Supporting Information

Hartwig et al. 10.1073/pnas.1108359108

<

	V	
ZmDET2/NA1	-MPDQTSAAAAGGAABDRCLLALYLISPLTVLALRFISAPYGKLS-RPG-WGPVLPAP	55
SbDET2	-MSDPTAAAAAAAGDAVFERCLLALYLISPLTVLALRFISAPYGKLS-RPC-WGPVLPAP	57
OsDET2-1	MGGGGGD-DALYARCLVILYLISPITVFLLRFVSAPYGKLS-RPC-WGPAVPAA	51
OsDET2-2	MTDRQAMAMGSSDGDVLYARCLVTLYLLSPLNVFALWFMSAPYGKLS-RPG-WGPTVPA	58
AtDET2	MEEIAD-KTFBRYCLLTLIFAGPPTAVLLKFLQAPYGKHN-RTG-WGPTVSPP	50
PsDET2	MLNQKIFLQD-DSLENYALLTLFLIAPPTFISLAFLQAPYGKHH-RPG-WGPNLSPP	54
SIDET2	MFSSD-ENLENFIVFFILVMAFPTFILCOFFISPYGKHY-TSADSCTTISPP	50
HsSRD5A1	MATATGVAEERULAALAYLQCAVGCAVFARNRQTNSVYGRHALPSHRLRVPAR	53
HsSRD5A2	SGYGKH ESLKPATR PAR	50
	▲	
ZmDET2/NA1	LAWEIMESPTIWLPPIVILRWPPPPLSCPLAALPAALYAEHYVHRTIVHPLRIAR	110
SbDET2	LAWFIMPSPILWIPPILVILRSPPPRLSGPLAALPAALVAFHYVHRUVHPURLAC	112
OSDET2-1	DAVELMESPE CUPPINTSAAASSSALRAA LUPAALVAI HYVNRUNHPURULR	106
OSDET2-2	LAWCIMESPERATEPIAVESATTTSLSTPTLEVTTEPAALYMERVERTAVEPIREL.B	115
A+DET2	TAWEVMESPTIMUTTLUTEPEGRHALNEKSLITESPYLTHYEHRTTTYPIRUFR	103
PenET2	LAWETMES PUTWETLY FOR ONSSNEKSTITTTPETTY FOR TYPETTY	110
SIDET2	TAWA EMES PULATITI WERLCKNYINDLA EMITSPYLEHYTNET TYPEPIPES	103
HeSPD5A1	AMMU OFLIGSTAL DLYOYASESADD	100
HeSPD5A2	AMUSTORIES FAURACIDA PORTS	10/
noordone		104
ZmDET2/NA1	IRRAPAPUPULTAACALGENIUNAYIOARSDALHAGROASPFRC	154
SbDET2	IRRATAPVPLLIAACAPGPSLINAYVOARSFALHAGRPASPARC	156
OsDET2-1	LRRAPAPVPILVAAFAPGENLLNAYVOARSWALDAAAPHSTATATATPAAVARC	160
OsDET2-2	LRRAPAPMPILIIMFGRSFNLLNAYIOARSWALDAVPPATATPLTVARC	164
AtDET2	SSFPAGKNGFPITIAALAFTFNLLNGYIOAR-WVSHYKDDYEDGNWFWWRF	153
PsDET2	TKKKSTLSKTPSGEPESTALMARVENLLNSYVOAR-WVSHYKSYDDGLEEWVVP	163
SIDET2	RNTKNNER NLAVTARIENLLNAY OSR-WVSHYANYODDDWFWVRD	149
HsSRD5A1	KPMPLDACTMAIMPCTCNGYLOSRYLSHCAVYADDWVTDPRP	151
HsSRD5A2	REYEATLILRCTAECTGNCVLOCYYLIYCAEYPDGWYTDIRE	146
	* ** **	
ZmDET2/NA1	LAGLALFAWGMRTNIAADKELLRLKEAGGGYKIPRGGWFDLVTSPNYFGERVEWLGFAV	213
SbDET2	LAGLALFAWGMRTNIAADKELLRLKEAGGG-YKIPRGGWFDLVTSPNYFGETVEWLGFAV	215
OsDET2-1	LVGLALFAWGMRTNIAADKALLRLKEAGKG-YOIPRGGLFDVVTCPNYFGEAVEWLGYAL	219
OsDET2-2	LVGLALFVWGMWTNIAAD RELLRLKEAGKG-YOIPKDGLFDLVACPNYFGETVEWLGYAL	223
AtDET2	VICMVFITGMYINITSDRTLVRLKKENRGGYVIPRGGWFELVSCPNYFGEATEWLGWAV	213
PsDET2	FCCVLVFSCCMCINVWSDKELLRLKGECKC-YVVPKCCFFEFVSCPNYFCEIVEWFGWAL	222
SIDET2	GIGLVIFGSCMLINTWADGVLLCLKSOGGG-YKIPRGGLFDYVSSPNYLGFIMEWLGWAL	208
HsSRD5A1	LICECTWLTCMLINTHSDHILRNLRKPCDTCYKTPRCCLEEYVTAANYFCEIMEWCCYAL	211
HsSRD5A2	SLEVELEILGMGINTHSDYILROLSKPGEISYRTPOGGLETYVSGANELGEITEWIGYAL	206
	*	
ZmDET2/NA1	VAWTPAAWAFFLYTCANLGPRARD-HRRWYVOKPRGEYPASRKAFIPYIY	262
SbDET2	VAWTPAAWAFFLYTCANLGPRARD-HRRWYVOKFGGEYPASRKAFTPYTY	264
OsDET2-1	VAWTPAAWAFFLYTCSNLGPRARD-HRRWYVGKFGDKYPASRKAFVPYIY	268
OsDET2-2	VAWTPAAWAFFLYTCVNLGPRARD-ORLWYTSKFGDKYPASRKAFTPYTY	272
AtDET2	MTWSWAGIGFFLYTCSNLFPRARASH-KWYIAKFKEEYPKTRKAVIPFVY	262
PsDET2	MTWSWACLGFFAYTFANLGPRARANH-OWYLEKFGEDYPKKRKAVLPYLY	271
SIDET2	MTWSWAGLAFFVYTCANLVPRAVSNH-KWYLOKFGEDYPKNRKAVFPFLY	257
HsSRD5A1	ASWSVQGAAFAFETFCFLSGRAKEHH-EWYLRKF-EEYPKFRKIIIPFLF	259
HsSRD5A2	ATWSLPALAFAFFSLCFLGLRAFHHH-RFYLKMF-EDYPKSRKALIPEIF	254

Fig. S1. Amino acid alignment of NA1 to selected 5α -steroid reductases. Sequences were aligned with ClustalW2 (version 2.0.12; http://www.ebi.ac.uk/Tools/ clustalw2/), and similarity shading was applied using Boxshade (version 3.21). Dashes represent gaps introduced to maximize the alignment. A red bar above the residues indicates the predicted 3-oxo- 5α -steroid 4-dehydrogenase domains (pfam02544). Conserved glycine and histidine residues, important for steroi binding, are indicated by inverted triangles; asterisks indicate residues important for cofactor binding originally identified in a human DET2 ortholog (1, 2); black diamond marks a glutamic acid residue shown to be important for DET2 function in Japanese morning glory (*Ipomoea nil*) (3). Zm, *Zea mays*; Sb, *Sorghum bicolor*; Os, *Oryza sativa*; At, *Arabidopsis thaliana*; Ps, *Pisum sativum*; SI, *Solanum lycopersicum*; Hs, *Homo sapiens*.

- 1. Russell DW, Wilson JD (1994) Steroid 5 α-reductase: Two genes/two enzymes. Annu Rev Biochem 63:25-61.
- 2. Luo M, et al. (2007) GhDET2, a steroid 5α-reductase, plays an important role in cotton fiber cell initiation and elongation. Plant J 51:419-430.
- 3. Suzuki Y, et al. (2003) A dwarf mutant strain of Pharbitis nil, Uzukobito (kobito), has defective brassinosteroid biosynthesis. Plant J 36:401-410.



Fig. 52. Phylogenetic analysis of NA1 and selected 5α-steroid reductases. MEGA5 (1) was used to construct the phylogenetic tree based on the neighborjoining method (2). Alignment was performed using the ClustalW function in Mega5 with default settings. The optimal tree with the sum of branch length = 5.50177703 is shown. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the internal nodes (3). The evolutionary distances were computed using the JTT matrix-based method (4) and are in the units of the number of amino acid substitutions per site. All ambiguous positions were removed for each sequence pair. NA1 is highlighted in red. Monocotyledon cluster (yellow box), dicotyledon cluster (green box), and human 5α-steroid reductases (red box) are color-coded. Pt, *Populus tremuloides*; At, *Arabidopsis thaliana*; Gh, *Gossypium hirsutum*; Ps, *Pisum sativum*; Gm, *Glycine max*; Sl, *Solanum lycopersicon*; In, *Ipomoea nil*; Zm, *Zea mays*; Sb, *Sorghum bicolor*; Ta, *Triticum aestivum*; Bd, *Brachypodium distachyon*; Os, *Oryza sativa*; Pp, *Physcomitrella patens*; Hs, *Homo sapiens*.

- 1. Tamura K, et al. (2011) MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 28: 2731–2739.
- 2. Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-425.
- 3. Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.
- 4. Jones DT, Taylor WR, Thornton JM (1992) The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 8:275-282.



Fig. S3. Genetic complementation test of *na1-1* and *na1-2*. Pollen from homozygous *na1-1* plants was used to fertilize homozygous *na1-2* ears. (A) *na1-1* homozygous plant. (B) F1 plant of the cross between *na1-1* and *na1-2*. (Scale bars, 20 cm.)



Fig. 54. Additional phenotypes of *na1* mutants. (*A*) Mature *na1-1* siblings with leaves removed for better visibility of internodes. Red arrows indicate stem nodes. Internode length varied considerably between *na1* plants. (*B*) Length to width ratio for the first seven fully expended leaves of *na1-1* and WT. (*C*) Leaf seven of wild type (WT). (*D*) Twisting leaf seven of *na1-1*. (*E*) Light-grown WT and *na1-1* plants both treated with 360 μ M GA3 and 35 μ M UCZ. The increase and reduction in plant height due to GA3 and UCZ treatment, respectively, is similar between WT and *na1* plants. (*F*–*H*) Sex determination in ears is not altered in *na1* mutants. Ears of WT (*F*), *na1-1* (*G*), and *ts2* (*H*). Stamen primordia and the gynoecium of the LF did not mature in WT and *na1* mutant ear spikelets, resulting in evenly rowed kernels. In contrast, *ts2* ears display irregular kernel placement due to the development of the normally suppressed gynoecium of the LF (1). (Scale bars, 1 cm.) (*B* and *E*) Data are means \pm SD; *n* = 10 (*B*) and *n* = 9 (*E*).

1. DeLong A, Calderon-Urrea A, Dellaporta SL (1993) Sex determination gene TASSELSEED2 of maize encodes a short-chain alcohol dehydrogenase required for stage-specific floral organ abortion. Cell 74:757-768.



Fig. S5. Light and dark development of wild-type, *d5*, and *na1* seedlings. (*A* and *B*) Wild-type seedlings grown in the light (*A*) or dark (*B*). (*C* and *D*) GA deficient *d5* (1) grown in the light (*C*) or dark (*D*). (*E* and *F*) *na1-1* grown in the light (*E*) or dark (*F*). Red arrows indicate the position of first (coleoptile) node. Plants were grown as described in *Chemical Treatments*. (Scale bar, 10 cm.)

1. Fujioka S, et al. (1988) Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, dwarf-1, dwarf-2, dwarf-3, and dwarf-5 seedlings of Zea mays L. Plant Physiol 88:1367–1372.

Table S1. Characterization of na1 alleles

NANG

Allele	Mutation	Position	Orig. genetic background	Seed source
na1-1	<i>Mu</i> insertion; 9 bp target site duplication (5'-CCGCACGCT-3')	298–306	Mixed Mu-active	This work
na1-2	G to C transversion; results in nonsense substitution W163 > STOP	489	Unknown	G. Neuffer/Maize COOP
na1-3	Mu insertion; 9 bp target site duplication (5'-CGCGCAAGG-3')	758–766	Mixed Mu-active	This work
na1-4	Mu insertion; 9 bp target site duplication (5'-AGATCCCGA-3')	557–565	Mixed Mu-active	This work

To determine the position of a mutation in the na1 gene, the A of the na1 translational start (ATG) was counted as +1. Sequences of na1 mutant alleles were deposited in GenBank (na1-1, JN020029; na1-2, JN020030; na1-3, JN020031; na1-4, JN020032).

Table S2. Induction of tassel floret feminization after exogenous application of PCZ

Line	PCZ, μM	No. of plants	Dwarf phenotype	Staminate tassel florets	Up to 50% of tassel florets feminized	>50% of tassel florets feminized
Wild type	0	13	0	13	0	0
na1-1	0	13	13	7	5	1
Wild type	500	13	13	0	11	2
na1-1	500	13	13	0	4	9

Plants were grown as described in *Chemical Treatments* in Turface and watered with fertilizer solution supplemented with 500 µM PCZ. A dwarf phenotype was scored if the total height did not exceed one-third of wild-type height and the secondary *na1* mutant phenotypic features (dark-green, short, twisting leaves) were present.

Table S3. Endogenous BR profiles of wild-type and na1-1 plants for each independently grown biological replicate

BR intermediates	WT replica1, ng per g of fw	WT replica2, ng per g of fw	<i>na1</i> replica1, ng per g of fw	<i>na1</i> replica2, ng per g of fw
24-Methylenecholesterol	981	737	1,040	864
Campesterol	52,400	28,300	40,400	29,500
4-en-3-one	152	126	693	623
3-one	6.8	8.8	1.1	0.8
Campestanol	944	709	2.9	64.9
6-Deoxocathasterone	0.2	0.34	n.d.	0.05
6-Deoxoteasterone	0.01	0.05	n.d.	0.02
3-Dehydro-6-deoxoteasterone	0.35	0.21	n.d.	n.d.
6-Deoxotyphasterol	1.8	1.98	n.d.	0.23
6-Deoxocastasterone	5.18	6.26	n.d.	0.47
6-Oxocampestanol	8.1	19.9	4.9	14.4
Cathasterone	n.d.	n.d.	n.d.	n.d.
Teasterone	n.d.	n.d.	n.d.	0.09
Typhasterol	0.04	0.05	0.15	0.13
Castasterone	1.03	1.25	n.d.	0.13
Brassinolide	n.d.	n.d.	n.d.	n.d.

A pool of 20 g of fresh weight (WT replicate1, 20.71 g fw; WT replicate2, 20.05 g fw; *na1-1* replicate1, 20.57 g fw; and *na1-1* replicate2, 20.88 g fw) from 10 individuals was analyzed for each replicate. n.d., not detected.

Sequence ID	Organism	GenBank accession (Gene ID)	
AtDET2	Arabidopsis thaliana	NP_181340 (At2g38050)	
BdDET2	Brachypodium distachyon	(Bradi2G55110)	
GhDET2	Gossypium hirsutum	AAZ83346	
GmDET2	Glycine max	AAG35638	
HmSRD5A1	Homo sapiens	NP_001038	
HmSRD5A2	Homo sapiens	NP_000339	
InDET2	Ipomoea nil	BAC87862	
OsDET2-1	Oryza sativa	NP_001044822 (Os01g0851600)	
OsDET2-1	Oryza sativa	NP_001176391 (Os11g0184100)	
PpDET2	Physcomitrella patens	XP_001769717	
PsDET2	Pisum sativum	AAT76665	
PtDET2-1	Populus temuloides	XP_002323554	
PtDET2-2	Populus temuloides	XP_002319902	
PtDET2-3	Populus temuloides	XP_002328371	
SbDET2	Sorghum bicolor	JN020028 (Sb03g040050)	
SIDET2	Solanum lycopersicon	CAH05260	
TaDET2	Triticum aestivum	CBH32517	
ZmDET2/NA1	Zea mays	NP_001149816	

Γable S4.	5α-steroid reductase	s used in the	phylogenetic	analysis of	ZmDET2/NA1
-----------	----------------------	---------------	--------------	-------------	------------

PNAS PNAS