

Supporting Information

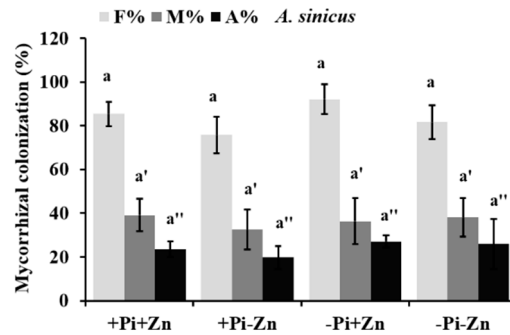


Figure S1. Effect of the external Pi and Zn availabilities on the arbuscular mycorrhizal symbiosis in *A. sinicus*. *A. sinicus* were grown in pot cultures treated with 300 μ M Pi and 50 μ M Zn (+Pi+Zn), 300 μ M Pi and 0.5 μ M Zn (+Pi-Zn), 30 μ M Pi and 50 μ M Zn (-Pi+Zn) or 30 μ M Pi and 0.5 μ M Zn (-Pi-Zn). 14-d-old *A. sinicus* seedling roots were colonized by *R. irregularis* at 42 dpi, and colonization level was quantified in roots of 56-d-old mycorrhizal plants after WGA488 staining. F%, the total colonization frequency; M%, the percentage of mycorrhizal intensity; A%, the percentage of arbuscule abundance. Error bars represent the SD for means of three biological replicates. The same letters indicate non-significant differences at $P < 0.05$, based on the Duncan's multiple range test.

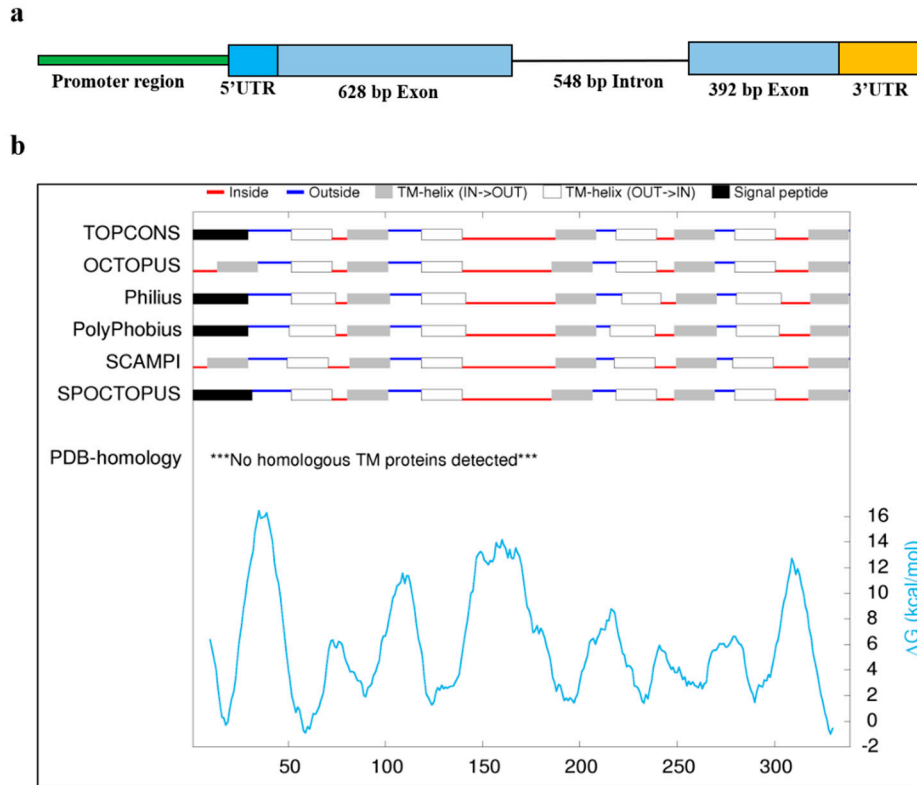


Figure S2. Gene structure and topology analysis of *A. sinicus* AsZIP2. (a) The AsZIP2 gene corresponds to MZ636517 and consists of two exons (light blue boxes). The dark blue and orange boxes are represented 5'UTR and 3'UTR regions of AsZIP2, respectively. The green line upstream of the 5'UTR represents the promoter region of AsZIP2. (b) The encoded membrane protein AsZIP2 from *A. sinicus* comprises 339 amino acids and 8 transmembrane domains (TM-helices). The TOPCONS program (<http://topcons.cbr.su.se/>) predicts that AsZIP2 (QYE52148) is a membrane intrinsic protein containing 8 TM domains.

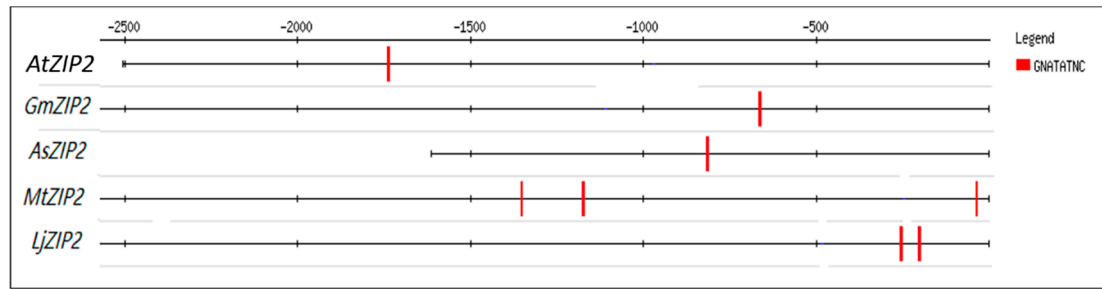


Figure S3. The P1BS-motif is present in the promoters of the *AsZIP2* from *A. sinicus* as well as the ZIP2 subfamily genes from other plant species. The P1BS-motif (GNATATNC), which is the binding site of the PHR1 and PHR1-like transcription factors in plants, was screened in the promoter regions of the plant ZIP2 subfamily genes by the DNA-pattern matching analysis (<http://rsat.ulb.ac.be/rsat/>). The GNATATNC motif is found in the promoter regions of *AtZIP2* (in *Arabidopsis thaliana*), *GmZIP2* (in *Glycine max*), *AsZIP2* (in *A. sinicus*), *MtZIP2* (in *Medicago truncatula*), and *LjZIP2* (in *Lotus japonicus*), whereas these promoter sequences do not contain the RTGTCGACAY motif, which is directly activated by the bZIP19/23 transcription factors during Zn starvation.

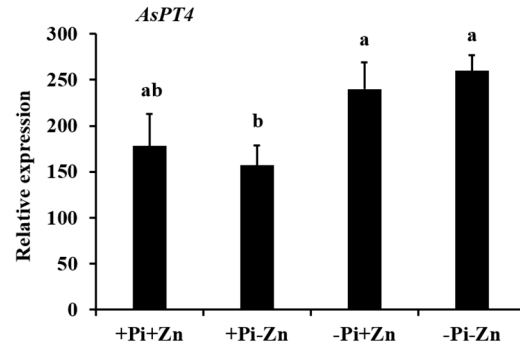


Figure S4. Transcription of the arbuscular mycorrhiza-specific *AsPT4* in mycorrhizal roots of *A. sinicus* in response to Pi and Zn availabilities. The 56-d-old *A. sinicus* roots with *R. irregularis* were harvested and transcription levels of *AsPT4* was estimated by real-time qRT-PCR. *AsActin* for *A. sinicus* was used as the house-keeping gene for normalization. Error bars represents SD from three biological replicates. The different letters indicate statistically significant differences among treatments at $P < 0.05$, based on Duncan's multiple range test.

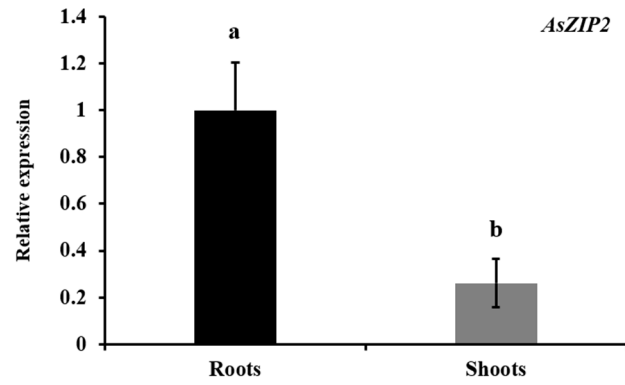


Figure S5. Transcription profiles of *AsZIP2* in the roots and shoots of *A. sinicus*. *A. sinicus* plants were grown under standard (300 μ M Pi and 1 μ M Zn) conditions. 56-d-old roots and shoots of *A. sinicus* plants were collected separately and transcript levels of *AsZIP2* was quantified by real-time qRT-PCR. The *AsActin* gene for *A. sinicus* was used as the internal control. Error bars mean standard deviation from three biological replicates. The different letters are statistically significant differences between roots and shoots at $P < 0.05$, based on Student's t-test.

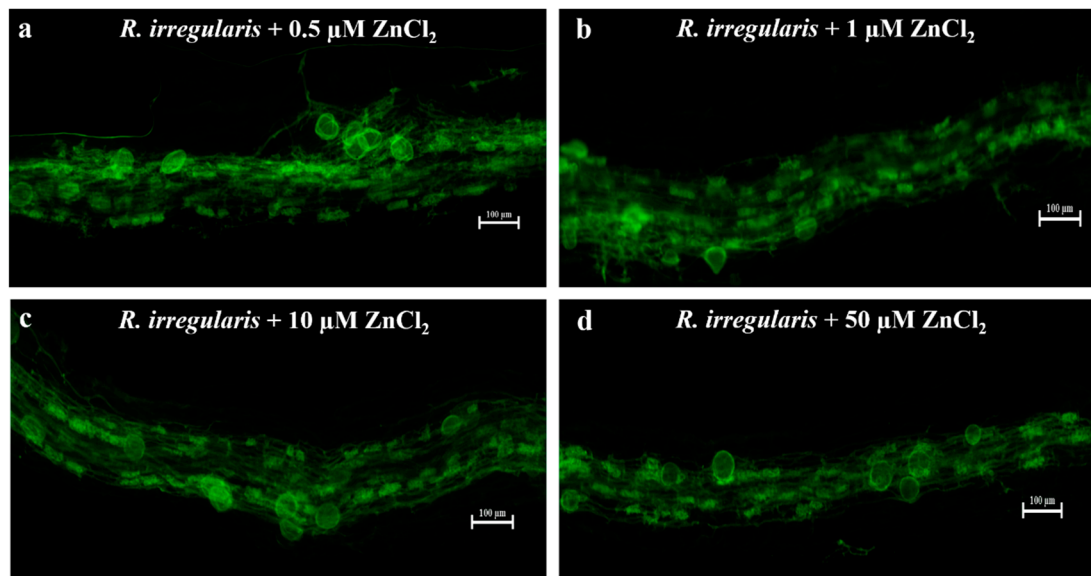


Figure S6. Effect of the external Zn concentrations on the AM symbiosis in *A. sinicus* roots. (a-d) The 56-day-old *A. sinicus* plants were grown in cultures supplied with 0.5, 1, 10, or 50 μM ZnCl_2 concentrations, and these *A. sinicus* roots were colonized by *R. irregularis*. Fluorescence images of *R. irregularis* within roots of *A. sinicus* exposed to different Zn concentrations as indicated above. AM roots were stained with WGA488. Scale bars, 100 μm .

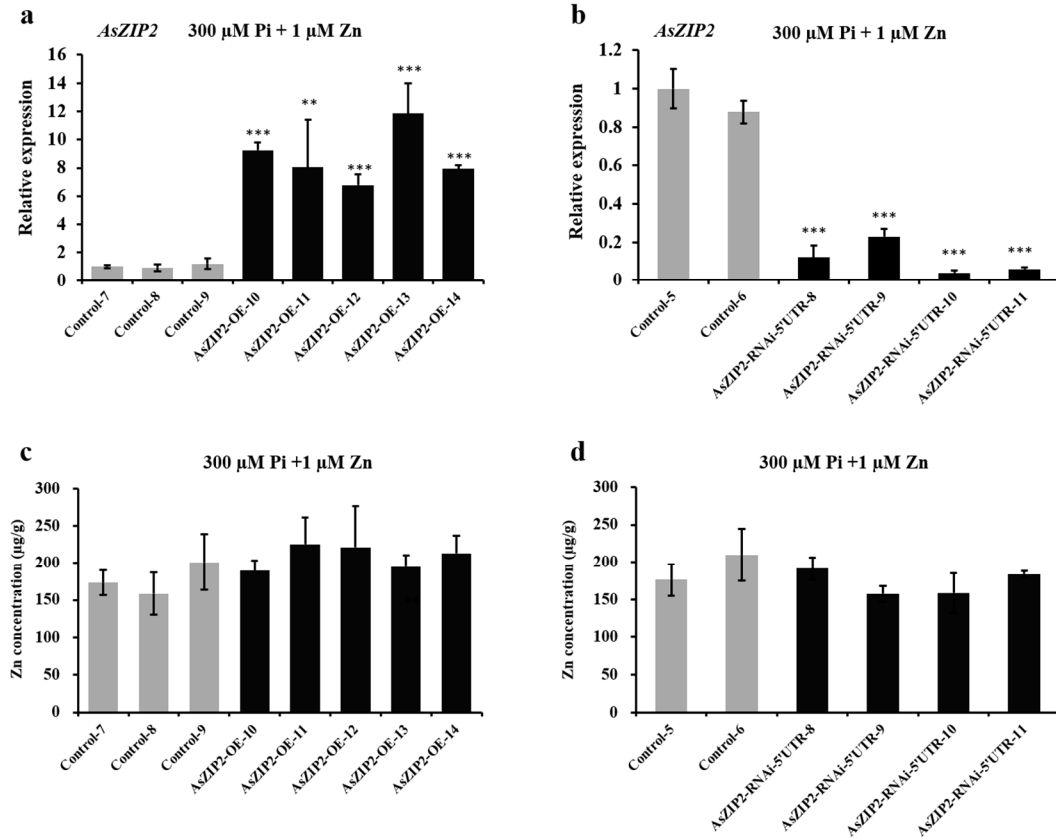


Figure S7. Effect of the *AsZIP2*-overexpression (*AsZIP2*-OE) and *AsZIP2*-silencing (*AsZIP2*-RNAi) on the Zn concentration in roots of *A. sinicus* under standard growth conditions. (a and b) Molecular phenotypes in the transgenic *A. sinicus* roots during standard growth (300 μM Pi and 1 μM ZnCl₂) conditions. Real time qRT-PCR analysis of *AsZIP2* expression in the *AsZIP2*-OE (a) and *AsZIP2*-RNAi (b) lines compared with the control lines. *AsActin* from *A. sinicus* served as the endogenous control. (c) The Zn concentrations in the control and *AsZIP2*-OE roots of 56-d-old *A. sinicus* plants under standard growth conditions. (d) The Zn concentrations in the control and *AsZIP2*-RNAi roots of 56-d-old *A. sinicus* plants under standard growth conditions. Error bars represented the SD for means of three technical replicates. Significant differences between the *AsZIP2*-OE (or *AsZIP2*-RNAi) and controls: ***, $P < 0.001$; **, $P < 0.01$; Student's t-test.

Table S1. A list of the primers used in this study.

Primer names	Primer sequences (from 5' to 3')	Use for
<i>AsZIPF1</i>	CAAAACCCTAAAGTCAAC	Degenerate primers
<i>AsZIPR1</i>	TCAATCCCAAATCATGACAAC	Degenerate primers
<i>AsZIPF4</i>	GTGACACTATTCTTCTCATCCTTGC	RT-PCR primers
<i>AsZIPR3</i>	CTCCATGCTTCCCCTTTAGTACCTG	RT-PCR primers
<i>AsZIPF4</i>	GTGACACTATTCTTCTCATCCTTGC	3'RACE primers
<i>AsZIPF5</i>	CTTAGGATGTTACCAAAG	3'RACE primers
<i>AsZIPR4</i>	CCACAACCTTATCTTCCCTCTG	TAIL-PCR primers
<i>AsZIPR5</i>	GAAGAAAAGTCTCATTCCAACC	TAIL-PCR primers
<i>AsZIPR6</i>	CTGAATCACCGTGTCTCCATGACC	TAIL-PCR primers
<i>AsZIPPF</i>	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> CAAGTCGCGTCTGCATCT AGAGC	Promoter analysis primers
<i>AsZIPPR</i>	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> GAATGTGTGTACGTATTT TTCTCTC	Promoter analysis primers
<i>AsInvF1</i>	GGTGGTGTGTCTCCTTACTTC	Inverse PCR primers
<i>AsInvF2</i>	CTTGAGTGATTCCAATGAAACCT	Inverse PCR primers
<i>AsZIPR7</i>	CTGAATCACCGTGTCTCCATGACC	Inverse PCR primers
<i>AsZIPR8</i>	TTGGTTTTGGAGGATGAAGCC	Inverse PCR primers
<i>AsZIP2-OE-F</i>	GGGGTACCATGGCTTCATCCTCCAAAACCAAAG	Overexpression primers
<i>AsZIP2-OE-R</i>	CGCGGATCCTCAATCCCAAATCATTACAAC	Overexpression primers
<i>AsZIP2-ORFF1</i>	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> ATGGCTTCATCCTCCAAA ACCAAAG	Subcellular localization in tobacco
<i>AsZIP2-ORFR1</i>	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> TATCCCAAATCATTACAA C	
<i>AsZIP2-Ri-5'-F</i>	<u>GGGGACAAGTTTGTACAAAAAAGCAGGCT</u> CTAGTACAAGTAATACAA TTGTAC	RNAi primers
<i>AsZIP2-Ri-5'-R</i>	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> CCAAAACCAAAGCCCTAA AGTC	RNAi primers
AD1	ATGGACTCCAGAGCGGCCGC (G/C/A)N(G/C/A)NNNGGAA	TAIL-PCR long adapters
AD2	ATGGACTCCAGAGCGGCCGC (G/C/T)N(G/C/T)NNNGGTT	TAIL-PCR long adapters
AD3	ATGGACTCCAGAGCGGCCGC (G/C/A)(G/C/A)N(G/C/A)NNNCCAA	TAIL-PCR long adapters
AD4	ATGGACTCCAGAGCGGCCGC (G/C/T)(G/A/T)N(G/C/T)NNNCGGT	TAIL-PCR long adapters
<i>AsZIP2-qF</i>	GTGACACTATTCTTCTCATCCTTGC	Real time qRT-PCR
<i>AsZIP2-qR</i>	CTCCATGCTTCCCCTTTAGTACCTG	Real time qRT-PCR
<i>AsPT2-qF</i>	TCCTGCTGCTCTTACCTTCTACTG	Real time qRT-PCR
<i>AsPT2-qR</i>	CCTCACTCAACTCCTGCACCTT	Real time qRT-PCR
<i>AsPT3-qF</i>	CCTTTGGATTCTCTATGCTC	Real time qRT-PCR
<i>AsPT3-qR</i>	TTTTCTTTAGATTTCAGGCAC	Real time qRT-PCR
<i>AsPT4-qF</i>	CAGAAACAAAGGGAAGATCATTGG	Real time qRT-PCR
<i>AsPT4-qR</i>	CATGTAATTCAGATCCTTCACACTG	Real time qRT-PCR
<i>AsActin-qF</i>	GTTCTTTTCCAGCCTTCTATGA	<i>A. sinicus</i> housekeeping gene
<i>AsActin-qR</i>	ATGTTTCCGTACAGATCCTTTC	for qRT-PCR

Table S2. The accession numbers of the plant ZIP family proteins used in this study.

Gene names	Accession Numbers	Species
AsZIP2	QYE52148	<i>Astragalus sinicus</i>
MtZIP2	XP_003597387.1	<i>Medicago truncatula</i>
LjZIP2	AFK49261.1	<i>Lotus japonicus</i>
AtZIP2	NP_200760.1	<i>Arabidopsis thaliana</i>
OsZIP1	XP_015633357.1	<i>Oryza sativa Japonica Group</i>
OsZIP2	XP_015628224.1	<i>Oryza sativa Japonica Group</i>
PtZIP11	XP_002300374.2	<i>Populus trichocarpa</i>
PtZIP12	XP_006369999.2	<i>Populus trichocarpa</i>
SlZIP2	NP_001234349.1	<i>Solanum lycopersicum</i>
AtZIP11	NP_564703.1	<i>Arabidopsis thaliana</i>
MtZIP11	XP_013460015.1	<i>Medicago truncatula</i>
GmZIP11	XP_003532170.1	<i>Glycine max</i>
GmZIP13	XP_006602141.1	<i>Glycine max</i>
GmZIP14	XP_003551465.1	<i>Glycine max</i>
GmZIP7	XP_003547009.1	<i>Glycine max</i>
GmZIP3	NP_001304414.2	<i>Glycine max</i>
GmZIP2	XP_003543520.3	<i>Glycine max</i>
MtZIP6	XP_003615761.1	<i>Medicago truncatula</i>
GmZIP6	XP_003544893.1	<i>Glycine max</i>
PtZIP6	XP_002299993.1	<i>Populus trichocarpa</i>
SlZIP6	XP_010321288.1	<i>Solanum lycopersicum</i>
AtZIP6	NP_180569.1	<i>Arabidopsis thaliana</i>
OsZIP6	XP_015640855.1	<i>Oryza sativa Japonica Group</i>
AtZIP7	NP_178488.1	<i>Arabidopsis thaliana</i>
GmIRT1	XP_003520144.1	<i>Glycine max</i>
MtIRT2	XP_024628929.2	<i>Medicago truncatula</i>
SlIRT3	XP_019067417.1	<i>Solanum lycopersicum</i>
GmZIP10	NP_001240277.1	<i>Glycine max</i>
GmZIP12	NP_001274385.1	<i>Glycine max</i>
MtIRT1	XP_003607852.1	<i>Medicago truncatula</i>
AtZIP8	Q8S3W4.1	<i>Arabidopsis thaliana</i>
AtIRT1	NP_567590.3	<i>Arabidopsis thaliana</i>
SlIRT2	NP_567590.3	<i>Solanum lycopersicum</i>
AtZIP10	NP_174411.2	<i>Arabidopsis thaliana</i>
SlIRT1	NP_001234248.1	<i>Solanum lycopersicum</i>
OsZIP10	XP_015641360.1	<i>Oryza sativa Japonica Group</i>
OsZIP7	XP_015637712.1	<i>Oryza sativa Japonica Group</i>
SlZIP4	XP_004245100.1	<i>Solanum lycopersicum</i>
AtZIP9	NP_195028.1	<i>Arabidopsis thaliana</i>
AtZIP4	NP_001318977.1	<i>Arabidopsis thaliana</i>
PtZIP4	XP_002324173.2	<i>Populus trichocarpa</i>
MtZIP4	XP_003603149.3	<i>Medicago truncatula</i>
GmZIP4	XP_006581291.1	<i>Glycine max</i>
GmZIP5	XP_003526000.2	<i>Glycine max</i>
MtZIP5	XP_013461166.1	<i>Medicago truncatula</i>
MtZIP8	XP_013461171.1	<i>Medicago truncatula</i>
MtZIP5.1	XP_003601469.1	<i>Medicago truncatula</i>
MtZIP8.1	XP_024635724.1	<i>Medicago truncatula</i>
SlZIP5	XP_004243896.1	<i>Solanum lycopersicum</i>
SlZIP7	XP_004243649.1	<i>Solanum lycopersicum</i>
SlZIP8	XP_004231600.1	<i>Solanum lycopersicum</i>
PtZIP8	XP_002307860.3	<i>Populus trichocarpa</i>
PtZIP2	XP_002312231.2	<i>Populus trichocarpa</i>
PtZIP3	XP_002315075.2	<i>Populus trichocarpa</i>
AtZIP1	NP_187881.1	<i>Arabidopsis thaliana</i>
MtZIP1	XP_013464193.1	<i>Medicago truncatula</i>
GmZIP1	XP_003531480.1	<i>Glycine max</i>

GmZIP8	XP_040865466.1	<i>Glycine max</i>
GmZIP9	XP_003546826.1	<i>Glycine max</i>
OsZIP8	XP_015644611.1	<i>Oryza sativa Japonica Group</i>
OsZIP9	XP_015637508.1	<i>Oryza sativa Japonica Group</i>
OsZIP5	XP_015637510.1	<i>Oryza sativa Japonica Group</i>
OsZIP3	XP_015635611.1	<i>Oryza sativa Japonica Group</i>
OsZIP4	XP_015650399.1	<i>Oryza sativa Japonica Group</i>
