M e OLDEN LI E o e se ce ou t o e ce ce by o o t s o t c os e

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Abstract

Drou ht h s become o e o the most severe b ot c stresses exper e ced r cultur l product o cross the orld. Pl ts respo d to ter de c t v stom t l moveme ts the le ves, h ch re m ly re ul ted by bsc s c c d B . prev ous study rom our l b sho ed th t co st tut ve express o o m e L. OLDEN - L E L tr scr pt o ctors r ce L. c mprove stom t l co duct ce d pl t photosy thet c c p c ty u der eld co d t o s. the prese t study, e u covered u ct o o Zm L re ul t o o stom t l moveme t r ce dur drou ht stress. We ou d th t elev ted drou ht toler ce r ce pl ts overexpress or s co erred by r p d B - med ted stom t l closure. Comp r t ve ly s s o RN - seque c RN - seq d t rom the r ce le ves d DN ty pur c t o seque c D P seq results obt ed v tro reve led th t Zm L s pl yed roles re ul t B - rel ted d stress respo s ve p th ys. Four upre ul ted e es closely u ct o b ot c stress toler ce th stro b d pe s the D P seq d t ere de t ed s put t ve t r et e es o Zm L 1 d Zm 2 r ce. These results demo str ted th t m e L s pl y mport t role re ul t stom t l moveme ts to coord te photosy thes s d stress toler ce. Th s tr t s v lu ble t r et or breed drou ht toler t crop pl ts thout comprom s photosy thet c c p c ty.

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deficit or are exposed to other environmental stimuli e.g. low light intensity, low air humidity, high CO_2 levels, and pathogens, stomata are rapidly closed, especially in angiosperms (Seril et al. 2018). This dynamic movement is driven by turgor pressure changes in the guard cells, as a result of the activation of anion channels and the inhibition of inward-rectifying K^+ channels, which are coded by and genes (et al. 2010). The efflux of anions and small metabolites, including Cl^-, NO_3^-, and malate, causes membrane depolarization to activate the outward-rectifying K^+ channel and facilitates K^+ efflux, further reducing turgor pressure inside the guard cells and leading to the stomatal closure (P et al. 2007). Under water deficit conditions, the phytohormone abscisic acid (ABA) plays as the primary regulator of stomatal movement to prevent water loss, which endogenous ABA levels are controlled by a precise balance between biosynthesis and catabolism, which also influenced by transport and conjugation process (ushiro et al. 2007; ue et al. 2011). ABA is initially synthesized from C_{40} carotenoids to form xanthophylls (e.g. 9′-violaxanthin and 9′-neoxanthin; a C_{15} intermediate, xanthoxin), is formed in the plastids via oxidative cleavage catalyzed by 9-cis-epoxycarotenoid dioxygenase (NCED). Xanthoxin is then exported to the cytosol and converted to ABA through a two-step reaction via short-chain dehydrogenase/reductase 1 (SDR1/ABA2) and aldehyde oxidase 3 (AAO3) (Seo and Koshiba 2002; and Zhu 2003).

Transcription factors (TFs) are crucial regulators of many biological processes, including responses to environmental signals and hormone regulation. These regulatory functions are accomplished through binding to specific elements in the promoter regions of target genes (Tod et al. 2019). Numerous abiotic stress-responsive TFs have been identified in plants; for instance, WR, MB, and DREB/CBF TFs have all been reported as key regulators of plant stress responses (M et al. 2011). OLDEN-LIKE TFs generally act as transcriptional activators of chloroplast development and biogenesis (Ross et al. 2001; Wang et al. 2013) and play important roles in regulating nuclear photosynthesis-related genes (Chen et al. 2016). Maize L. genes, and , have shown differential expression patterns between mesophyll cells and the bundle sheath (Hall et al. 1998; Chang et al. 2019). Ectopic overexpression of maize genes in rice induces chloroplast development in bundle sheath cells and activates intercellular plasmodesmatal connections, considering the key step in forming intermediate proto-Kranz anatomy in the transition from C_3 to C_4 photosynthesis (Wang et al. 2017). In a previous study from our lab showed that constitutive expression in rice leads to increased xanthophyll content and further mitigates the photoinhibition under high light conditions, resulting in enhanced photosynthetic capacity with higher stomatal conductance and improved biomass and grain yield in the field (Li et al. 2020). Moreover, L s also function in abiotic stress responses (hmad et al. 2019) and pathogen resistance (Murmu et al. 2014); for example, L s affect stomatal movement in rabidopsis when exposed to ozone (Nagatoshi et al. 2016).

In this study, we uncovered the dual function of maize L s, and that ectopic overexpression of and in rice conferred improved drought tolerance by promoting stomatal closure in response to water deficit while maintaining high stomatal conductance to obtain efficient photosynthesis when sufficient water is available. We further showed that rapid stomatal movement is mediated by ABA-involved pathway under drought conditions. These results suggest that genes may be promising candidates for breeding rice varieties with high stomatal flexibility and sustainable yield, which could strongly improve agricultural production and increase food security in the context of climate change.

Results

ZmGLK1 and ZmG2 conferred improved drought tolerance in rice

In our previous study, field-grown transgenic rice lines constitutively expressing or driven by the maize promoter performed improved photosynthetic rates and higher stomatal conductance (Li et al. 2020). We further explored the stomatal responses of transgenic rice plants to water deficit in pot experiments in the growth chamber. Surprisingly, transgenic rice plants exhibited stronger drought tolerance than wild type (WT) plants after recovery from a 10 d drought treatment (Fig. 1A). Specifically, the survival rates of : and : plants were 53.0% to 67.0% after the 6 d recovery period, which were significantly higher than the WT (17.3%; Fig. 1B). Moreover, the relative water content (RWC) in the leaves of WT and transgenic plants ranged from 97.7% to 95.3% before drought but decreased to 73.1% in the WT after water was withheld for 7 d. In comparison, : and : plants maintained a relatively high RWC, especially : , ranging from 86. % to 90.9%. After 10 d of drought stress, the RWC values of WT and : plants decreased to 11.6% to 19.9%, which were significantly lower than those of : plants (17.5% to 18.6%; Fig. 1C). These results indicated Zm L 1 and Zm 2 both conferred higher capacities for water conservation and thus drought tolerance.

We next tested the growth performance of WT, : , and : rice plants to PEG-induced osmotic stress as a drought simulation. After growth in 20% PEG 6000 for 10 d, : and : rice plants showed less wilting and chlorosis compared to the WT (Supplemental Fig. S1). The maximum quantum efficiency of photosystem II (PSII; F_v/F_m) was measured as an important indicator of plant physiological state under stress conditions, and that

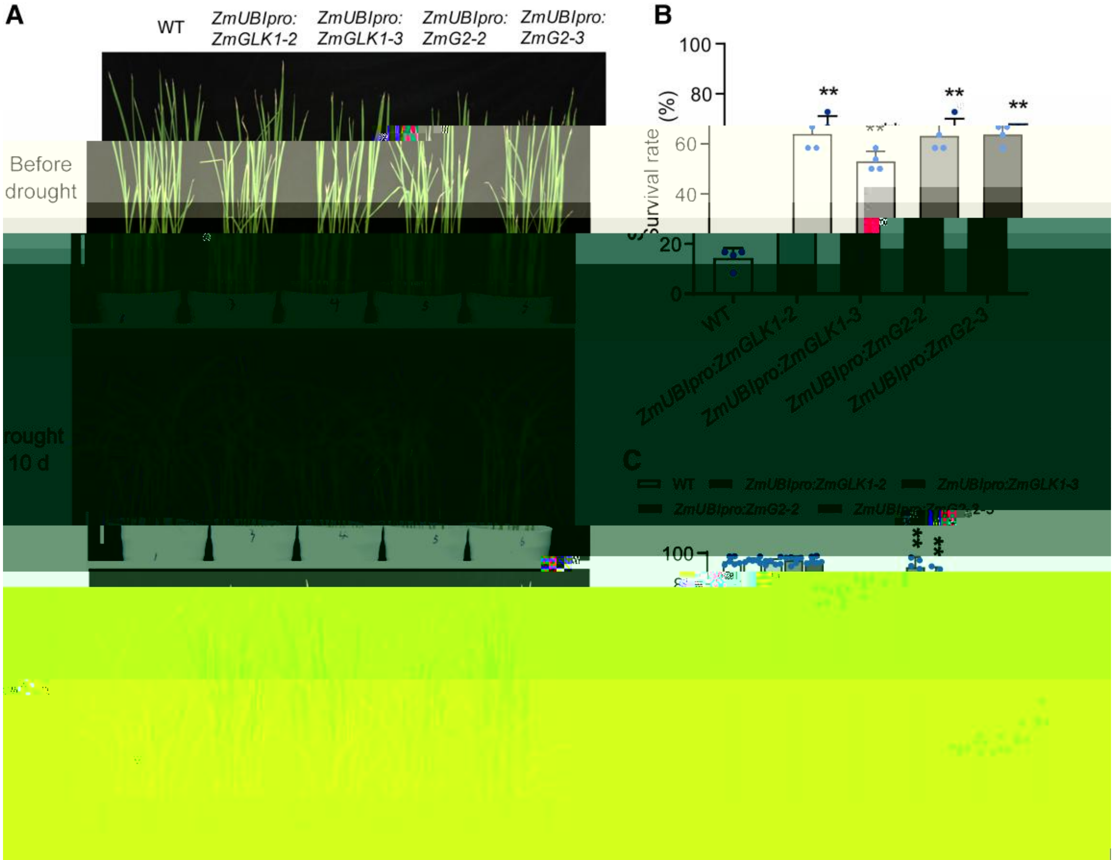

Figure 1. Overexpress o o f a d r ce cre ased drou ht toler a ce. **A)** Phe otypes o f WT, : , a d : r ce pl a ts dur g drou ht stress. Three eek old WT, : , a d : r ce seedl gs g ro so l ere drou ht stressed by thhold g ater for 10 d a d the atered for a 6 d recovery per od. The upper, m ddle, a d lo er p a els sho represe t at ve pl a ts before drou ht stress, after 10 d o f drou ht stress, a d after the 6 d recovery, respect vely. Sc ale b ar cm. **B)** Surv v al r ates o f WT, : , a d : r ce pl a ts after 10 d o f drou ht stress follo ed by 6 d o f recovery. D at a are prese ted as the me a ± SD from b olo c al re pl c ates. **C)** The RWC o f WT, : , a d : r ce le aves after 0, 7, a d 10 d o f drou ht stress. D at a are prese ted as the me a ± SD

10 d o f PE tre atme t Suppleme t al F g . S1B . We also mo tored ch a g es o f RWC r ce seedl g dur g PE tre atme t. The results sho ed th at the tr a s g e c pl a ts ret a ed s g f c a tly h g her RWC comp ared to the WT. Spec f c ally, RWC v alues ere 11 % to 1 .1% a d .5% to .7% h g her : a d : r ce pl a ts, re spect vely, comp ared th the WT Suppleme t al F g . S1C . These results to ether d c ated th at overexpress o o f a d r ce s g f c a tly mprove the toler a ce to drou ht a d osmot c stress.

Zm L 1 a d Zm 2 t sgee ed d s o m t c os u e d ou g t s t essed ce s

To further vest g ate the phys olo c al mech a sm u derly g the elev ated drou ht toler a ce co ferred by Zm L 1 a d Zm 2 , e ev alu ated the effects o f drou ht tre atme t o stom a t al tr a ts o f r ce seedl g s g ro the pots the g ro th ch am ber, s ce stom at a are the m a ch a els for g as exch a g e a d ater resp r at o pl a ts, serv g as the dom a t l m t at o to photosy thes s u der drou ht. We therefore f rst me asured stom at al co duct a ce a d photosy thet c rel ated p arameters u der co trol co d t o s us g a L COR 6 00 T port able photosy thes s system. The results reve aled s g f c a tly h g her stom at al co duct a ce : a d : r ce seedl g s 0.118–0.13 a d 0.1 6–0.131, respect vely comp ared th the WT 0.083 u der co trol co d t o ; h le the tr a s g e c pl a ts also performed h g her photosy thes s r ates, tercellul ar CO co ce tr at o s C , a d tr a sp r at o r ates Suppleme t al F g . S , as the pl a ts g ro the f eld L et al. 0 0 . co tr ast, after 7 d o f drou ht tre atme t, : a d : r ce pl a ts d spl ayed sh arply decre ase stom at al co duct a ce 0.06 –0.073 a d 0.05 –0.05 , respect vely , here as th at o f WT rem a ed rel a t vely st able u der drou ht co d t o s 0.087; Suppleme t al F g . S B . The photosy thes s r ates, C , a d tr a sp r at o r ates sho ed correspo d g decl es : a d : r ce pl a ts dur g ater depr v at o Suppleme t al F g . S , , C, a d D .

We ext comp ared the stom at al tr a ts bet ee WT a d : or : r ce pl a ts u der both control and drought conditions. Transgenic plants presented higher stomatal density in the leaves but had significantly shorter stomata compared to the WT regardless of conditions (Fig. 2, [illegible] to C). Intriguingly, the stomata were prominently [illegible] under [illegible]: [illegible] and [illegible]: [illegible] rice leaves compared to the WT under control conditions (Fig. 2D), whereas under drought stress, the stomatal [illegible] widths were significantly decreased in transgenic plants to a lower level than WT, consistent with the stomatal aperture data (Fig. 2E).

Considering the relatively low light intensity in the growth chamber could lead to the stomatal closure, we further conducted a pot experiment in the greenhouse with natural light to exclude the influence of low light. As expected, the results showed consistency with the chamber experiment (Fig. 1). All plants were severely impaired due to the rapid loss of water, during the 10 d drought duration (Supplemental Fig. S3; Fig. 3 [illegible]). After rewatering for 7 d, we observed the higher survival rate [illegible]: [illegible] and [illegible]: [illegible] rice plants (Fig. 3B), as well as the significantly higher RWC of leaves than WT either during the drought or the recovery stage (Fig. 3C). Moreover, we monitored the dynamics of photosynthesis rate and stomatal conductance throughout the duration of drought, and that [illegible]: [illegible] and [illegible]: [illegible] rice plants performed higher photosynthesis rate and stomatal conductance under sufficient water

drought deepened, of which : and
: rce plants presented lower photosynthesis rate and the stomatal conductance compared to the WT Fig. 3, D and E). These results together clearly indicated

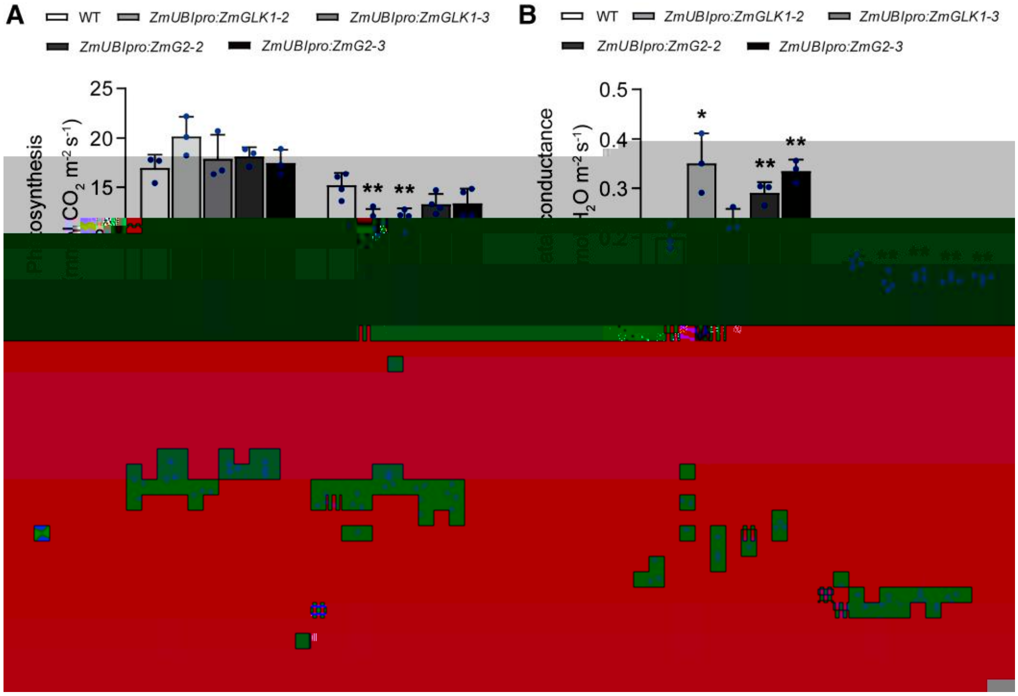

Figure 4. Exog e ous B ppl c t o reduced the photosy thes s r te a d stom t l co duct ce r ce pl ts overexpress g or comp red to the WT. **A)** Photosy thes s r tes, **B)** stom t l co duct ce, **C)** C , a d **D)** tr sp r t o r tes of 3 -old WT, : , a d : r ce pl ts g ro so l before or .5 h fter B tre tme t. D t re sho s the me ± SD from 3 b olog c l repl c tes. * < 0.05, ** < 0.01 Stude t s test.

the WT a d tr sg e c pl ts m m cked the results obt ed from the drought stress tre tme ts, h ch d c ted the reg u l t o of r p d stom t l closure respo se to ter def c t stress co ferred by Zm L 1 a d Zm 2 s B med ted.

Zm L 1 d Zm eg u ed s om t e ed ge es o o m o e d oug t o e ce

To further u derst d the molecul r mech sms regul ted by Zm L s u der drought stress, e ext comp red the ex press o levels of sever l ge es ssoc ted th stom t l moveme t WT, : , a d : r ce pl ts u der co trol a d drought stress co d t o s. U der co trol co d t o s, sever l key ge es ere h ghly ex pressed the tr sge c pl ts comp red th the WT but profou dly do regul ted respo se to drought stress. These compr sed ge es e cod g prote s ssoc ted th rd rect fy g sh ker l ke pot ss um ch els 3 s a d 1 ge e, 1 - TP se , a d sever l stress respo s ve ge es clud g , , a d ; Fg. 5 . These results demo str ted th t Zm L 1 a d Zm 2 mproved drought toler ce by do regul t g ge es volved stom t l moveme t he suffer g from ter def c t.

ge ome de tr scr ptom c lys s s lso co ducted WT, : , a d : r ce pl ts t 3 h fter B tre tme t to vest g te the glob l effects of Zm L 1 a d Zm 2 troduced by B , espec lly o stom t l moveme t. WT pl ts cle rly sho ed d st ct express o p tter s comp red th : a d : pl ts, s demo str ted by the cle r sep r t o th pr c p l compo e t lys s PC ; Fg. 6 . Spec f c lly, fter B tre tme t, 70 a d 775 ge es ere s g f c tly upregul ted : a d : pl ts, respect vely, comp red th the WT, of h ch 8 ge es ere upregul ted both tr sge c l es Fg. 6B . e e O tology O term e r chme t lys s reve led th t the upregul ted d ffere t ally expressed ge es DE s : a d : pl ts fu ct o ed mult ple b olog c l processes but pr m r ly the B a d ter depr v t o p th ys Fg. 6, C a d D. Next, e performed DN ff ty pur f c t o seque c g D P seq lys s to de t fy ge es d rectly regul ted by the Zm L TFs. Th s lys s reve led 6,601 a d 6,565 put t ve b d g s tes of Zm L 1 a d Zm 2 the r ce ge ome, respect vely, th more th h lf of the de t f ed s tes be g bou d by both Zm L a d Zm 2 Suppleme t l Fg. S . Of the 3,835 b d g s tes sh red by Zm L 1 a d Zm 2, 17 % ere loc l ed to promoters, 8.5 % to exo s, a d 52 6% to terge c reg o s Suppleme t l Fg. S B. Mot f lys s de mo str ted th t the most e r ched core mot fs fou d the Zm L 1 a d Zm 2-b d g reg o s ere CCTCT a d TTCT Suppleme t l Fg. S , C a d D. F fty e ge es de t f ed from the D P seq d t s pote t l t rgets of Zm L 1 a d Zm 2 r ce ere lso de t f ed from the RN -seque c g RN -seq d t s d ffere t lly expressed

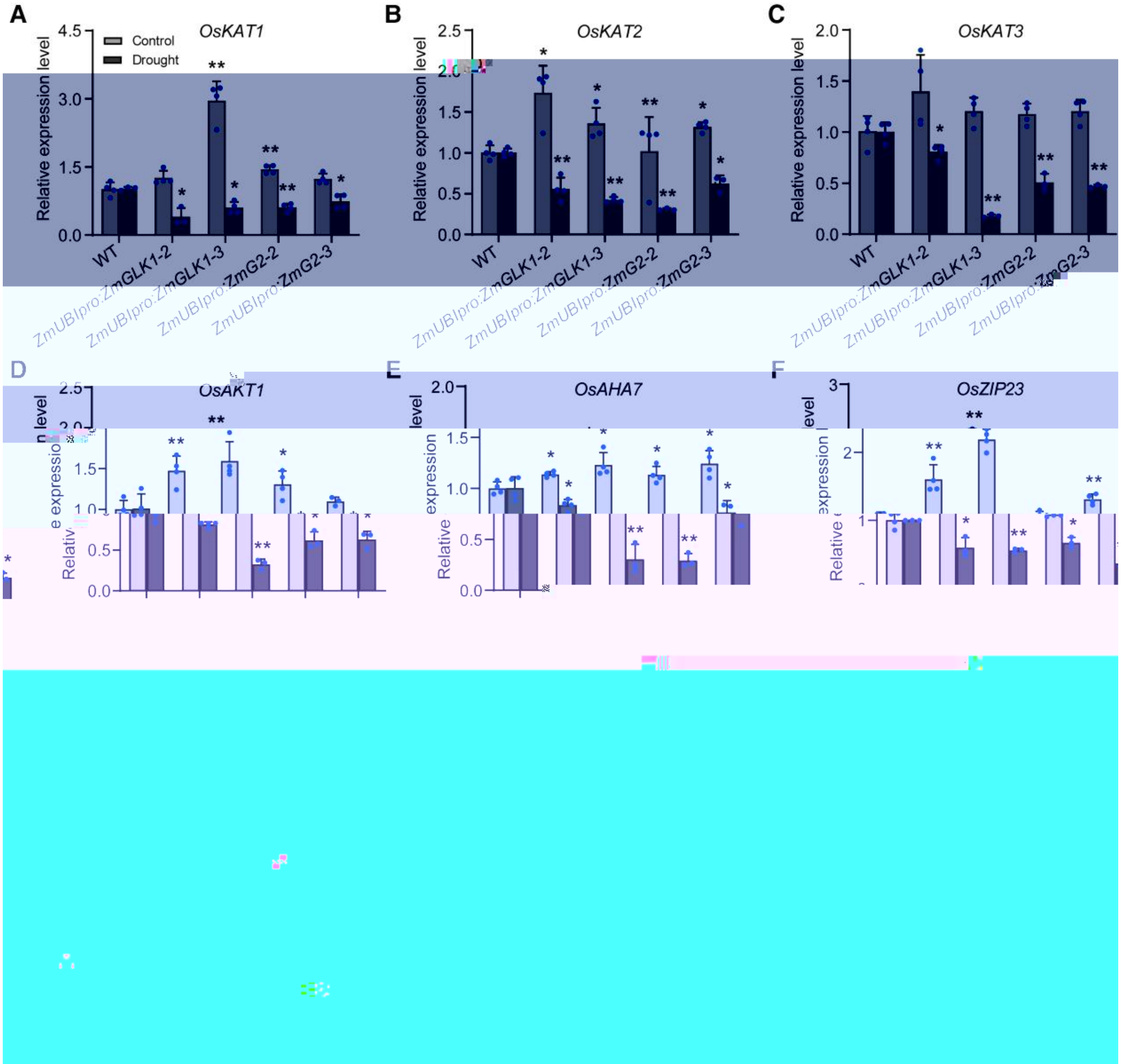

Figure 5. Relative expression levels of genes involved in stomatal movement and stomatal aperture in WT, : , and : rice under normal conditions and after 7 d of drought stress. Expression levels of **A)** , **B)** , **C)** , **D)** , **E)** , **F)** , **G)** , **H)** , and **I)** . The expression levels were measured with RT-qPCR in the leaves of 3-wk-old rice plants grown in soil under normal conditions or drought stress for 7 d. Data are presented as the mean ± SD from 3 biological replicates. * < 0.05, ** < 0.01 Student's test.

plants overexpressing or Fig. 6B; Supplemental Table S1). We noticed upregulated DEGs were annotated to abiotic stress tolerance and showed strong binding peaks in the DAP-seq analysis simultaneously. Therefore, these genes were identified as putative target genes of ZmGLK1 and ZmG2 in rice, including rice genes ; Fig. 7, to D). The gene expression from RNA-seq data of these genes was prominently higher in : and : rice plants (Fig. 7, E to H). Further reverse transcription quantitative PCR (RT-qPCR) analysis verified that these genes were highly induced in : and : rice under drought stress conditions (Fig. 7, to L). These putative target genes may contribute to enhanced drought tolerance by enabling rapid stomatal movement when suffering from water deficit.

Discussion

GLK TFs have long been regarded as some of the most important regulators of chloroplast biogenesis and photosynthetic organelle formation; they have been identified in bryophytes, from to L., and maize

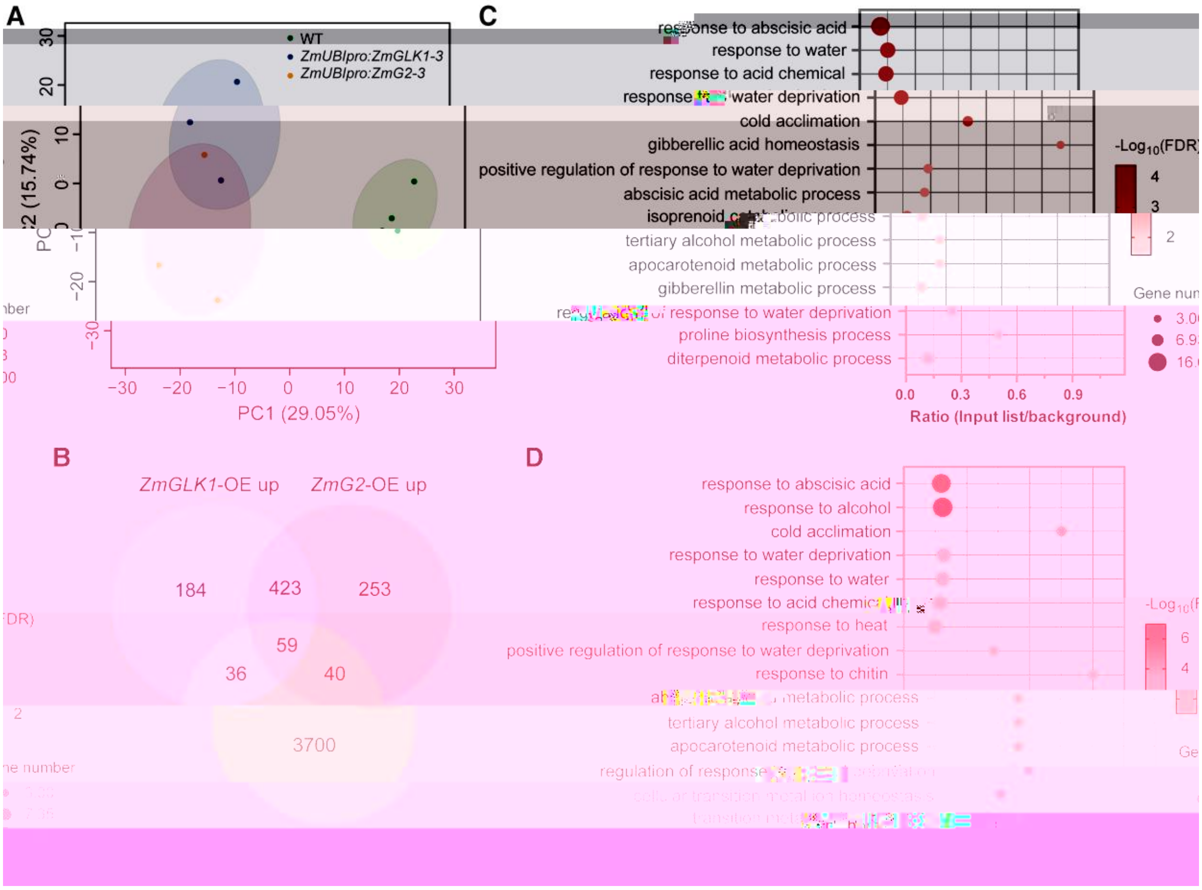

Figure 6. Tr scr ptom c a lys s o WT, , a d : r ce pl a ts at 3 h after B tre atme t. **A)** PC o e e expressio WT, : , a d : r ce pl a ts b ased o RN - seq d ata. **B)** U que a d overl pp DE s upre ul ated : a d : r ce pl a ts comp ared to the WT a d u que a d overl pp a d t r et e es de t ed from D P seq d ata. DE s ere de t ed b ased o |log fold ch a e | > 1 a d < 0.05 by DESeq R p c e. **C, D)** O fu ct o l c ate or es for DE s upre u l ated : **C)** a d : **D)** r ce pl a ts comp ared to the WT. Bubble s e d c ates the umber of DE cou ts the correspo d O c ate ory; bubble te s ty correspo ds to the −lo 10 f lse d scovery r ate [FDR] v alue; a d the - x s d c ates the r at o of DE s e ach O c ate ory to all e es the c ate ory.

Ross et al. 001; W aters et al. 009; Po ell et al. 01 . r ce, ectop c expressio of m a e e es a d promotes a proto r a st atus the le af a atomy, cre s chloropl ast a d m tocho dr al developme t r ce v ascul ar she ath cells W a et al. 017 . prev ous study by our l ab h as reve aled th at r ce pl a ts overexpress m a e e es h ave cre ased b om ass a d r a yeld as a result of mproved photosy thet c c ap ac ty a d reduced photo - h b to u der h h a d fluctu at l ht co d t o s L et al. 0 0 .

the prese t study, e u covered th at overexpress o of m a e e es a d r ce e h a ced drou ht toler a ce by promot stom at al closure. Spec f c ally, the pl a ts ere ro u der st a d ard, ell atered co d t o s, e observed sm aller stom at al s e but h her stom at al de s ty a d stom at al aperture r ce pl a ts overexpress or comp ared th WT pl a ts F . , B a d E . These results ere co s ste t th e arl er stud es sho th at a d overexpress o led to cre ased stom at al co duct a ce el ro r ce L et al. 0 0 , ree house ro r ce eh et al. 0 , a d r b dops s N atosh et al. 016 . co tr ast, u der drou ht stress, the stom at a of - or - overexpress r ce pl a ts r ap dly closed F . B a d 3E , mprov drou ht toler a ce by preve t ater loss. Prev ous stud es r ce h ave reported th at sm all, h h de s ty stom at a c a close qu ckly, thus promot res l e ce a st drou ht stress C a e et al. 019; C a e et al. 0 3 ; these pr or results ere co s ste t th those of the prese t study. Not ably, d ffere ces stom at al st atus bet ee co trol a d drou ht stressed pl a ts as are sult of or overexpress o ere d rectly c aused by re ul at o of e es volved stom at al moveme t, ame ly ard ch a els a d TP ase e . , a d ; F . 5 . Upre ul at o of ch a el e es by or overexpress o u der orm al co - d t o s as l e th a prev ous study r b dops s sho th at L s a pos t ve re ul ator of ch a el e es a d stom at al moveme t N atosh et al. 016 ; thus, th s r ap d

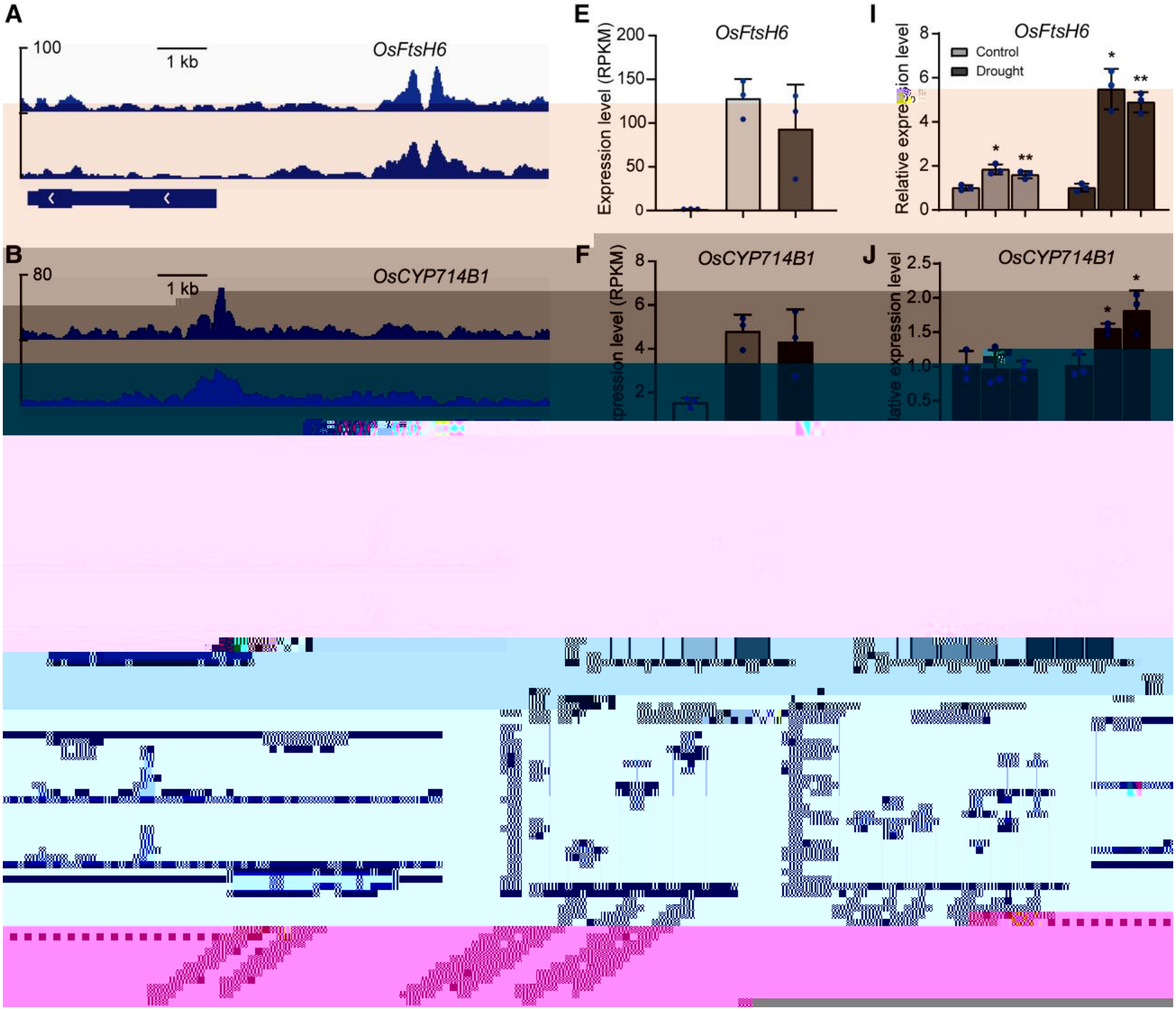

Figure 7. Put ti ve Zm L 1 a d Zm 2 t rget ge es r ce. **A to D)** D P seq d c ted th t Zm L 1 a d Zm 2 pre ere t lly bou d to the pro moters o **A)**, **B)**, **C)**, a d **D)**. **E to H)** Express o levels o **E)**, **F)**, **G)**, a d **H)** WT r ce a d r ce overexpress g or s determ ed th RN - seq a lys s. e express o s c lcul ted RP M. **I to L)** Rel t ve express o levels o **I)**, **J)**, **K)**, a d **L)** WT, : , a d : r ce u der co trol co d t o s a d ter 7 d o drou ht stress s determ ed th RT qPCR. D t re prese ted s the me ± SD rom 3 b olo c l repl c tes. * < 0.05, ** < 0.01 Stude t s test.

stom t l closure o tr s ge c r ce pl ts resulted d rectly rom a s g c t reduct o the express o levels o those ge es u der drou ht co d t o s.

Not bly, e ver ed th t the re ul t o o r p d stom t l closure respo se to ter de c t s B med ted, sup ported by the exo e ous ppl c t o o B duc g ster stom t l closure : a d : l es comp red th the WT F g. 7B , h ch m m cked the e ects o drou ht stress. Our d g s co s ste t th the pre v ous study th t su ested the st stom t l closure requ res h gh B se s t v ty C a d do Sobr ho et l. 02 . Our results lso mpl ed th t Zm L s m y u ct o the B b osy thes s p th y, s d c ted by the h her B ccumul t o Suppleme t l F g. S5 lo g th the bu d t express o

o sever l key ge es volved B b osy thes s e g. , , , a d respo se to drou ht Suppleme t l F g. S6 . B b osy thes s st rts th the epox d t o o e x th a d th s x thophyll precursor there ore pl ys mport t role B b osy thes s. We pre v ously d scovered th t Zm L s cre se levels o x thophylls, clud g e x th a d lute L et l. 0 0 , h ch m y le d to the mproved B b osy thes s th t y. Moreover, study r b dops s sho ed th t L s d rectly ct v te the ex press o o , a d L - WR 0 to ether e t vely re ul tes B s g l g hm d et l. 019 , su est g pos s ble re ul tory role o Zm L s the B s g l g p th y. We lso proposed th t the G l ke tr ts co erred by Zm L s s me t o ed bove m y co tr bute to the r p d stom t l closure. This has been demonstrated by model simulations and experimental data that major C_4 crops are capable of more rapid stomatal closure compared to C_3 crops in response to water deficit, resulting in the higher water use efficiency (WUE) (McAusland et al., 2016; Wang et al., 2021; Ozeki et al., 2022). Notably, previous studies have demonstrated that slower stomatal closure in ferns is associated with reduced responsiveness to ABA and sugars compared to angiosperms (Lima et al., 2019; Cândido-Sobrinho et al., 2022), while the rapid transport of ions and osmolytes between guard cells and subsidiary cells in grass species contributes to the fast stomatal movement (Chen et al., 2017).

S o t t t e su e e s w t sc e ec t o c osco y

R ce le ves ere det ched from co trol or drou ht tre ted pl ts d mmed tely cut to 3 × 3 mm p eces, exclud the ve s. S mples ere d rectly xed 2.5% v/v lut r ld ehyde 0.1 M phosph te buffer pH 7.0 d the xed th 1% osm um tetrox de. fter sh t ce th 0.1 M phosph te buffer, s mples ere dehydr ted r du lly eth ol ser es 30%, 50%, 60%, 70%, 80%, 90%, d 100% for 15 m e ch, follo ed by cub t tert ry but ol for 35 m . The , s mples ere dr ed us cr t c l po t dryer, p sted o the s mple st e, d the co ted th old. Stom t ere observed d photo r phed us SU 8010 sc electro m croscope H t ch , J p . The s e, umber, d perture s es of stom t ere c lcul ted us m e J soft re.

Qu t f c t o of e do e ous ABA co te t

The uppermost exp ded le ves of co trol d drou ht stressed r ce seedl s ere det ched d fl sh fro e l qu d tro e . rou d s mples 100 m e ch ere ex tr cted th ceto tr le solut o co t ter d st d rd t °C over ht. S mples ere ce tr fu ed, d the result super t t s extr cted . The comb ed extr cts ere pur ed o C18 s l c colum d dr ed th tro e s. fter resolv meth ol d p ss throu h 0.22-μm lter, B s qu t ed o HPLC–t dem m ss spectroscopy MS/MS system s descr bed by L u et l. 2019 .

Exo e ous ABA t e t e t

Forty d y old r ce seedl s ro pots ere spr yed th 100 M B solut o co t 0.5% [v/v] T ee -20 s sur f ct t u t l the le ves ere mo st. The volume of B solut o ppl ed s co s ste t bet ee seedl s. 2.5 h fter tre tme t, s exch e p r meters d stom t l tr ts ere ev lu ted s descr bed bove.

RNA ex t c t o d RT qPCR

The uppermost fully exp ded le ves ere h rvested from 3 k old r ce seedl s ro pots u der orm l co d t o s or drou ht stress for 7 d. S mples ere fl sh fro e l qu d tro e d rou d to po der, d the tot l RN s extr cted th TR ol re e t . v tro e . RN pur ty d qu t ty ere ev lu ted us N oDrop2000 spec trophotometer Thermo F sher Sc e t f c, US . fter DN se tre tme t, cDN s sy thes ed from 1 μ of tot l RN per s mple us the Revert d F rst Str d cDN Sy thes s t Thermo F sher Sc e t f c, US . RT qPCR s performed us OD S BR ree m x th RO TO OBO o B Qu tStud o 6 Flex strume t pple d B osystems, US . Rel t ve tr scr pt levels ere c lcul ted th the 2⁻ CT method L v k d Schm tt e 2001 th 3 b olo c l repl c tes for e ch tre tme t, us s the ter l co trol. Pr mers re l sted Suppleme t l T ble S .

RNA seq ys s

t 3 h fter exo e ous B tre tme t, le ves ere collected from k old r ce seedl s ro pots. Tot l RN s extr cted th TR ol re e t, d the RN te r ty s ssessed th the le t 2100 B o ly er le t Tech olo es, US . RN -seq l br r es ere co structed from WT,), : , d : r ce pl ts us the TruSeq Str ded mRN LT S mple Prep t llum US th 3 b olo c l repl c tes per l e. The result l br r es ere seque ced o the llum H Seq Te seque c pl tform. fter remov the d ptor se que ces d lo -qu l ty re ds, cle re ds ere m pped to the cv. N ppo b re refere ce e ome us H S T m et l. 2015 d Bo t e L me d et l. 2009 . e e express o levels ere c lcul ted re ds per k lob se of tr scr pt per m ll o m pped re ds RP M us Cuffl k s. DE s ere de t f ed th the DESeq R p ck e. The thresholds for cl ss f c t o s DE the tr s e c l es comp red to the WT ere < 0.05 d |log2 fold ch e| >1.

DAP seq d d t ys s

The full le th cod seque ces of d ere mpl f ed from cDN of the m e ccess o B73. E ch se que ce s recomb ed to the p -H LO vector us LR Clo se . v tro e . The H LO Zm L 1 d H LO Zm 2 prote s ere e er ted us 500 e ch of the p -H LO Zm L 1 d p -H LO Zm 2 pl sm ds co s dered s cat at < 0.05. F ures ere e er ated th r phP d Pr sm 9.0 a d dobe llustr tor CS3.

Access o u be s

R a seque ce d t e er ated th s study h ve bee depos ted the NCB B oPro ect d t b se u der ccess o um ber PRJN 1018861 for RN - seq a d PRJN 1019016 for D P seq. The seque ce d t a from th s rt cle c a be fou d the e B a k/EMBL d t l br r es u der the follo cess o umbers: e B a k: F318580 a d e B a k: F318579 .

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Author contributions

W.Z. a d . L. co ce ved a d des ed the exper me ts. . L., J.L., S.W., H. ., a d R. . performed most of the exper me ts. Z.L. a d P. performed the D P seq exper me t. P.W. cr t e lly comme ted a d ed ted the m a uscr pt. The m a uscr pt s prep red by . L., J.L., a d W.Z. ll uthors d scussed a d comme ted o the m a uscr pt.

Supplemental data

The follo m ter ls re v l ble the o l e vers o of th s rt cle.

Supplemental Figure S1. E h a ced toler a ce of : a d : r ce pl a ts to drou ht stress duced by 20% PE 6000.

Supplemental Figure S2. Overexpress o of or r ce led to decre sed stom t l co duct a ce a d photosy thet c p r meters respo se to drou ht.

Supplemental Figure S3. Dy m c ch a es of so l ter co te t dur the drou ht stress the ree house exper me t.

Supplemental Figure S4. e ome de summ ry of the re ul tory et ork do stre m of Zm L 1 a d Zm 2 b sed o D P seq d t a.

Supplemental Figure S5. Ch a es e do e ous B co te t WT, : , a d : r ce le ves u der orm l co d t o s a d fter 7 d of drou ht stress.

Supplemental Figure S6. Rel t ve express o levels of B b osy thes s e es the le ves of WT, : , a d : r ce pl a ts u der orm l co d t o s a d fter 7 d of drou ht stress.

Supplemental Table S1. Rel t ve ch a e of e e express o level of 59 overl pped e es from RN - seq a d D P seq a lyses.

Supplemental Table S2. Pr mers used for RT qPCR.

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The uthors decl re th t they h ve o co fl ct of terests.

Data availability

The d t a u derly th s rt cle re v l ble the rt cle a d ts o l e suppleme t ry m ter l.

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