggtttgttgtcaccag |
| segment 3 | gtaccctatggtcctgggtgagc | cgacgaccatggtggaggcgaagc |
| segment 4 | gcttcgcctccaccatggtcgtcg | ccgtcagttgctcggcgcatc |
| segment 5 | cagcccctgcaaggccaacgttgagc | gcgggctgtacaccgttacgcccg |
| segment 6 | gtggtgaagatcccggcgggtctgg | cagaagcgcaggcagcctcgag |
| <i>RT-qPCR</i> | | |
| Bmr6 | gaggtgctccagttctgcg | cagcgcctcgttcacgtac |
| SbCAD4 | atgctggacttcgcaggaga | ctcgatcgctgtgttgacgta |
| eIF4a1 | tggcgaaggatggtttctaagt | ggagcggcacatagcctactc |
| <i>E.coli</i> | | |
| <i>expression</i> | | |
| Bmr6 | gatctgggtaccatggggagcctggcgtccgagagg | ggacgcaagctttcagttgctcggcgcatcagcgg |
| SbCAD4 | gatctgggtaccatggatatggagcagggtctgc | ggccgcaagcttttaagcaacggctcctccatc |
| CAPS | cacaaccactccactactgcgaac | gtcaccacaaggcatccatacg |

Table S1. Primer sequences

These primers were used to amplify genomic DNA and cDNA for the experiments in this manuscript.