Supplementary Data

Article title: *ZAXINONE SYNTHASE 2* regulates growth and arbuscular mycorrhizal symbiosis in rice

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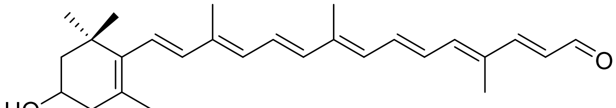
The following Supporting Data is available for this article:



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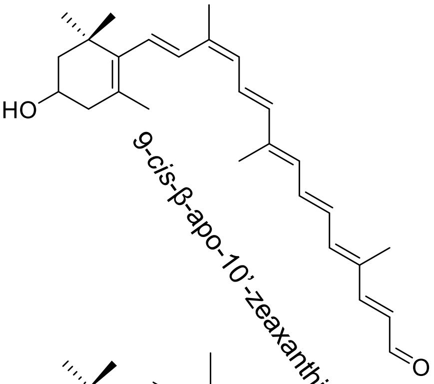

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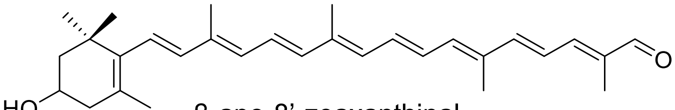

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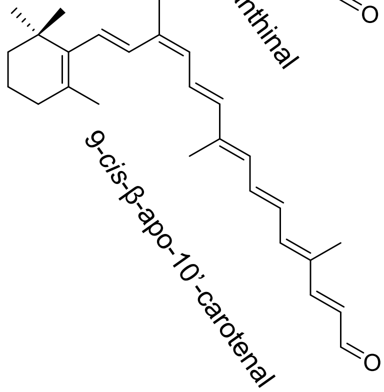

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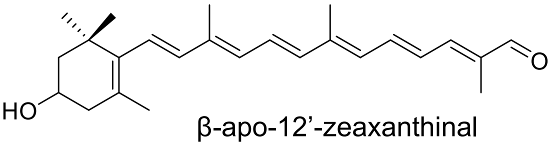

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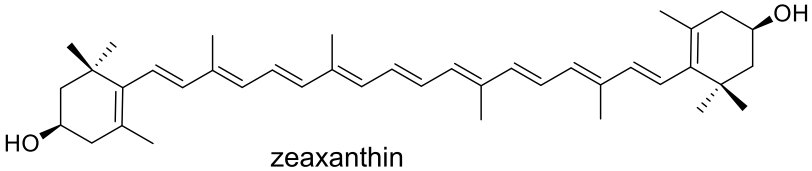
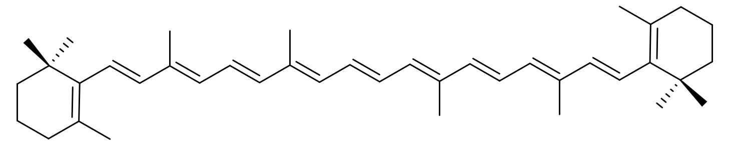
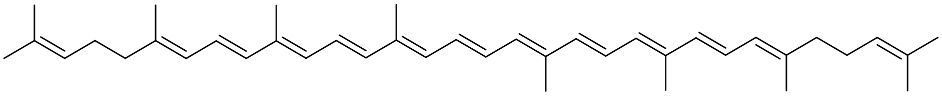


zeaxanthin

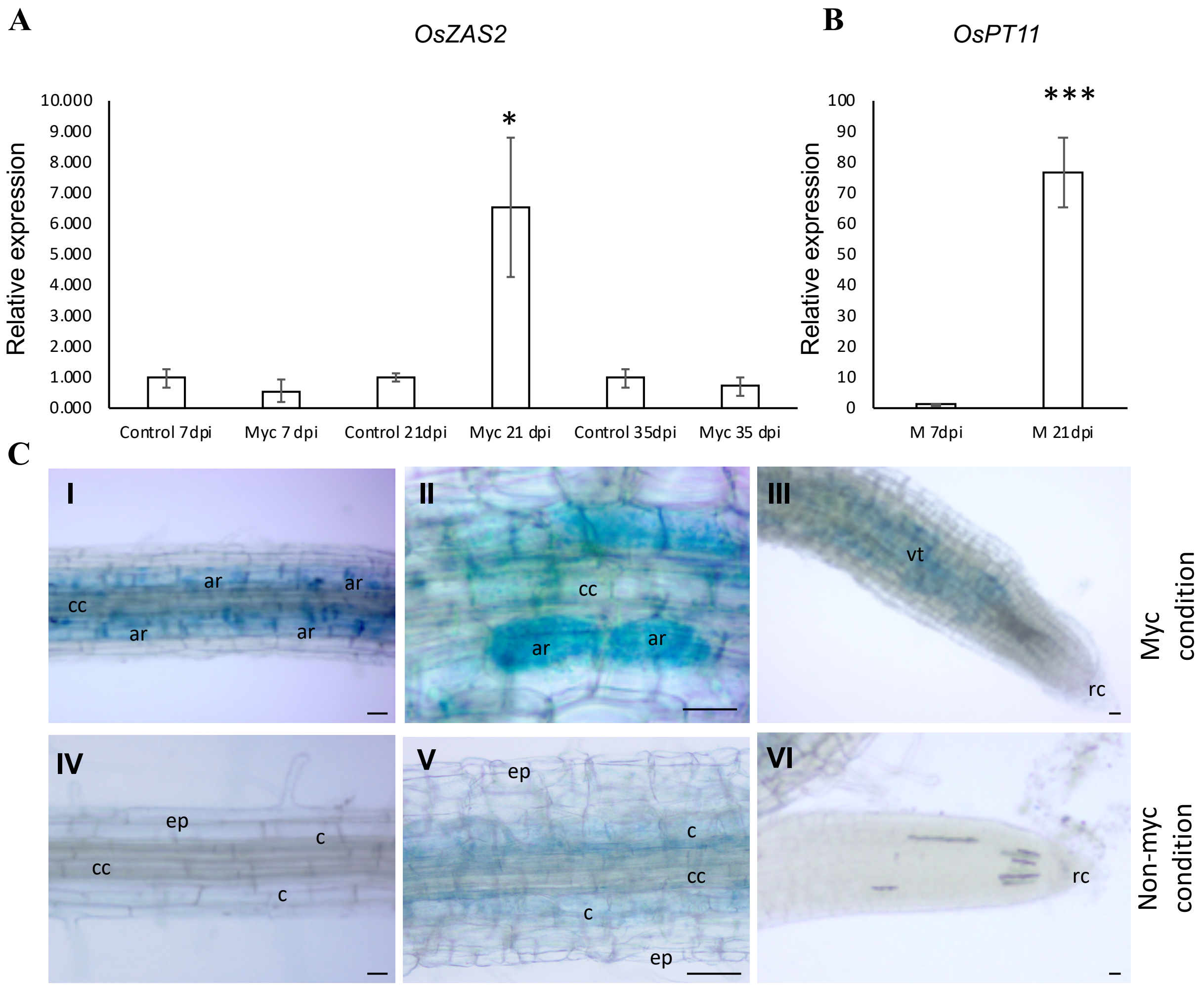


β-carotene

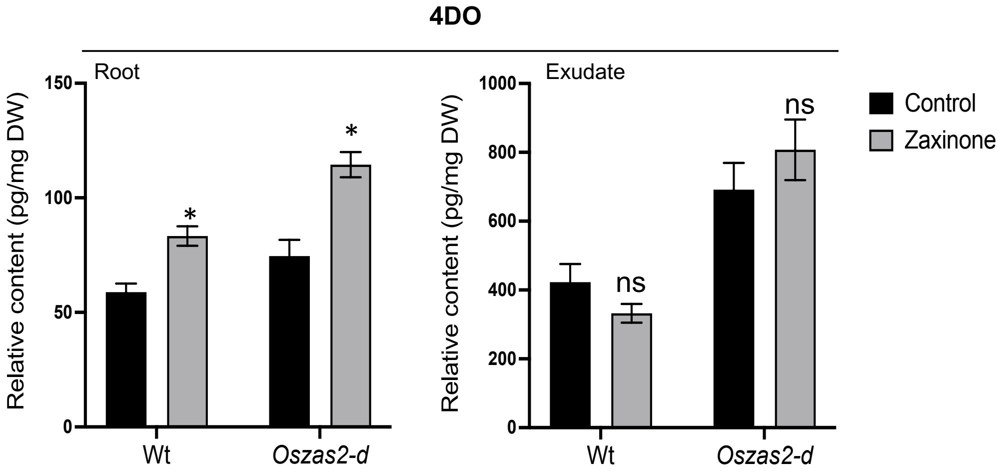


lycopene

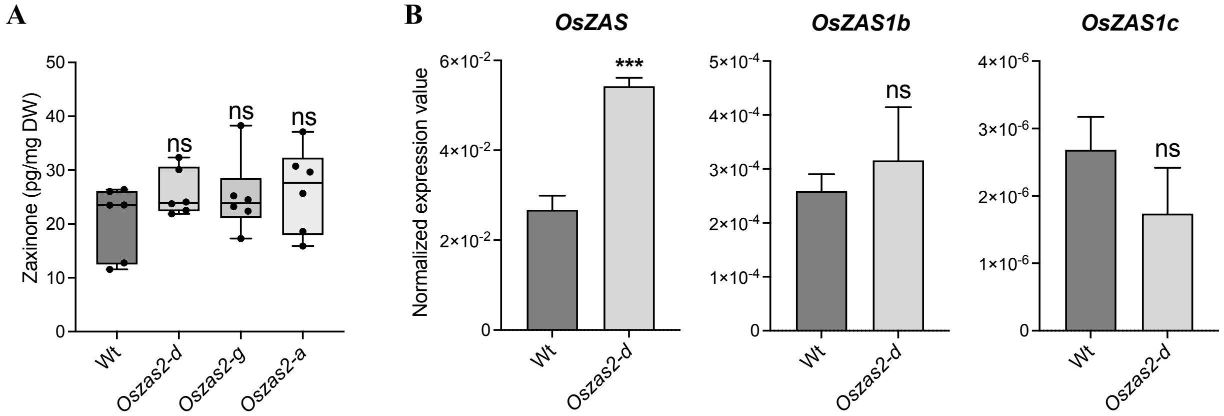
Supplemental Figure S1. Structures of carotenoids and apocarotenoids used as substrates in OsZAS2 *in vitro* and *vivo* assays.



Supplemental Figure S2. *OsZAS2* expression during arbuscular mycorrhizal (AM) establishment. A, *OsZAS2* expression pattern at a different stage of mycorrhizal colonization (*R. irregularis*). B, *OsPT11 (*AM marker gene*)* expression pattern at a different stage of mycorrhizal colonization (*R. irregularis*). C, GUS staining analysis of roots of *pZAS2:GUS-L11* reporter line inoculated (I, II, III) for 35 days with *F. mosseae* and non-inoculated (IV, V, VI). cc, central cylinder; c, non-colonized cortical cells; ep, epidermal cells; vt, vascular tissue; ar, arbuscule containing cells; rc, root cap; dpi, day post-inoculation. Bars in all panels correspond to 50 µm. Values in (A-B) are means ±SD ($n \geq 3$) and student's t-test was applied for the statistical analysis (*$P \leq 0.05$).



Supplemental Figure S3. Relative content of 4DO after zaxinone (5 µM) treatment in root tissue and exudate of Wt and *Oszas2* mutant. DW, dry weight. Values are means ±SD (n ≥ 5). Student's t-test was applied for the statistical analysis (*P ≤ 0.05; **P ≤ 0.01; ns: not significant).



Supplemental Figure S4. Zaxinone quantification and *OsZAS* genes expression analysis in *Oszas2* mutants under low Pi conditions. A, Quantification of zaxinone content in Wt and *Oszas2* mutants roots under low Pi condition. Boxes in boxplots represent the median, first and third quartile. The minimum and maximum values are showed with the length of the whiskers. Dots represent the biological replicates. B, *OsZAS* genes expression analysis in Wt and *Oszas2-d* under low Pi condition. Values in (A-B) are means ±SD ($n \geq 3$) and student's t-test was applied for the statistical analysis (***$P \leq 0.001$; ns, not significant).

Ref (Wt): MFRAGGCSSQVTRCIHLGKSPADQASSLSPPNAVKVQSSVTGWNKQPLMQELTKALKSVSSDLLDRFIDSVYKFSEQPYLNE/---/PQRVPYGFHGNFFYK* 593 aa

Oszas2-d: MFRAGGCSSQVIHLGKSPADQASSLSPPNAVKVQSSVTGWNKQPLMQELTKALKSVSSAGPIHRLRVQVF* 71 aa

Oszas2-g: MFRAGGCIHLGKSPADQASSLSPPNAVKVQSSVTGWNKQPLMQELTKALKSVSSDLLGASSTPCTSFLSSLI* 73 aa

Oszas2-a: MFRAGGCSSQMHSSREEPS* 20 aa

Supplemental Figure S5. Truncated amino acid sequences of *Oszas2* mutant lines after CRISPR-Cas9 genome editing. **aa, amino acid.**

OsZAS2 (tRNA-gRNA1-tRNA-gRNA2)

Standard synthesis of *OsZAS2* (tRNA-gRNA1 -tRNA-gRNA2), including added 5'UTR (GGATGGGCAGTCTGGGCA) and 3'UTR (GTTTGCTGTCGCTCATCC) sequences, with sequence verification and custom cloning into pRGEB32 (Kanamycin) via 5’BsaI and 3’BsaI; delivered as a mini-scale DNA sample.

OsZAS2 (tRNA-gRNA1-tRNA-gRNA2)|Length: 391 |Vector: pRGEB32

GGATGGGCAGTCTGGGCAAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGGTACAGACCCGGGTTC
GATTCCCGGCTGGTGCAGATGAATGCATCTAGTGACCGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGT
TATCAACTTGAAAAAGTGGCACCGAGTCGGTGCAACAAAGCACCAGTGGTCTAGTGGTAGAATAGTACCCTGCCACGG
TACAGACCCGGGTTCGATTCCCGGCTGGTGCAATCAGTCTCTTCAGATCTGCGTTTTAGAGCTAGAAATAGCAAGTTAAA
ATAAGGCTAGTCCGTTATCAACTTGAAAAAGTGGCACCGAGTCGGTGCTTTTTTTTTGTTTGCTGTCGCTCATCC

The sequences are annotated as follows:
Fok I site: green background and restriction site underlined
gRNA scaffold: yellow background
pre-tRNA: turquoise background
gRNA spacer: red letters

Supplemental Figure S6. gRNA targets of OsZAS2 were fused to tRNA sequences (Xie et al., 2015).

Supplemental File S1. Clustal alignment of ZAS members.

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                             20                  40                  60                  80
NaZAS4     MTFCSYTFQ---VN------------------------------------------------------------------ 11
NtaZAS4a   MAFCSYTFQ---VN------------------------------------------------------------------ 11
NtaZAS4a   MAFCSYTFQ---VN------------------------------------------------------------------ 11
NtoZAS4    -------------------------------------------------------------------------------- –
SlZAS4     MAFCSYTCK---VN------------------------------------------------------------------ 11
SpZAS4     MAFCSYTRK---VN------------------------------------------------------------------ 11
StZAS4b    MALCSYTCK---VN------------------------------------------------------------------ 11
StZAS4a    MALRSYTFK---VN------------------------------------------------------------------ 11
SpZAS3b    MAAYIYACQ---VN------------------------------------------------------------------ 11
SlZAS3b    MATYIYACQ---VN------------------------------------------------------------------ 11
StZAS3b    -------------------------------------------------------------------------------- –
SpZAS3a    MAVCSYTCQ---VN------------------------------------------------------------------ 11
SlZAS3a    M------------------------------------------------------------------------------- 1
StZAS3a    M------------------------------------------------------------------------------- 1
CanZAS3    MVACIYACQ---VN------------------------------------------------------------------ 11
NaZAS5     MAAYSYAL-QVNKC------------------------------------------------------------------ 13
NtaZAS5    M------------------------------------------------------------------------------- 1
StZAS5     MTANSYALLQVVTK------------------------------------------------------------------ 14
BdZAS1a    MCYKLRSNSSHDCRKIRRVSI------------------------RML--TTSSPRTFRPSRTC-RPA---FSLSARTSS 50
AtaZAS1a   MEV---NRPPHDCRRIRLCHYQKS---------------------RMLKATTSPPNALRPSRAYYRPA---FSLSATTSS 53
OsZAS      MM----TASLHPC------------------------------------VCKASP--------AFRPA---SSLGARTQP 29
SbZAS1a    MVLKASAS-----------------------------------------FSPSLPLPLSCRINGGRPS-MSMSAGARTAA 38
ZmZAS1     M--KASAP-----------------------------------------FSPSQPL--SCRTHGGRPSSMSMSAGARTAT 35
OsZAS1b    MCIQGDQKR-----------------------CHPCIPF-------NHAYASPAPGHPSPLK--------LLVYLIRKHP 42
AtaZAS1b   MAVCRDTTSNFVGSSVLVLHGITNATNLEAIACREGLSLAQDLMLQNFVITSDSKQVVNDIKRANRGGYGAVIIEIRQCL 80
OsZAS1c    MVVQKEQHY---------------------------------WNTKT--------INHKP-------------------- 19
BdZAS1c    MVVAAYMPVSSLGQ-------------------RMSFPRIRPWPANTRPAATSSPMASVP-------------------- 41
ZmZAS2     MPLLSLPACCGYDAMFRAGLKPTTSSSRCRSSRLIRSHGGAPDHAPP-FT------------------------------ 49
SbZAS2a    M--------------FRAGLKPTTTSS---SSRCIHSRR-APDHALP-FN------------------------------ 31
SbZAS2b    M--------------FRDGLKPTNSCR----CRCIRSHRAPADHALPCFT------------------------------ 32
OsZAS2     M--------------FRAG------GCSSQVTRCIHLGKSPADQA-SSLS------------------------------ 29
ObZAS2     M--------------FRAA------GCRSQVTRCIHLGKG-TDRS-SSLS------------------------------ 28
AcZAS2     MNSMSMIK-----------------------ARSIH--KSPPT---STLT------------------------------ 22
Consensus  MA--SYT-------------------------------------------------------------------------
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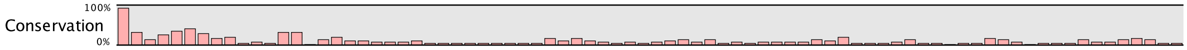

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NtaZAS4a   -----CSYQRPSIPSKVQDLKVSISSALNP-LSRDLLHFPIVAADIPKAI-KETSFKLLDAFVDLVFEFVDQPLLPSQSN 84
NtaZAS4a   -----CSFQRPSVPSKVQDLRGSISSALNP-ISRELVHFPIV--NIPKAI-KETSFKLLDAFVDLAFEFVDQPLLPSQSN 82
NtoZAS4    --------------------------------------------------------------------FVDQPLLPSQSN 12
SlZAS4     -----CFFQRPSVPSKVEDLKDSISSALKP-FSRDLVHFSIVA-DIPKAV-KETSIKLLDAFVDSVFEFVDQPLLPSQSN 83
SpZAS4     -----CFFQRPSVPSKVEDLKASISSALKP-FSRDLVHFSIVA-DIPKAV-KETSIKLLHAFVDSVFEFVDQPLLPSQSN 83
StZAS4b    -----CSFQRPSIPSKVEDLKDSISSALKP-FSRDLVHFSIVA-DIPKVV-KETSFKLLDAFVDFVFEFVDQPLLPSQSN 83
StZAS4a    -----CSIQRPSIPTKDEDLKASISSALKP-FSRNLVHFPVVA-DIPKVV-KETSIKLLDAFVDLVFEFVDQPLLPSQSN 83
SpZAS3b    -----YCCLKKPLIPKFKDLRASVSSTFKP-FLSNLEN-----LDVQKVI-KEISFKLLDNFVDFTFEFLDQPLLPSQSN 79
SlZAS3b    -----FCCLKKPLIPKFKDLRASVSSTFKP-FLSNLEN-----LDVQKVI-KEISFKLLDNFVDFTFEFVDQPLLPSQSN 79
StZAS3b    -----------------------------------------------------------------------QPLLPSQSN 9
SpZAS3a    -----YCCLQRHVIPKFKDLRASVSSNFKP-FLTNLEHFPLI-MDVPKVILKEVIFKLLDNFIDLTFEFVDQPLSPSQSN 84
SlZAS3a    --------------------------------------------DVPKVILKEVIFKLLDNFIDLTFEFVDQPLSPSQSN 37
StZAS3a    --------------------------------------------DGMKLGIGAISGRNL--------EVGWRPPIRAEHK 29
CanZAS3    -----YCCSKKPLIPKFKDLKASISSTLKP-FLTNLEHFPLI-MDVPKAT-KETSFKLLDTFVDLIFEFVDQPLLPSKSN 83
NaZAS5     -----SSPQRPSLPQKIEHFSNPLSTSFKP-LLRGLDQFPNISVDVTKAI-KKTSCKLLDAFVDFIFEFVGQPLLPSQRN 86
NtaZAS5    -----------------------------------------------------------------VWSIIA--------- 7
StZAS5     -----CSLQRPSLPPKLEHSKNPLSSTFKPILLKELDHFPNISLDVPKAI-KDTSFNLLEAFVDFIYEFVDQPLLPCQSN 88
BdZAS1a    SSITA-----NSKKPLFQELQRQLSLKI----------------DDASQILKNAPHRLLDAIVDSTFKFTAQALLPSESN 109
AtaZAS1a   SSLTS-----NPKKSLIQELRRQLSSTI----------------DDASQVLKNAPQKLLDAIVDSTFKFTHQALLPSESN 112
OsZAS      KSTAT-----NPKRPLFQELQRRLSFRI----------------DEASKALETAKQGLLDALVDSTFKFSDQPMLPSENN 88
SbZAS1a    -SVGT-----SSQKPLLGELLGNLSSKM----------------DRASKALKDVPQRFLDVLVDATFKFTDEALNPSESN 96
ZmZAS1     -SVGT-----SSKRPWFGDLLGRLSSTM----------------DGASRALKDAPQRFLDALVDATFRFTDQALNSAESN 93
OsZAS1b    L---------------EREIQVHLSSKLG----------------EASNAMNSTYQQLLDSFVDHTFTFKCQPLRPTESN 91
AtaZAS1b   LAFNCIITFKGTTANNDADMLAKFAHSLGTC----------DVFNSSSNALNYASQRLLDAFVGTVFTLSHQPLRPTEGN 150
OsZAS1c    ---------------HFKEFQQQLTTKLG----------------EASAAVTMASSLLLDAFVDSTFTFSHQSLRPTESN 68
BdZAS1c    ---------------YFKELQQQITSKLA----------------EASER-------LLDAFVDSTFTFSNQSLRPTESN 83
ZmZAS2     -------------QPNSVKVPPS------PGLKKQ-----ALSVQDMSRALKSLSSDLLQRFVDRAYSFSEEPSL-NEGN 104
SbZAS2a    -------------PPNAIK-----------------------------------------------------------GN 39
SbZAS2b    -------------PPNAIKVPPS------PGLKKQ-----ALILQGISQALKSVSSNLLERFIDRAYRFSEQPSL-NEGN 87
OsZAS2     -------------PPNAVKVQSS-----VTGWNKQ-----PL-MQELTKALKSVSSDLLDRFIDSVYKFSEQPYL-NEGN 84
ObZAS2     -------------PPNAIKVQSS-----VTGWKKQ-----TL-IQELTKTLKSVSLDLLDRFIDSAYKFSEQPFL-NEGN 83
AcZAS2     -------------PHNFISSLSSPFKTLLKEWEKL-----PLRI-EVSNSMKAASLNLVDAFVDWMYKFSDLPAL-IEEN 82
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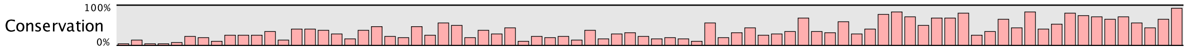
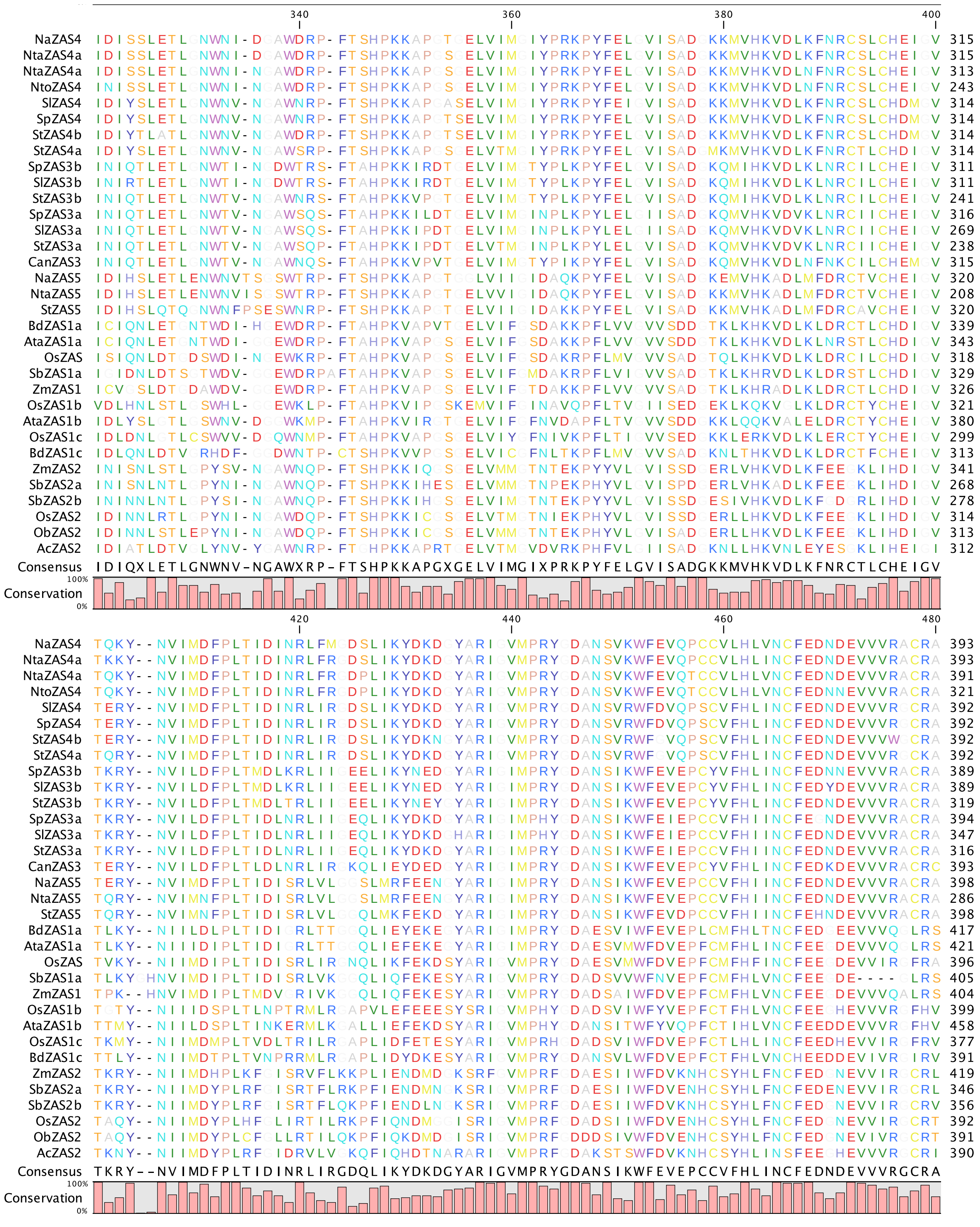



180
200
220
240
Consensus FAPVEEIGEAVL--VTTIEGE--IPDDFPEGVYIRNGSNPLFGGLKSTKSIFGKSSHIWVEGEGMLHALYFTKEKG-GX-
Conservation
100%
0%
260
280
300
320
Consensus WNISYKNKYVQTDTFKMEKDRKKP--GFLPAIEGDSPAILVAYILNVLRFGKENKYLSNTNVFEHAKKYYSIAENHLPQE
Conservation
100%
0%



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NtaZAS4a    IDISSLETLGNWNI-DGAWDRP-FTSHPKKAPGSGELVIMGIYPRKPYFELGVISADGKKMVHKVDLKFNRCSLCHEIGV 315
NtaZAS4a    IDISSLETLGNWNI-NGAWDRP-FTSHPKKAPGSGELVIMGIYPRKPYFELGVISADGKKMVHKVDLNFNRCSLCHEIGV 313
NtoZAS4     INISSLETLGNWNI-NGAWDRP-FTSHPKKAPGSGELVIMGIYPRKPYFELGVISADGKKMVHKVDLNFNRCSLCHEIGV 243
SlZAS4      IDIYSLETLGNWNV-NGAWNRP-FTSHPKKAPGASELVIMGIYPRKPYFEIGVISADGKKMVHKVDLKFNRCSLCHDMGV 314
SpZAS4      IDIYSLETLGNWNV-NGAWNRP-FTSHPKKAPGTSELVIMGIYPRKPYFELGVISADGKKMVHKVDLKFNRCSLCHDMGV 314
StZAS4b     IDIYTLATLGNWNV-NGAWDRP-FTSHPKKAPGTSELVIMGIYPRKPYFELGVISADGKKMVHKVDLKFNRCSLCHDMGV 314
StZAS4a     IDIYSLETLGNWNV-NGAWSRP-FTSHPKKAPGSGELVTMGIYPRKPYFELGVISADGMKMVHKVDLKFNRCTLCHDIGV 314
SpZAS3b     INIQTLETLGNWTI-NGDWTRS-FTAHPKKIRDTGELVIMGTYPLKPYFELGVISADGKQMIHKVDLKLNRCILCHEIGV 311
SlZAS3b     INIRTLETLGNWTI-NGDWTRS-FTAHPKKIRDTGELVIMGTYPLKPYFELGVISADGKQMIHKVDLKLNRCILCHEIGV 311
StZAS3b     INIQTLETLGNWTV-NGAWNRS-FTAHPKKVPGTGELVIMGTYPLKPYFELGVISADGKQMIHKVDLKLNRCILCHEIGV 241
SpZAS3a     INIQTLETLGNWTV-NGAWSQS-FTAHPKKILDTGELVIMGINPLKPYLELGIISADGKQMVHKVDVKLNRCIICHEIGV 316
SlZAS3a     INIQTLETLGNWTV-NGAWSQS-FTAHPKKIPDTGELVIMGINPLKPYLELGIISADGKQMVHKVDVKLNRCIICHEIGV 269
StZAS3a     INIQTLETLGNWTV-NGAWSQP-FTAHPKKIPDTGELVTMGINPLKPYLELGVISADGKQMVHKVDVKLNRCIICHEIGV 238
CanZAS3     INIQTLETLGNWTV-NGAWNQS-FTAHPKKVPVTGELVIMGTYPIKPYFELGVISADGKQMVHKVDLNFNKCILCHEMGV 315
NaZAS5      IDIHSLETLENWNVTSGSWTRP-FTSHPKKAPGTGGLVIIGIDAQKPYFELGVISADGKEMVHKADLMFDRCTVCHEIGV 320
NtaZAS5     IDIHSLETLENWNVISGSWTRP-FTSHPKKAPGTGELVVIGIDAQKPYFELGVISADGKKMVHKADLMFDRCTVCHEIGV 208
StZAS5      IDIHSLQTQGNWNFPSESWNRP-FTSHPKKAPGTGELVIIGIDAKKPYFELGVISADGKKMVHKADLMFDRCAVCHEIGV 320
BdZAS1a     ICIQNLETGNTWDI-HGEWDRP-FTAHPKVAPVTGELVIFGSDAKKPFLVVGVVSDDGTKLKHKVDLKLDRCTLCHDIGV 339
AtaZAS1a    ICIQNLETGNTWDI-GGEWDRP-FTAHPKVAPGSGELVIFGSDAKKPFLVVGVVSDDGTKLKHKVDLKLNRSTLCHDIGV 343
OsZAS       ISIQNLDTGDSWDI-NGEWKRP-FTAHPKVAPGSGELVIFGSDAKRPFLMVGVVSADGTQLKHKVDLKLDRCILCHDIGV 318
SbZAS1a     IGIDNLDTSGTWDV-GGEWDRPAFTAHPKVAPGSGELVIFGMDAKRPFLVIGVVSADGTKLKHRVDLKLDRSTLCHDIGV 329
ZmZAS1      ICVGSLDTGDAWDV-GGEWDRP-FTAHPKVAPGSGELVIFGTDAKKPFLVVGVVSADGTKLKHRADLKLDRCTLCHDIGV 326
OsZAS1b     VDLHNLSTLGSWHL-GGEWKLP-FTAHPKVIPGSKEMVIFGINAVQPFLTVGIISEDGEKLKQKVGLKLDRCTYCHEIGV 321
AtaZAS1b    IDLYSLGTLGSWNV-DGGWKMP-FTAHPKVIRGTGELVIFGFNVDAPFLTVGVVSDDGKKLQQKVALELDRCTYCHEIGV 380
OsZAS1c     IDLDNLGTLCSWVV-DGQWNMP-FTAHPKVAPGSGELVIYGFNIVKPFLTIGVVSEDGKKLERKVDLKLERCTYCHEIGV 299
BdZAS1c     IDLQNLDTVGRHDF-GGDWNTP-CTSHPKVVPGSGELVICGFNLTKPFLMVGVVSADGKNLTHKVDLKLDRCTFCHEIGV 313
ZmZAS2      INISNLSTLGPYSV-NGAWNQP-FTSHPKKIQGSGELVMMGTNTEKPYYVLGVISSDGERLVHKVDLKFEEGKLIHDIGV 341
SbZAS2a     INISNLNTLGPYNI-NGAWNQP-FTSHPKKIHESGELVMMGTNPEKPHYVLGVISPDGERLVHKADLKFEEGKLIHDIGV 268
SbZAS2b     ININNLNTLGPYSI-NGAWNQP-FTSHPKKIHGSGELVIMGTNTEKPYYVLGVISSDGESIVHKVDLKFGDGRLIHDIGV 278
OsZAS2      IDINNLRTLGPYNI-NGAWDQP-FTSHPKKICGSGELVTMGTNIEKPHYVLGVISSDGERLLHKVDLKFEEGKLIHDIGV 314
ObZAS2      IDINNLSTLEPYNI-NGAWDQP-FTSHPKKICGSGELVMMGTNTEKPHYVLGVISADGERLLHKVDLKFEEGKLIHDIGV 313
AcZAS2      IDIATLDTVGLYNV-YGAWNRP-FTGHPKKAPRTGELVTMGVDVRKPHFVLGIISADGKNLLHKVNLEYESGKLIHEIGI 312
Consensus   IDIQXLETLGNWNV-NGAWXRP-FTSHPKKAPGXGELVIMGIXPRKPYFELGVISADGKKMVHKVDLKFNRCTLCHEIGV
Conservation 100% 0%

                                    420                 440                 460                 480
NaZAS4      TQKY--NVIMDFPLTIDINRLFMGDSLIKYDKDGYARIGVMPRYGDANSVKWFEVQPCCVLHLVNCFEDNDEVVVRACRA 393
NtaZAS4a    TKKY--NVIMDFPLTIDINRLFRGDSLIKYDKDGYARIGVMPRYGDANSVKWFEVQPCCVLHLINCFEDNDEVVVRACRA 393
NtaZAS4a    TQKY--NVIMDFPLTIDINRLFRGDPLIKYDKDGYARIGVMPRYGDANSVKWFEVQTCCVLHLVNCFEDNDEVVVRACRA 391
NtoZAS4     TQKY--NVIMDFPLTIDINRLFRGDPLIKYDKDGYARIGVMPRYGDANSVKWFEVQTCCVLHLVNCFEDNNEVVVRACRA 321
SlZAS4      TERY--NVIMDFPLTIDINRLIRGDSLIKYDKDGYARIGVMPRYGDANSVRWFDVQPSCVFHLINCFEDNDEVVVRGCRA 392
SpZAS4      TERY--NVIMDFPLTIDINRLIRGDSLIKYDKDGYARIGVMPRYGDANSVRWFDVQPSCVFHLINCFEDNDEVVVRGCRA 392
StZAS4b     TERY--NVIMDFPLTIDINRLIRGDSLIKYDKNGYARIGVMPRYGDANSVRWFGVQPSCVFHLINCFEDNDEVVVWGCRA 392
StZAS4a     TQRY--NVIMDFPLTIDINRLIRGDSLIKYDKDGYARIGVMPRYGDANSVRWFGVQPSCVFHLINCFEDNDEVVVRGCKA 392
SpZAS3b     TKRY--NVILDFPLTMDLKRLIIGEELIKYNEDGYARIGIMPRYGDANSIKWFEVEPCYVFHLINCFEDNNEVVVRACRA 389
SlZAS3b     TKRY--NVILDFPLTMDLKRLIIGEELIKYNEDGYARIGIMPRYGDANSIKWFEVEPCYVFHLINCFEDYDEVVVRACRA 389
StZAS3b     TKRY--NVILDFPLTMDLTRLIIGEELIKYNEYGYARIGIMPRYGDANSIKWFEVEPCYVFHLINCFEDNDEVVVRACRA 319
SpZAS3a     TKRY--NVILDFPLTIDLNRLIIGEQLIKYDKDGYARIGIMPHYGDANSIKWFEIEPCCVFHIINCFEGNDEVVVRACRA 394
SlZAS3a     TKRY--NVILDFPLTIDLNRLIIGEQLIKYDKDGHARIGIMPHYGDANSIKWFEIEPCCVFHIINCFEDNDEVVVRACRA 347
StZAS3a     TKRY--NVILDFPLTIDLNRLIIGEQLIKYDKDGYARIGIMPRYGDANSIKWFEIEPCCVFHIINCFEDNDEVVVRACRA 316
CanZAS3     TERY--NVILDFPLTLDLNRLIRGKQLIEYDEDGYARIGIMPRYGDANSIKWFEVEPCYVFHLINCFEDKDEVVVRACRC 393
NaZAS5      TERY--NVIMDFPLTIDISRLVLGGSLMRFEENGYARIGIMPRYGDANSIKWFEVEPCCVFHIINCFEDNDEVVVRACRA 398
NtaZAS5     TQRY--NVIMNFPLTIDISRLVLGGSLMRFEENGYARIGIMPRYGDANSIKWFEVEPCCVFHIINCFEDNDEVVVRACRA 286
StZAS5      TQRY--NVIMNFPLTIDISRLVLGGQLMKFEKDGYARIGIMPRYGDANSIKWFEVDPCCVFHIINCFEHNDEVVVRACRA 398
BdZAS1a     TLKY--NIILDLPLTIDIGRLTTGGQLIEYEKEGYARIGIMPRYGDAESVIWFEVEPLCMFHLTNCFEDGEEVVVQGLRS 417
AtaZAS1a    TLKY--NIIIDIPLTIDIGRLTTGGQLIEFEKEGYARIGIMPRYGDAESVMWFDVEPFCMFHLINCFEEGDEVVVQGLRS 421
OsZAS       TVKY--NIIMDIPLTIDISRLIRGNQLIKFEKDSYARIGVMPRYGDAESVMWFDVEPFCMFHFINCFEEGDEVVIRGFRA 396
SbZAS1a     TLKYGHNVIMDIPLTIDISRLVKGGQLIQFEKESYARIGVMPRYGDADSVVWFNVEPFCMFHLVNCFEEGDE----GLRS 405
ZmZAS1      TPK--HNVIMDIPLTMDVGRIVKGGQLIQFEKESYARIGVMPRYGDADSAIWFDVEPFCMFHLVNCFEEGDEVVVQALRS 404
OsZAS1b     TGTY--NIIIDSPLTLNPTRMLRGAPVLEFEEESYSRIGVMPHYGDADSVIWFYVEPFCTFHLVNCFEEGHEVVVRGFHV 399
AtaZAS1b    TTMY--NIILDSPLTINKERMLKGALLIEFEKDSYARIGVMPHYGDANSITWFYVQPFCTIHLVNCFEEDDEVVVRGFHV 458
OsZAS1c     TKMY--NIIMDMPLTVDLTRILRGAPLIDFETESYARIGVMPRHGDADSVIWFDVEPFCTLHLINCFEEDHEVVIRGFRV 377
BdZAS1c     TTLY--NIIMDTPLTVNPRRMLRGAPLIDYDKESYARIGVMPRYGDANSVLWFDVEPFCTFHLVNCHEEDDEVIVRGIRV 391
ZmZAS2      TKRY--NIIMDHPLKFGISRVFLKKPLIENDMDGKSRFGVMPRFGDAESIIWFDVKNHCSYHLFNCFEDGNEVVIRGCRL 419
SbZAS2a     TKRY--NIIMDYPLRFGISRTFLRKPFIENDMNGKSRIGVMPRFGDAESIIWFDVENHCSYHLFNCFEDENEVVIRGCRL 346
SbZAS2b     TKRY--NIIMDYPLRFGISRTFLQKPFIENDLNGKSRIGVMPRFGDAESIIWFDVKNHCSYHLFNCFEDGNEVVIRGCRV 356
OsZAS2      TAQY--NIIMDYPLHFGLIRTILRKPFIQNDMGGISRIGVMPRFGDADSIIWFDVENHCSYHLFNCFEDGNEVVIRGCRT 392
ObZAS2      TAQY--NIIMDYPLCFGLLRTILQKPFIQKDMDGISRIGVMPRFGDDDSIVWFDVENHCSYHLFNCFEDGNEVIVRGCRT 391
AcZAS2      TKNY--NIIMDYPLRFGIDRVLVGKQFIQHDTNARARIGVMPRFGDAKSTSWFDVKNHCSYHLINSFEEGHEVVVRGCRI 390
Consensus   TKRY--NVIMDFPLTIDINRLIRGDQLIKYDKDGYARIGVMPRYGDANSIKWFEVEPCCVFHLINCFEDNDEVVVRGCRA
Conservation 100% 0%
```

```
                                                500                 520                 540                 560
                                                 |                   |                   |                   |
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  NtaZAS4a  HESVLPR--------QQSFKSSKEISSI--ENNNESSEEPF-FVHVCEWRLNMRTGEVKEKIATTE---FSMEFPMINEK 459
  NtaZAS4a  RESVLPR--------QQSFKGSKEISSI--ENNNESSVEPF-FVHVCEWRLNMRTGEVKEKNATTE---FSMEFPVINEK 457
   NtoZAS4  RESVLPR--------QQSFKGSKEISSI--ENNNESSVEPF-FVHVCEWRLNMRTGEVKEKNATTE---FSMEFPVINEK 387
    SlZAS4  RESVLPRPGSKDEKYKRFFEESEETSST--KINNESLEESF-FYRVCEWRLIMRTGEVKEKNVITN---FFMEFPMINEK 466
    SpZAS4  RESVLPRPGSKDEKYKRFFEESEETSST--KNNNESLEELF-YFRVCEWRLNMRTGEVKEKNVITK---FFMEFPMINER 466
   StZAS4b  RESVLPRPGYKDKKSKRFFEGFEETSST--KNNNESLEESF-FFRVCEWRLNMRTGEVKEKNVVTK---FFMEFPMINEK 466
   StZAS4a  RESVLPRPGSKVKEFERFSKGSEETSST--KINNESLEESF-FYRVCEWRLNMRTGEVKEKNVITN---FFMEFPMINEK 466
   SpZAS3b  RRWIIPRPN----ESELSLGTLNETSFS--KDNTESSKDLFFFFNVCEWRLNMKTGEVKMKNLTTNDHRFIMEFPMINEH 463
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   StZAS3b  RRWIIPRPN----ESELSLDTLNETSFN--KNNIESSKDLFSFFNVCEWRLNMKTGEVKMKNLTKNNHRFIMEFPMINEH 393
   SpZAS3a  RKSIIPRPDIIVDDLGLSLDALNEMSFD--KDNIKTSKDLFSFSKVCEWRLNMKTGEVKMKNLTTNDHRFLMEFPMINEH 472
   SlZAS3a  RKSIIPRPDIIVDELGLSLDALNEMSFS--KDNIKTSEDLFSFSKVCEWRLNMKTGEVKMKNVTTNDHRFIMEFPMINEH 425
   StZAS3a  RRSIIPRPDIIVDEFELSLDELDEMNFS--KNNVQMLKEIFSFFRVCEWRLNMKTGEVKLKDSTTTDHQFIMEFPMINEH 394
   CanZAS3  RRWIIPRPNLTIDEAELSLDSSNETSFS--KDDVESSKDLFSFFNICEWRLNMITSAVKMKNQTTADHQFIMEFPIINEH 471
    NaZAS5  RQSAIPGPNSRVNHFE---KWLRGRSSK--DDRIEFTKES-TFPRVCEWRLNMKTGEVRVKKNLSGD-EFAMEFPSINEN 471
   NtaZAS5  RQSVIPGPNSRVNHFE---KWLKGRSSK--DERIEFTKES-TFPRVCEWRLNMKTGEVREKKNLSGD-EFAMEFPLINEK 359
    StZAS5  RQSVIPGPSSSVNHLETLSKWIKRETSSIDDDSHEFTKES-TFPRVCEWRLDMKTGEIKEKKNLSGD-EFAMEFPIINEK 476
  BdZAS1a  ADSIIPGPRLRLSKHDMPPERSELTRYG--KTMKQGINEKL-FSRLYEWRLNLKTKTISGEYLTGTE--FSLEFPMINNH 492
 AtaZAS1a  ADSVIPGPRL--NKQEILPERSELKKDG--KAMKQGINEKL-FSRLFEWRLNLKTKTVSGEYLTGTE--CSLEFPMINNH 494
     OsZAS  ADSIIPGPRISLNKNDLLSDPSKC-------SVKQGINEEF-FSRLYQWRLNTKTKAVSGQYLSGTE--FSMEFPVINDH 466
  SbZAS1a  PDSIIPGPRLAPNKCD--SKMSELTEDD--KP-NEGTTKEF-FFRLYQWRLNLKTKSVSGEYLTGTE--FSFEFPIINNQ 477
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 AtaZAS1b  PGSIILGPRIK-------SN--------------QGLTKEC-FSRLYEWRLNLKTRTVVGKCLTGKE--VALKFPVMNDK 514
  OsZAS1c  PGSIITGITLEHTANEEPAN--------------QGPSEKS-FPRLYEWRLNMKSRAVTGKYLTGTD--VALEFPVINNK 440
  BdZAS1c  PPSVLVGLNQAHLTSAN--D--------------QGTDEEY-FSRLYEWRLNLKTGAVKGKYITGKD--VALEFPVINDQ 452
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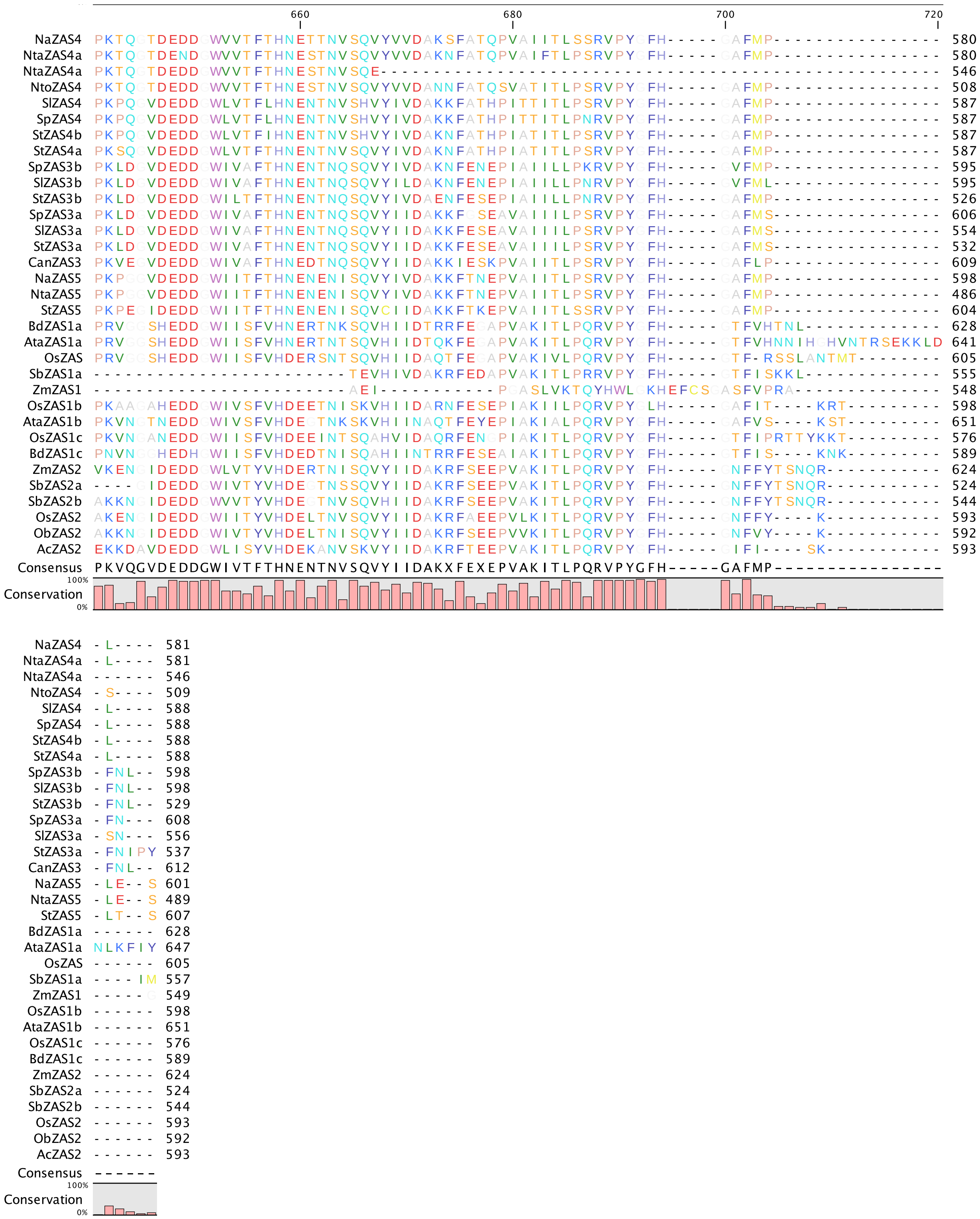
   SbZAS2a  LGSIIPSGRHRVDKSKWYGRAFLQPDKD--SEDFDPSLDGTLFSRPYEWRLNLENGSVHEGYITSE--KVAMDFPVISDK 422
   SbZAS2b  LASLIPSGH-----------------ED--SEDFDPSLDGTLFSRPYEWRLNLENSIVCEDYITSE--KIAMDFPVINDK 415
    OsZAS2  LDSVLSSASHDDDKSKCSGRAFLQPDKN--SEGFDPSVDGTLFSRPYEWRLNLKSGTTKEGYLTDE--KVAMDFPVINED 468
    ObZAS2  LDSVIPSGRHNADKSKCYGRAFLPADKN--LQGFDPSVDGTLFSRPYEWRLNLKDGTTKEGYLTNE--NVAMDFPVINEN 467
    AcZAS2  LCSVIPGPDHKTDKAKWYQRAFLQPKKD--SKEFDPSVDGILFSRPYEWRLDLVTGEVEEGYITCE--EVAMDFPAINNN 466
 Consensus  RESXIPRPR---DKSELSSKASEETSSD--KNNNESSKELF-FSRVCEWRLNMKTGEVKEKYLTTE---FAMEFPMINEK
      100%
Conservation
        0%
                                                580                 600                 620                 640
                                                 |                   |                   |                   |
    NaZAS4  FVGLRNKFGYLQVVDLEASS------ISEGLAKYGGLAKLHFQE------------YEELVEVEYHMFPKGTFCSGATFV 521
  NtaZAS4a  FVGLRNKFGYLQVVDLEACS------ISEGLAKYGGLAKLHFQE------------YEELVEVEYHMFPKGTFCSGATFV 521
  NtaZAS4a  FVGLRNRFGYLQVVDLEASS------ISEGLAKYGGLAKLHFKE------------DEELAKVEYHMFPKGTFCSGATFV 519
   NtoZAS4  FVGLRNRFGYLQVVDLEASS------ISEGLAKYGGLAKLHFKE------------DEELAKVEYHMFPKGTFCSGATFV 449
    SlZAS4  FIGLKNKFCYLQVVDIEASS------ISDGLVKYGGLAKFQFED------------DMELIKVEYHMLAEGNFCSGTTFV 528
    SpZAS4  FIGLRNKFCYLQVVDIEASS------ISDGLVKYGGLAKFQFEE------------DMELIKVEYHMLAEGNFCSGTTFV 528
   StZAS4b  FIGLRNKFCYLQVVDREASS------ISDGLVKYGGLVKLQFEE------------DMELIKVEYHMLPEGNFCSRTTFV 528
   StZAS4a  FIGLKNKFCYLQVVNNEVSS------ISDGLVKYRGLAKLQFEE------------DVELIKVEYHMLPEGNFCSGTTFV 528
   SpZAS3b  FIGIKNKFGYQQLINSETFSNS-AKNLSGGFAKFEGLAKLNFEEI----SKDDKKG--DLIKVEYHIFPKNTFCSGATFV 536
   SlZAS3b  FIGLKNKFGYQQLINLETFSNS-AKNVSGGFAKFGGLAKLNFEEI----SKDDKKG--DLIKVEYHIFPKNTFCSGATFV 536
   StZAS3b  FISLKNKFGYQQLVNSETFFNS-AKNLSGGFAKFGGLAKLHFEEI----SKDIKEEE-DLIKVEYHIFPKNTFCSGATFV 467
   SpZAS3a  FIGIKNKFGYLQLIDSEAFSNYFAKTLSGGFAKFGGLAKLNFEEI----SKDIKEEE-DLIKVEHHIFPKNTFCSGATFV 547
   SlZAS3a  FIGLKNKFGYLQLIDSEAFSNYF-----GGFAKFGGLAKLNFEEI----SKDFKEEE-DLIKVEYHIFPKNTFCSGATFV 495
   StZAS3a  FIGIKNKFCYLQLIDSEAFSNYFAKNLSGGFAKFGGLAKLHFEERHVENSKDNKEEE-DLIKVEYHIFPKNTFCSGATFV 473
   CanZAS3  FIGIKNKFGYVQLFNSETYSNS-GKYLPGGFAKFGGLAKLHYEERQFEISKDDKQEEYDLIKVEYHIFPKNTFWSGAAFV 550
    NaZAS5  FTGFRNKFAYLQVVESTEIS-------GSGFTRFGGLTKLHFEEKR-IPT----EEEDELVKAEYHMLPNNTFCSGASFV 539
   NtaZAS5  FTGFRNKFAYLQVVESTEIS-------GSGFTRFGGVAKLHFEEKR-IPT----EEEDELIKAEYHMLPQNTFCSGASFV 427
    StZAS5  FSGFRNKFAYLQVVESTELS-------GSGFTRFSGLVKLHFEEKKTFNM----DEEDELIKAEYHMFPKNTFCSGTSFV 545
  BdZAS1a  YTGVHHSYAYAQIVDSLTRPDGSHEKVL---PKYGGFAKICLADRQNVNKE--TSE-EDLIKMEKHWLGEDQFCSGASFV 566
 AtaZAS1a  YTGVRHSYGYAQIVDSLTRSASSSEKVL---PKYGGFAKLCLEERENT--E--TSA-EDLIKMEIHRLSEDEFCSGASFV 566
     OsZAS  YTGLHHSYAYAQVVDSLESSYGVNEKVI---LKYGGLAKLCLEEADNVIAE--TS--EDLIKTEYHGFGEDQFFSGAAFV 539
  SbZAS1a  YTGLQHSYAYAQIVDSCENCG----KVN---PKYGGFAKFYLDERNN--------------------------------- 517
    ZmZAS1  YTGLQHRYAYAQVVDSCGNCG----KVN---PKYGGFAKFYLDERSN--------------------------------- 517
  OsZAS1b  FSGLRHRYAYVQVADCSACFGGGHEIAR---PKFIGFAKLCLEEKQNIATK--QIDREDLIKVEYHQLAKNQFCSGVTFV 536
 AtaZAS1b  YVGLPHKYAYAQVANSPANLAGGHHVVR---PKFGGFAKFCLHEKEDATTK--NKGREDRIKVKYHHLARNQFCSGATFV 589
  OsZAS1c  YAGLHHKYAYAQVIDVQGSLEGGCGTVR---PKFGGFAKLHLQDNNKVFGG-------DLINVEYHHLGKNQFCSGATFV 510
  BdZAS1c  FAGLHHSYAYAQVVHSTASLAGGSGTVR---PKFGGFAKLYLEEAVSKYSE--LAEKEDLINVEYHHLNANQFCSGATFV 527
    ZmZAS2  FMGIQNKYGYAQVADSLETS-----KT--GLFKFNMIAKLHFDTPD--------KENEQLIFVEYHTLQEQQFCSGVQFV 560
   SbZAS2a  FVGVQNKYGYAQVADSLATS-----KT--GLFKFKMIAKLHFNMPD--------KKN----------------------- 464
   SbZAS2b  FIGIQNKYGYAQVADSLATS-----KT--GLFKFKMIAKLHFNMPD--------KENKELISVEYHTLKEKQFCSGVQFV 480
    OsZAS2  FVGVKNNYGYAQVVDSVATS-----EI--GLFKYNRIAKVHFDRQD--------KENKQLKSVEYHVLKEKTFCSGVQFV 533
    ObZAS2  FVGIENKYGYAQVVDSVATS-----KI--GLFKYNMIAKVHFDMED--------KENQELKLVEYHVLPEKSFCSGVQFV 532
    AcZAS2  FIGIRNKYAYTQVVDSLATS-----NSIAGLTKYKMLAKLHFDIKN--------EDDKNFINVDYHVLEENHFCSGVQFV 533
 Consensus  FIGLRNKFGYLQVVDSEASS------IS-GLAKFGGLAKLHFEE-----------EEEDLIKVEYHMLPKNTFCSGATFV
      100%
Conservation
        0%
```



Supplemental Table S1 Primer sequences used in this study.

Experiment	Primer name	Sequence (5′–3′)
RT-qPCR analyses	UBQ-Q-F	GCCCAAGAAGAAGATCAAGAAC
	UBQ-Q-R	AGATAACAACGGAAGCATAAAAGTC
	OsD27-Q-F	CTTCCAAGCTACATCCTCAC
	OsD27-Q-R	CCCAACCAACCAAGGAAA
	OsCCD7-Q-F	CAGTCTCCAAGCACAGATG
	OsCCD7-Q-R	GTTCTTTGGCACCTCTAGTT
	OsCCD8-Q-F	TGGCGATATCGATGGTGA
	OsCCD8-Q-R	GACCTCCTCGAACGTCTT
	OsCO-Q-F	ATTGTCAGCGATCCACTTC
	OsCO-Q-R	GCGCCGTTCTTGAAATTG
	OsZAS-Q-F	CAGCCAAAATCCACAGCCAC
	OsZAS-Q-R	AAGCCCCTGTTTTGCTGTCT
	OsZAS1a-Q-F	GACCAAAAACGGTGCCATCC
	OsZAS1a-Q-R	CTCCCTTTCCAGTGGATGCT
	OsZAS1b-Q-F	GCAGCAGCTTACCACCAAAC
	OsZAS1b-Q-R	GTGCGAAATTGCTCTCGGTG
	OsZAS2-Q-F	CATCTAGGGAAGAGCCCAGC
	OsZAS2-Q-R	TGGCTGCTTATTCCACCCAG
	RiEFα-F	GCTATTTTGATCATTGCCGCC
	RiEFα-R	TCATTAAAACGTTCTTCCGACC
	OSPt11-Q-F	GAGAAGTTCCCTGCTTCAAGCA
	OSPt11-Q-R	CATATCCCAGATGAGCGTATCATG
	RiPEIP1-Q-F	AAGAAAGTAAACGTGTGGCT
	RiPEIP1-Q-R	TAACACTCATCTCGGGACTG
	OsRubQ1-F	GGGTTCACAAGTCTGCCTATTTG
	OsRubQ1-R	ACGGGACACGACCAAGGA
Oszas2 mutant screening	gOsZAS2-F	ACAAGCTCGAACTAGCAGGC
	gOsZAS2-R	GTTGCTCGTCGACTTCACAAAT
pOsZAS2:GUS reporter	pOsZAS2-F	TGTACGCGGCTGGCCTATTC
	pOsZAS2-F-CACC	CACCTGTACGCGGCTGGCCTATTC
	pOsZAS2-R	GGCTTGCTCAGGTTATTCGATCC
In situ hybridization	OsZAS2-mid-F	CTCCGCACCCTAGGACCTTA
	OsZAS2-mid-R	TAGGCATCACTCCAATGCGT
	OsZAS2-SP6-R	CTATTTAGGTGACACTATAGACTCCGCACCCTAGGACCTTA
	M13-F	GTAAAACGACGGCCAG
In vitro assay	OsZAS2-CDS-F	ATGTTCAGAGCTGGAGGGT
	OsZAS2-CDS-R	TCACTTGTAGAAAAAATTTCCATG
	OsZAS2-MBP-F	CCAGGGAGCAGCCTCGatgttcagagctggagggtgca
	OsZAS2-MBP-R	GCAAAGCACCGGCCTCGtcacttgtagaaaaaatttccatgaaacccatat
Subcellular localization	OsZAS2-CDS-Box2-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGTTCAGAGCTGGAGGGTG
	OsZAS2-CDS-Box2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTTGTAGAAAAAATTTCCATGAAACCCATATGGAAC

Supplemental Table S2 Distribution of ZAS members across monocot and dicot plants. Abreviation: SN, shorten name of the species used in phlygenetic trees of this study. Columns from 4th to 8th represent numbers of Clade I-V orthologues in each species' genome. This table adopted from Wang et al., 2019.

SN	Species	RefSeq Assembly Accession	Clade I	Clade II	Clade III	Clade IV	Clade V
Monocot							
Os	*Oryza sativa*	GCF_001433935.1	3	1	0	0	0
Zm	*Zea mays*	GCF_000005005.1	1	1	0	0	0
Ob	*Oryza brachyantha*	GCF_000231095.1	0	1	0	0	0
Bd	*Brachypodium distachyon*	GCF_000005505.2	2	0	0	0	0
Ata	*Aegilops tauschii subsp. tauschii*	GCF_001957025.1	2	0	0	0	0
Sb	*Sorghum bicolor*	GCF_000003195.2	1	2	0	0	0
Ac	*Ananas comosus*	GCF_001540865.1	0	1	0	0	0
Dicot							
Na	*Nicotiana attenuata*	GCF_001879085.1	0	0	0	1	1
Nto	*Nicotiana tomentosiformis*	GCF_000390325.2	0	0	0	1	0
Nta	*Nicotiana tabacum*	GCF_000715135.1	0	0	0	2	1
Can	*Capsicum annuum*	GCF_000710875.1	0	0	1	0	0
St	*Solanum tuberosum*	GCF_000226075.1	0	0	2	2	2
Sp	*Solanum pennellii*	GCF_001406875.1	0	0	2	1	0
Sl	*Solanum lycopersicum*	GCF_000188115.3	0	0	2	1	0
			9	6	7	8	4

Supplemental Table S3 Protein accession of ZAS members in different organisms.

Clades	Gene Symbol	Protein Accession (NCBI)
Clade I	BdZAS1a	XP_003576413.2
	AtaZAS1a	XP_020201457.1
	OsZAS	XP_015611401.1
	SbZAS1a	XP_002462200.1
	ZmZAS1	NP_001152477.1
	OsZAS1b	XP_015648368.1
	AtaZAS1b	XP_020149972.1
	OsZAS1c	XP_015649272.1
	BdZAS1c	XP_010235077.1
Clade II	ZmZAS2	XP_008659260.1
	SbZAS2a	XP_002437880.1
	SbZAS2b	XP_002437880.1
	OsZAS2	XP015641132.1
	ObZAS2	XP_015693936.1
	AcZAS2	XP_020098001.1
Clade III	SpZAS3a	XP_015058049.1
	SlZAS3a	XP_019066509.1
	StZAS3a	XP_015158838.1
	SpZAS3b	XP_015057245.1
	SlZAS3b	XP_004251419.1
	StZAS3b	XP_015158843.1
	CanZAS3	XP_016546962.1
Clade IV	NtoZAS4	XP_009601557.1
	NtaZAS4a	XP_016482181.1
	NaZAS4	XP_019242827.1
	NtaZAS4a	XP_016506620.1
	StZAS4a	XP_006359778.1
	StZAS4b	XP_006359779.1
	SlZAS4	XP_004245397.1
	SpZAS4	XP_015084337.1
Clade V	NaZAS5	XP_019231151.1
	NtaZAS5	XP_016506622.1
	StZAS5	XP_006359776.1