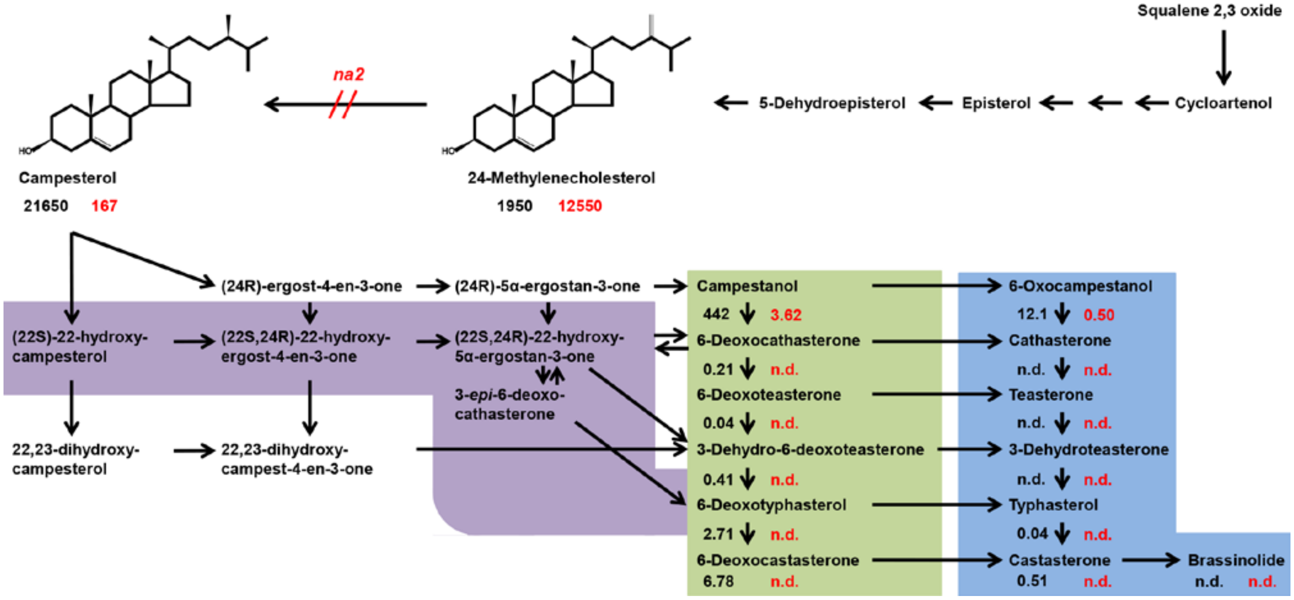

Figure S1. The proposed brassinosteroid pathway and measured intermediates from *na2-1* and wild-type plants.

Brassinosteroid intermediates in ng/g fresh weight from 28d old wild-type and *na2-1* plants are presented in black and red text, respectively. The early C-22 oxidation pathway is highlighted in purple, the late C-6 oxidation pathway is highlighted in green, and the early C-6 oxidation pathway is highlighted in blue. Data are means of two independent experiments of 10 plants each. n.d., not detected. Proposed brassinosteroid pathway modified from Nakamura et al., 2009; Ohnishi et al., 2012; Verhoef et al., 2013; Chung and Choe, 2013.

Chung Y, Choe S (2013) The regulation of brassinosteroid biosynthesis in Arabidopsis. Crit Rev Plant Sci 32: 396–410

Nakamura A, Fujioka S, Takatsuto S, Tsujimoto M, Kitano H, Yoshida S, Asami T, Nakano T (2009) Involvement of C-22-hydroxylated brassinosteroids in auxin-induced lamina joint bending in rice. Plant Cell Physiol 50: 1627–1635

Ohnishi T, Godza B, Watanabe B, Fujioka S, Hategan L, Ide K, Shibata K, Yokota T, Szekeres M, Mizutani M (2012) CYP90A1/CPD, a brassinosteroid biosynthetic cytochrome P450 of Arabidopsis, catalyzes C-3 oxidation. J Biol Chem 287: 31551–31560

Verhoef N, Yokota T, Shibata K, De Boer GJ, Gerats T, Vandenbussche M, Koes R, Souer E (2013) Brassinosteroid biosynthesis and signalling in *Petunia hybrida*. J Exp Bot 64: 2435–2448

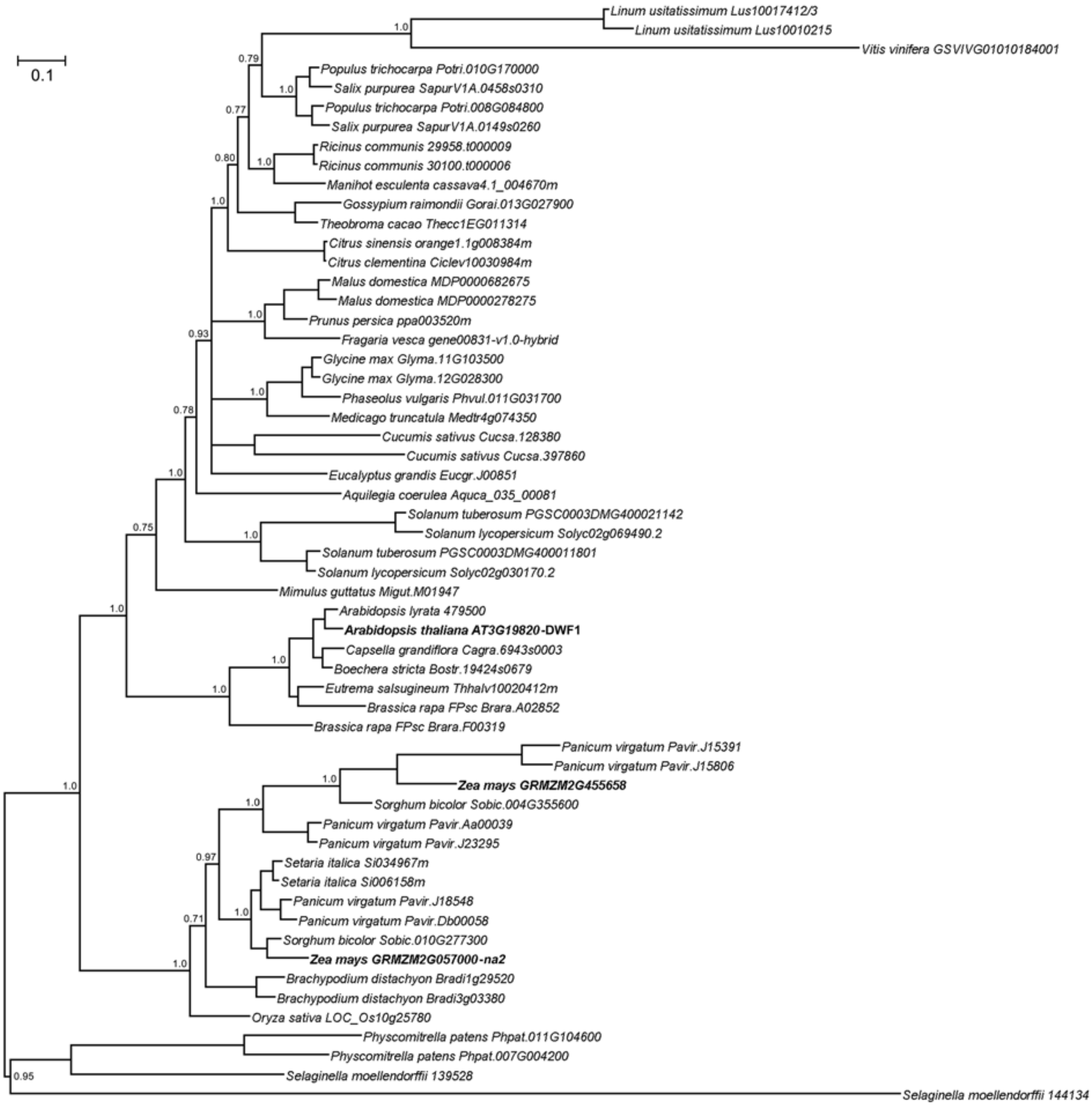

Figure S2. Phylogenetic tree of presumed Δ^{24}-sterol reductases from 36 taxa.

Presented is a maximum likelihood, Bayesian MCMC, phylogenetic consensus tree of conceptually-translated protein sequences derived from the genes encoding likely Δ^{24}-sterol reductases identified within plant whole genome assemblies in Phytozome. All genes annotated as similar to GRMZM2G057000 with an alignment score greater than 1500 and sequence similarity greater than 50% were included in the analysis. Scale bar indicates the number of amino acid changes per orthologous site. Posterior probabilities for selected nodes are indicated. The Arabidopsis *DWF1*, maize *na2*, and the gene encoded by the maize paralog of *na2* are indicated by bold text.

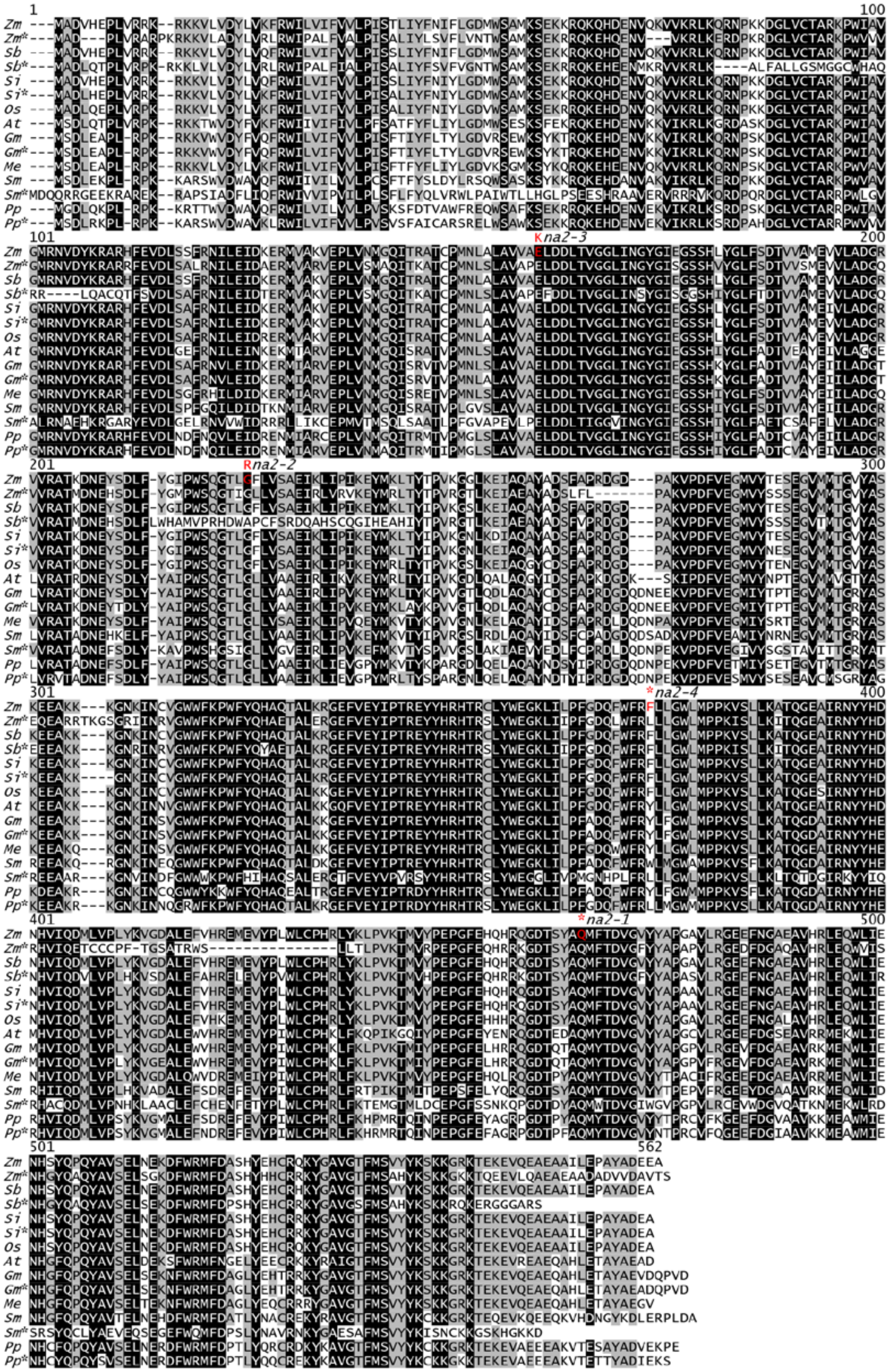
Zm
Zm*
Sb
Sb*
Si
Si*
Os
At
Gm
Gm*
Me
Sm
Sm*
Pp
Pp*
1
100
101
200
K na2-3
201
300
R na2-2
301
400
*na2-4
401
500
*na2-1
501
562

Figure S3. Multiple sequence alignment of *Zea mays* NA2 protein sequence with orthologous sequences.

Red amino acids indicate the SNP induced amino acid changes and are denoted above the sequence in *na2-1*, Q to *; *na2-2*, G to R; *na2-3*, E to K; and *na2-4*, G to *. Black background indicates identity conservation of ≥90%, grey indicates conservation of ≥50%, and white indicates conservation of ≤50%. Dashed lines indicate gaps in the alignment. *Zm, Zea mays* (GRMZM2G057000); *Zm**, *Zea mays* (GRMZM2G455658); *Sb*, *Sorghum bicolor* (Sobic.010G277300); *Sb**, *Sorghum bicolor* (Sobic.004G355600); *Si*, *Setaria italic* (Si006158m); *Si**, *Setaria italic* (Si034967m); *Os*, *Oryza sativa* (LOC_Os10g25780); *At*, *Arabidopsis thaliana* (AT3G19820); *Gm*, *Glycine max* (Glyma12g03210); *Gm**, *Glycine max* (Glyma11g103500); *Me*, *Manihot esculenta* (cassava4.1_004670m.g); *Sm*, *Selaginella moellendorfii* (139528); *Sm**, *Selaginella moellendorfii* (144134); *Pp*, *Physcomitrella patens* (Phpat.007G004200); *Pp**, *Physcomitrella patens* (Phpat.011G104600).

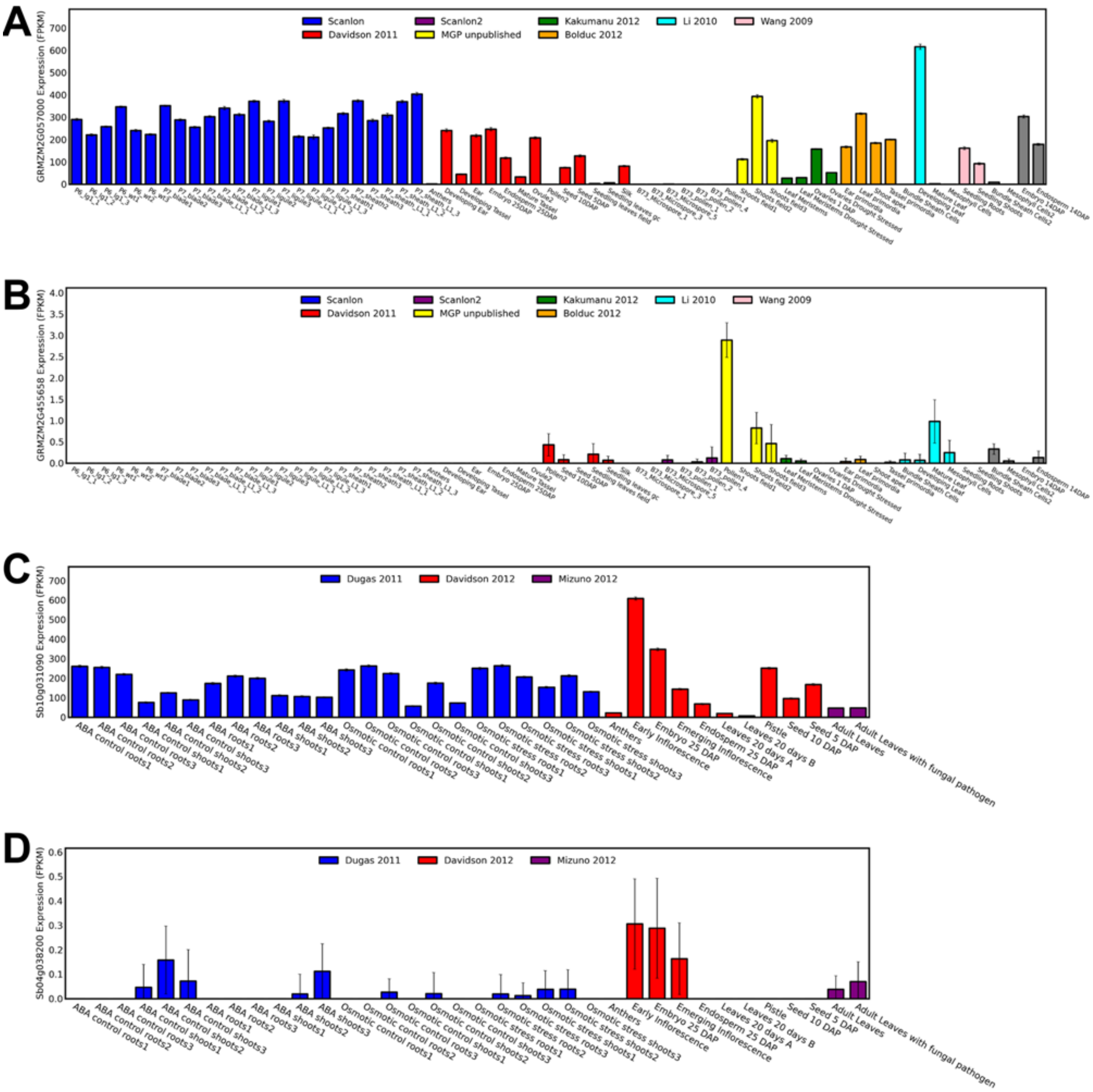

Figure S4. RNA expression levels of maize or sorghum genes from qTeller dataset.

RNA expression FPKM values from previously published RNA-seq experiments for (A) GRMZM2G057000 (*na2*), (B) GRMZM2G455658, (C) Sb10g031090 (Sobic.010G277300), and (D) Sb04g038200 (Sobic.004G355600). Data are taken from the from qTeller database. Figures can be regenerated using the following links:

GRMZM2G057000:

http://qteller.com/qteller3/bar_chart.php?name=GRMZM2G057000&info=

GRMZM2G455658:

http://qteller.com/qteller3/bar_chart.php?name=GRMZM2G455658&info=

Sb10g031090 (Sobic.010G277300):

http://qteller.com/sorghum/bar_chart.php?name=Sb10g031090&info=

Sb04g038200 (Sobic.004G355600):

http://qteller.com/sorghum/bar_chart.php?name=Sb04g038200&info=

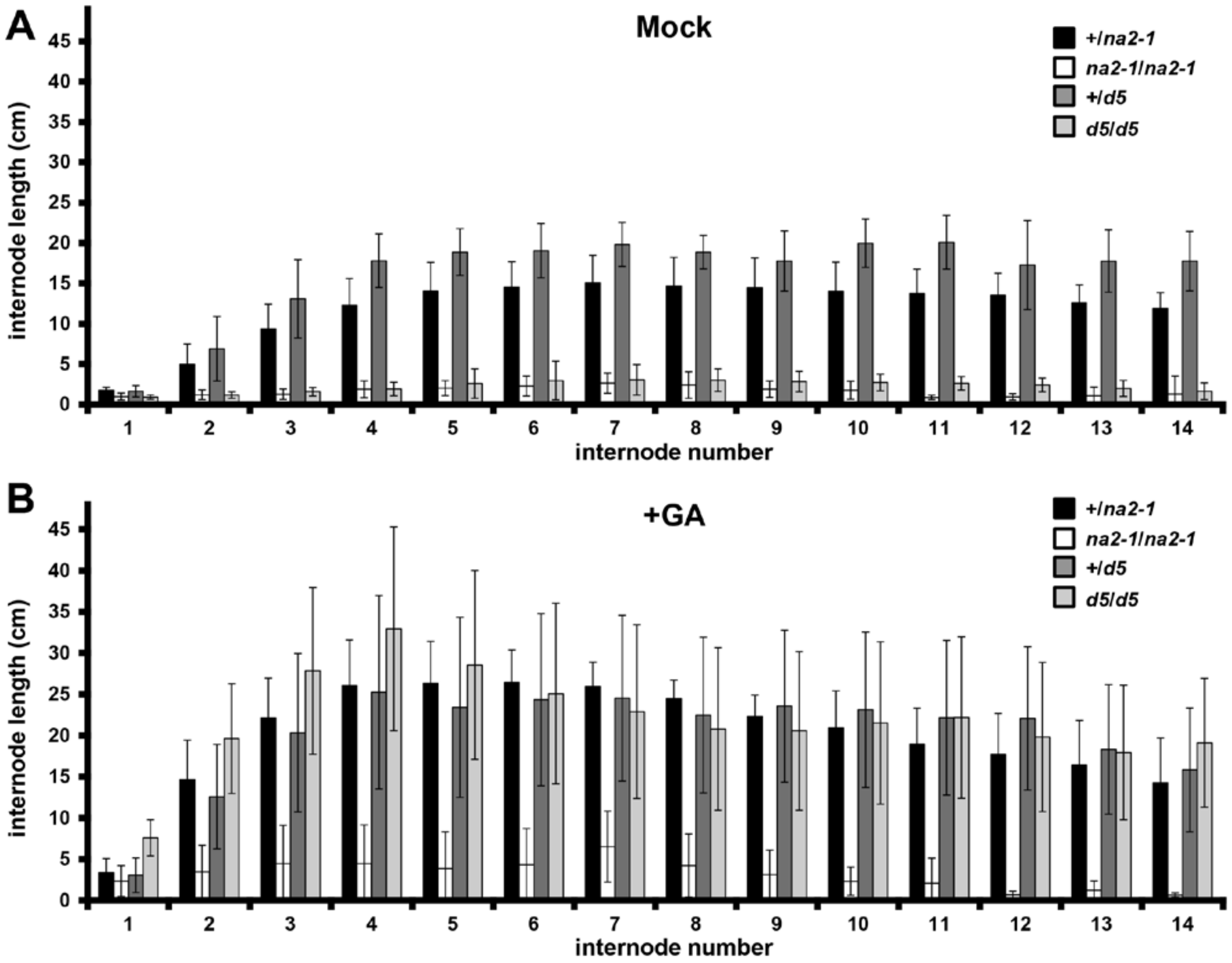

Figure S5. Internode lengths of *na2-1*, *d5*, and wild-type siblings with and without GA_3 application.

(A) Internode lengths in centimeters measured at maturity under mock conditions. (B) Internode lengths in centimeters measured at maturity treated with GA_3. 1 mL GA_3 application was applied to the leaf whorl at a concentration of 866 µM every third day, starting 25 d after sowing. (A-B) Errors bars represent ± standard deviation**.**

Table SI. Segregation ratios of crosses.

Site	Ear #	Mother	Father	WT	mut	TOT	χ^2 *p*-val
WL2011	CN833	+/*na1-1*	*na2-1*/*na2-1*	31	0	31	2.58E-08
WL2011	CN1393	+/*na2-3*	*na1-1*/*na1-1*	15	0	15	0.0001
WL2011	CN1394	+/*na2-3*	*na1-1*/*na1-1*	18	0	18	2.21E-05
WL2011	CN1395	+/*na2-3*	*na1-1*/*na1-1*	17	0	17	3.74E-05
WL2011	CN841	+/*na2-7*	*na1-1*/*na1-1*	32	0	32	1.54E-08
GH2011	MA45	+/*na2-506D*	*na2-1*/*na2-1*	40	31	71	0.2854
GH2011	MA46	+/*na2-506D*	*na2-1*/*na2-1*	33	28	61	0.5221
WL2011	CN857	+/*na2-1*	*na2-518C*/*na2-518C*	16	15	31	0.8575
WL2011	CN828	+/*na2-7*	*na2-506D*/*na2-506D*	18	14	32	0.4795
WL2011	CN939	+/*na2-7*	*na2-518C*/*na2-518C*	9	8	17	0.8084
WL2011	CN745	+/*na2-7*	*na2-1*/*na2-1*	17	15	32	0.7327
WL2010	CN560	+/*na2-7*	*na2-1*/*na2-1*	13	2	15	0.0045
WL2010	CN561	+/*na2-7*	*na2-1*/*na2-1*	6	8	14	0.5930
WL2010	CN578	+/*na2-7*	*na2-1*/*na2-1*	8	9	17	0.8084
GH2010	CN673	+/*na2-2*	*na2-7*/*na2-7*	19	16	35	0.6121
GH2010	CN674	+/*na2-2*	*na2-7*/*na2-7*	16	15	31	0.8575
GH2010	CN646	+/*na2-3*	*na2-7*/*na2-7*	15	13	28	0.7055
GH2010	CN648	+/*na2-3*	*na2-7*/*na2-7*	19	15	34	0.4927
GH2011	MA37	+/*na2-4*	*na2-506D*/*na2-506D*	4	2	6	0.4142
GH2011	MA38	+/*na2-4*	*na2-506D*/*na2-506D*	14	11	25	0.5485
GH2011	MA160	+/*na2-4*	*na2-506D*/*na2-506D*	28	26	54	0.7855
WL2011	CN1384	+/*na2-1*	*na2-6*/*na2-6*	13	11	24	0.6831

Table SII. Stomatal indices and densities of *na2-1*, *na2-2*, and wild-type plants.

	+/na2-1	*na2-1/na2-1*	*+/na2-2*	*na2-2/na2-2*
SI abaxial[a]	15.7 ±1.2	16.9 ±1.7	17.4 ±1.6	18.7 ±2.2
SD abaxial[b]	158.5 ±29.4	166.2 ±15.5	181.4 ±24.7	192.8 ±20.2
SI adaxial[a]	10.5 ±0.7	12.1 ±1.7	12.1 ±2.3	11.8 ±1.1
SD adaxial[b]	110.4 ±14.2	121.8 ±17.4	115.4 ±20.7	109.1 ±10.4
RS adaxial/abaxial[c]	0.696	0.733	0.636	0.566

[a]SI, stomatal index, [b]SD, stomatal density, and [c]RS, ratio of stomata, were measured at the widest portion of leaf 5 of 30 d old plants.

Data is presented as means with standard deviation of 6 biological replicates.

Table SIII. Source of maize genetic stocks.

Allele	Source	Stock Name	Genetic Background
na2-1	Maize COOP	4407D d*-N2374	Unknown
na2-2	Maize TILLING	PB03NM417	B73
na2-3	Maize TILLING	04INW22CW04351	W22
na2-4	Maize TILLING	04IAB73PS020G1	B73
na2-5	Identified in *Mu*-tagging	na2-*Mu*13 (*Mu*#8)	Unknown
na2-6	Maize TILLING	04CAB73SH0365	B73
na2-7	Maize COOP	4405B d*-N1074C	Unknown
na2-506D	Maize COOP	506D	Unknown
na2-518C	Maize COOP	518C	Hi27
na1-1	Maize COOP	310G	Unknown
d5	Carolina Bio Sup Co	177110	Unknown
d1	Maize COOP	302A d1-6016	Unknown

Table SIV. Primers used in this study.

Primer Name	Primer sequence- 5’ to 3’
Genomic	
ZmDWF1_-988FOR	GTGTCAACTGGTAGGATAATAGCCCA
ZmDWF1_-440FOR	GTGTGCATATTCTCAAGGCTGTGG
ZmDWF1_-163FOR	CAGATAACAATGTGCTTCCTTCAGC
ZmDWF1_-163REV	GCTGAAGGAAGCACATTGTTATCTG
ZmDWF1_-21FOR	AACGACCACGAGCTTTTAGGC
ZmDWF1_+144FOR	CTTCAACATCTTCCTGGGCGAC
ZmDWF1_+144REV	GTCGCCCAGGAAGATGTTGAAG
ZmDWF1_+675FOR	CACTGGGGTTCCTTGTCTCTGCG
ZmDWF1_+675REV	CGCAGAGACAAGGAACCCCAGTG
ZmDWF1_+1168FOR	GCTATCAGGAACTACTACCACGACAACC
ZmDWF1_+1168REV	GGTTGTCGTGGTAGTAGTTCCTGATAGC
ZmDWF1_+1243FOR	TACAATGGCTGGCTGGTGCT
ZmDWF1_+1243REV	AGCACCAGCCAGCCATTGTA
ZmDWF1_+1850REV	TGGATGTAGTGTGTGTCTGTCTCAAG
ZmDWF1_na2-1DC	GTAGTACACGCCCACGTCCGTGAACAGCT
MuExt22D	CCAACGCCAWSGCCTCYATTTC
MuInt19	GCCTCYATTTCGTCGAATC
qRT-PCR	
ZmDWF1qRT_FOR2	GAGCAGTGGCTGATCGAGAAC
ZmDWF1qRT_REV2	AACATGCGCCAGAAGTCCTT
ZmMOLqRT_FOR2	CTGTGTCCTCCGTGCTCCAT
ZmMOLqRT_REV2	AGGACTCCCGCATCTCCATA

Table SV. Two independent experiments showing BR intermediates in wild-type and *na2-1* plants.

	Exp. 1		Exp. 2	
Compound[a]	+/*na2-1*	*na2-1*/*na2-1*	+/*na2-1*	*na2-1*/*na2-1*
24-methylenecholesterol	2040	14000	1860	11100
campesterol	25400	190	17900	143
campestanol	555	6.10	329	1.13
6-oxocampestanol	12.5	0.35	11.6	0.64
6-deoxocathasterone	0.19	n.d.[b]	0.23	n.d.[b]
6-deoxoteasterone	0.02	n.d.[b]	0.05	n.d.[b]
3-dehydro-6-deoxoteasterone	0.55	n.d.[b]	0.27	n.d.[b]
6-deoxotyphasterol	3.09	n.d.[b]	2.32	n.d.[b]
6-deoxocastasterone	7.71	n.d.[b]	5.84	n.d.[b]
cathasterone	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]
teasterone	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]
3-dehydroteasterone	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]
typhasterol	0.03	n.d.[b]	0.04	n.d.[b]
castasterone	0.58	n.d.[b]	0.43	n.d.[b]
brassinolide	n.d.[b]	n.d.[b]	n.d.[b]	n.d.[b]

[a] ng/g fresh weight from 28 DAG plants.

[b] n.d., not detected.