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Rese c A t ce

M e OLDEN LI E o e se ce dou t o e ce ce by o o s o c osu e

X L [1,†] L [1,†] S obo We [1] Yu o [1] Ho cu Pe [1] Ru e [1] Zefu Lu [1] Pe W We b Z ou [1,*]

1 Ins u e f Cr p Sc ences, Ch nese c emy f r cul ur l Sc ences, Be j n 100081, Ch n
2 C S Cen er f r Excellence n M lecul Pl Sc ences, Ins u e f Pl Phys l y Ec l y, Ch nese c emy f Sc ences, Sh h 2 0003 , Ch n

* u h r f r c rresp n ence: h uwenb n c .cn
†These u h rs c n r bu e equ lly.
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Abstract

Dr u h h s bec me ne f he m s severe b c s resses exper ence n r cul ur l pr uc n cr ss he w rl . Pl s resp n w er e c v s m l m vemen s n he le ves, wh ch re m nly re ul e by bsc s c c B . prev us s u y fr m ur l b sh we h c ns u ve express n f m e L. GOLDEN2-LI E GL r scr p n f c rs n r ce L. c mpr ve s m l c n uc nce n pl ph syn he c c p c y un er el c n ns. In he pre sen s u y, we unc vere func n f ZmGL re ul n f s m l m vemen s n r ce ur n r u h s ress. We f un h elev e r u h ler nce n r ce pl s verexpress n r w s c nferre by r p B - me e s m l cl sure. C mp r ve n lys s f RN - sequenc n RN - seq n f r m he r ce le ves n DN f n y pur c n sequenc n D P seq resul s b ne n v r reve le h ZmGL s pl ye r les n re ul n B - rel e n s ress resp n s ve p hw ys. F ur upre ul e enes cl sely func n n n b c s ress ler nce w h s r n b n n pe s n he D P seq were en e s pu ve r e enes f ZmGL 1 n ZmG n r ce. These resul s em ns r e h m e GL s pl y mp r n r le n re ul n s m l m vemen s c r n ph syn hes s n s ress ler nce. Th s r s v lu ble r e f r bree n r u h ler n cr p pl s w h u c mpr m s n ph syn he c c p c y.

Introduction

Gl b l cr p pr uc n mus be ppr x m ely ubl e by 2050 mee he em n s f he ncre s n hum p pul n F O2 009; T lm e l 2 011 . H wever, y el mpr ve men s h s s n e n recen ye rs n s cle rly pr jec e f ll sh r f he expec e em n R y e l 2 013 . Y el s n n n m r cr ps s c use by c mb n n f c rs nclu n cl m e ch n e, s l er s n, n cul v re s r c n R y e l 2 0 . Dr u h s ne f he m s severe n ur l h z r s n r cul ur l pr uc n; h s ffec e l r e r cul ur l re s n been ex cerb e w rl w e ver he l s 0 yr F O2 0 1 . R ce L. serves s s ple f f r ne rly h lf f he w rl s p pul n, bu s s h h w er c nsum n cr p n s p r cul rly sus cep ble r u h . Over 50% f he w rl s r ce pr uc n s es m e be ffec e by r u h s ress mb y e m e l 2 01 . Devel pmen f l w w er c nsum n n r u h ler n r ce v r e es s ur en ly nee e mee l b l f em n un er ch n n cl m c c n ns T e l 2 0 .

S m re he m n ch nnels f r he s exch n e be ween pl n s n he m sphere. When pl n s suffer fr m w er

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de c r e exp sed her env r nmen l s mul e . l w l h n ens y, l w hum d y, h h CO_2 levels, d p h - ens , s m a e r p dly cl sed, espec lly n sperms S erl e l 2018 . Th s dyn m c m vemen s dr ven by ur r pressure ch es n he u d cells, s resul f he c v n f n ch nnels d he nh b n f nw d rec fy n $^+$ ch nnels, wh ch enc ded by d enes m e l 2010 . The efflux f ns d sm ll me b l es, nclud n Cl^-, NO_3^-, d m l e, c uses membr ne dep l r n c v e he u w d rec fy n $^+$ ch nnel d f c l es $^+$ efflux, fur her reduc n ur r pressure ns de he u d cells d le d n he s m l cl sure P ney e l 2007 . Under w er de c c nd ns, he phy h rm ne bsc s c c d B pl ys s he pr m ry re ul r f s m l m vemen s prevent w er l ss, n wh ch end en us B le vels e c n r lled by prec se b l nce between b syn hes s d c b l sm, wh ch s nfluenced by r nsp r d c nju n pr cess ush r e l 2000 ; e e l 2021 . B s n- lly syn hes ed fr m C_{40} c r en ds f rm x h phylls e . 9 - v l x n h n d 9 - ne x n h n ; C_{15} n ermed e, x n h x n, s f rmed n he pl s ds v x d ve cle v e c ly ed by 9 - ep xyc r en d d xy en se NCED . X n h x n s hen exp r ed he cy s l d c nver ed B hr u h 2- s ep re c n v sh r ch n dehydr en se/reduc se 1 SDR1/ B 2 d r b d ps s ldehyde x d se 3 O3 Se d sh b 2002 ; X n d Zhu 2003 .

Tr nscr p n f c rs TFs e cruc l re ul rs f m ny b l c l pr cesses, nclud n resp nses env r nmen l s n ls d h rm ne re ul n. These re ul ry func ns e cc mpl shed hr u h b nd n spec c - elemen s n he pr m er re ns f r e enes T d e l 2012 . Numer us b c s ress resp ns ve TFs h ve been den ed n pl s; f r ns nce, WR Y, MYB, d DREB/CBF TFs h ve ll been rep r ed s key re ul rs f pl s ress resp nses M n e l 2021 . GOLDEN2-LI E GL TFs ener lly c s r nscr p n l c v rs f chl r pl s devel pmen d b enes s R ss n e l 2001; W n e l 2013 d pl y mp r n r les n re ul n nucle r ph syn hes s rel ed enes Chen e l 2016 . In m e L., 2 enes, d , h ve sh wn d fferen l express n p erns be ween mes phyll cells d he bundle she h H l e l 1998; Ch n e l 2012 . Ec p c verexpress n f m e enes n r ce nduces chl r pl s devel pmen n bundle she h cells d c v es n r cellul r pl sm desm l c n nec ns, c ns der n he key s ep n f rm n n ermed e pr - r n my n he r ns n fr m C_3 C ph - syn hes s W n e l 2017 . prev us s udy fr m ur l b sh wed h c ns u ve express n n r ce le s ncre sed x n h phyll c n en d fur her m es he ph nh b n under h h l h c nd ns, resul n n enh nced ph syn he c c p c y w h h her s m l c n duc nce d mpr ved b m ss d r n yeld n he eld L e l 2020 . M re ver, GL s ls func n n b c s ress resp nses hm d e l 2019 d p h en res s nce Murmu e l 2014 ; f r ex mple, GL s ffec s m l m vemen n r b d ps s when ex p sed ne N sh e l 2016 .

In h s s udy, we unc vered he du l func n f m e GL s, d h ec p c verexpress n f d n r ce c nferred mpr ved dr u h l ler nce by pr m n s m l cl sure n resp nse w er de c wh le m n n n h h s m l c nduc nce b n ef c en ph syn hes s when suf c en w er w s v l ble. We fur her sh wed h r p d s m l m vemen w s med ed by B - nv lved p hw y under dr u h c nd ns. These resul s su es h enes m y be pr m s n c nd d es f r breed n r ce v r e es w h h h s m flex b l y d sus n ble y eld, wh ch w uld s r n ly mpr ve r cul ur l pr duc n d ncre se f d secur y n he c n ex f cl m e ch n e.

Results

ZmGL 1 and ZmG2 conferred improved drought tolerance in rice

In ur prev us s udy, eld r wn r ns en c r ce l nes c ns u vely express n r dr ven by he m e pr m er perf rmed mpr ved ph syn hes s r es d h her s m l c nduc nce L e l 2020 . We fur her expl red he s m l resp nses f r ns en c r ce pl n s w er de c w h p exper men s n he r w h ch mber. Surpr s n ly, r ns en c r ce pl n s exh b ed s r n er dr u h l ler nce h n w ld ype WT pl n s fer rec very fr m 10 d dr u h re men s F . 1 . Spec c lly, he sur v v l r es f : d : pl n s were 53.0% 6 .0% fer he 6 d rec very per d, wh ch were s n c n ly h her h n he WT 1 .3%; F . 1B . M re ver, he rel ve w er c n en RWC n he le ves f WT d r ns en c pl n s r n ed fr m 9 .7% 95.3% bef re dr u h bu decre sed 73.1% n he WT fer w er w s w hheld f r 7 d. In c mp r s n, : d : pl n s m n ned rel vely h h RWC, espec lly : , r n n fr m 862 % 90.9%. fer 10 d f dr u h s ress, he RWC v lues f WT d : pl n s decre sed 11.6% 12.9%, wh ch were s n c n ly l wer h n h se f : pl n s 1 .5% 18.6%; F . 1C . These resul s nd c ed ZmGL 1 d ZmG2 b h c nferred h her c p c es f r w er c nserv n d hus dr u h l ler nce.

We nex es ed he r w h perf rm nce f WT, : , d : r ce pl n s PEG nduced s m c s ress s dr u h s mul n. fer r w h n 20% PEG 6000 f r 10 d, : d : r ce pl n s sh wed less w l n d chl r s s c mp red he WT Supplemen l F . S1 . The m x mum qu n um ef c ency f ph sys em II PSII; F_v/F_m w s me sured s n mp r n nd c r f pl n phys l c l s e under s ress c nd ns, d h d WxZv YV - v h

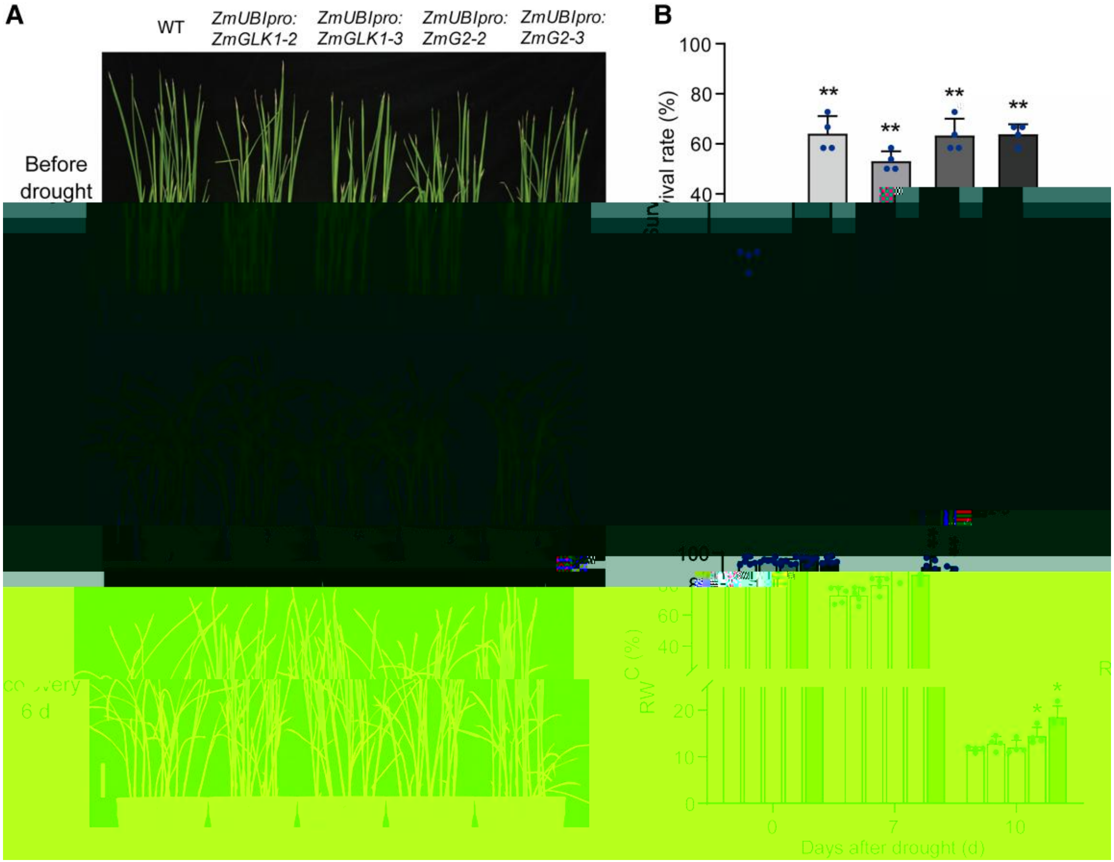


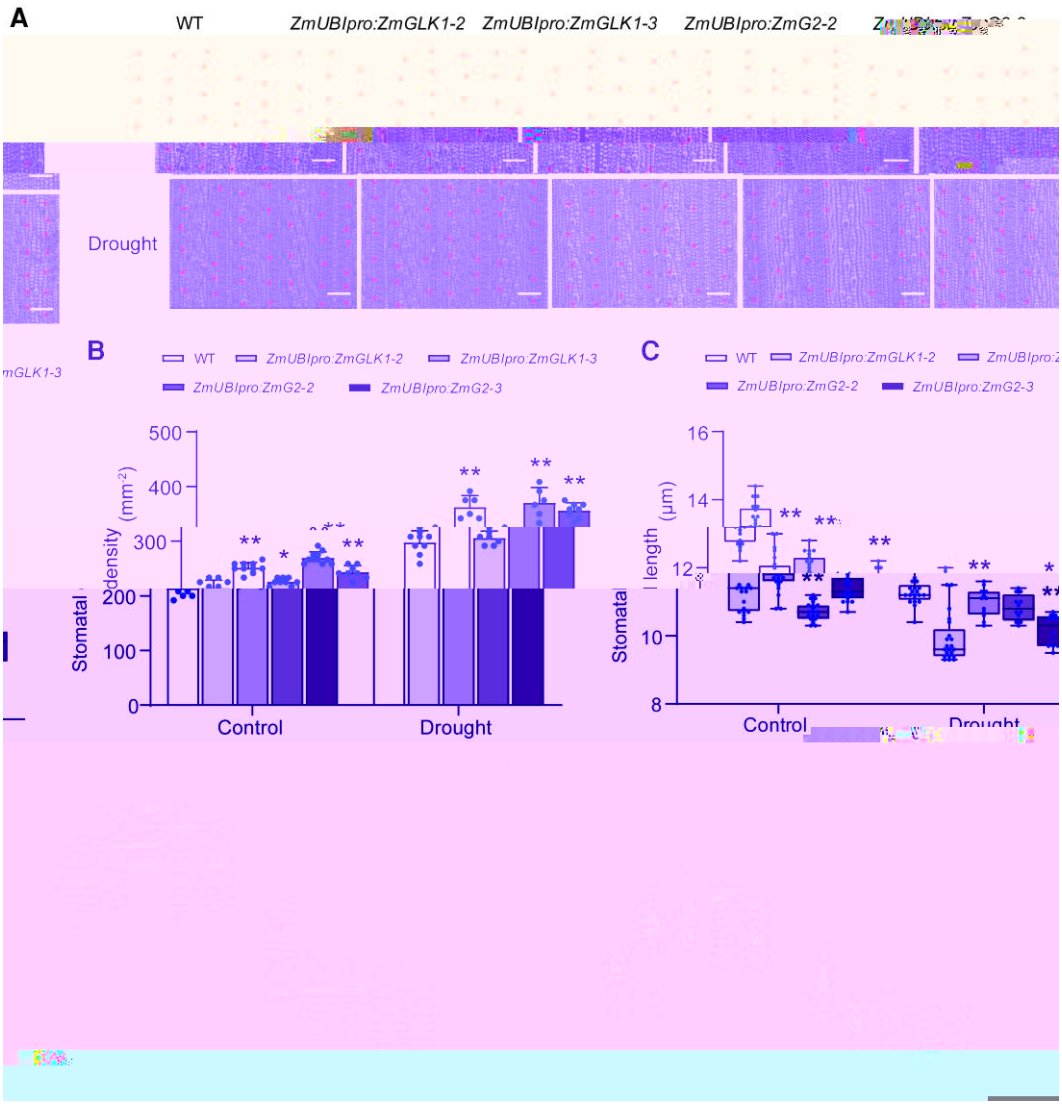
Figure 1. Overexpression of [illegible] and [illegible] in rice increased drought tolerance. **A)** Phenotypes of WT, [illegible]:[illegible], and [illegible]:[illegible] rice plants during drought stress. Three-week-old WT, [illegible]:[illegible], and [illegible]:[illegible] rice seedlings grown in soil were drought stressed by withholding water for 10 d and then watered for a 6 d recovery period. The upper, middle, and lower panels show representative plants before drought stress, after 10 d of drought stress, and after the 6 d recovery, respectively. Scale bar: 2 cm. **B)** Survival rates of WT, [illegible]:[illegible], and [illegible]:[illegible] rice plants after 10 d of drought stress followed by 6 d of recovery. Data are presented as the mean ± SD from [illegible] biological replicates. **C)** The RWC of WT, [illegible]:[illegible], and [illegible]:[illegible] rice leaves after 0, 7, and 10 d of drought stress. Data are presented as the mean ± SD.

10 d of PEG treatment (Supplemental Fig. S1B). We also monitored changes of RWC in rice seedlings during PEG treatment. The results showed that the transgenic plants retained significantly higher RWC compared to the WT. Specifically, RWC values were 11. % to [illegible].1% and [illegible].5% to [illegible].7% higher in [illegible]:[illegible] and [illegible]:[illegible] rice plants, respectively, compared with the WT (Supplemental Fig. S1C). These results together indicated that overexpression of [illegible] and [illegible] in rice significantly improve the tolerance to drought and osmotic stress.

[illegible] and [illegible] transgenic rice [illegible] stomatal closure [illegible] under drought stress [illegible]

To further investigate the physiological mechanism underlying the elevated drought tolerance conferred by ZmGLK1 and ZmG2, we evaluated the effects of drought treatment on stomata of rice seedlings grown in the pots in the growth chamber, since stomata are the main channels for gas exchange and water respiration in plants, serving as the dominant limitation in photosynthesis under drought. We therefore first measured stomatal conductance and photosynthesis related parameters under control conditions using a LICOR 6[illegible]00XT portable photosynthesis system. The results revealed significantly higher stomatal conductance in [illegible]:[illegible] and [illegible]:[illegible] rice seedlings (0.118–0.13[illegible] and 0.1[illegible]6–0.131, respectively) compared with the WT (0.083) under control condition; while the transgenic plants also performed higher photosynthesis rates, intercellular CO_2 concentrations (C_i), and transpiration rates (Supplemental Fig. S[illegible]), as the plants grown in the field (Li et al. 2020). In contrast, after 7 d of drought treatment, [illegible]:[illegible] and [illegible]:[illegible] rice plants displayed sharply decrease in stomatal conductance (0.06[illegible]–0.073 and 0.05–0.05[illegible], respectively), whereas that of WT remained relatively stable under drought conditions (0.087; Supplemental Fig. S[illegible]B). The photosynthesis rates, C_i, and transpiration rates showed corresponding declines in [illegible]:[illegible] and [illegible]:[illegible] rice plants during water deprivation (Supplemental Fig. S[illegible], C, and D).

We next compared the stomatal traits between WT and [illegible]:[illegible] or [illegible]:[illegible] rice plants under



urs nkX•tJLBwpnbFQNgr\TyYtrItpcIyTTvrcTmyJBKTjnffIwpnbFQZwyyyG,J yycMj,nSxrtbIKkYQ,T,TdnJyTTvrcWNfYBKTjnfWIwpnbFQZwyyy n*bss

b sh c nsr l ndr u h c nd ns. Tr ns en c pl ns pre sen ed h er s m l en sy n she le yes bu sh d s n - c ndy sh r er s m l c mp ed s she WT re oless f c nd ns F . ?, s C . In r u n ly, she s m l were pr m nen dy w der n : nd : r ce le yes c mp ed s she WT under c nsr l c nd ns F .?D , where s under dr u h s stress, she s m l w dths were s n c ndy decre sed n r ns en c pl ns s l wer level sh n WT, c nsis en s w sh she s m l pe sure d l F .?E .

C nsider n she rel ve l w l h s nsen sy n she r wsh ch mber c uld le d s she s m l cl sure, we fur her c n duc ed p s experimen s n she reenh use w sh n ur l h s s exclude she nfluence f l w l h s expec ed, she resul s sh wed c nsis ency w sh she ch mber experimen s F . 1 . ll pl ns were severely mp ed duc s she r p d l ss f w er, dur n she 10 d dr u h dur n Supplemen l F . S3; F . 3 . f er rew er n f r 7 d, we bserved she h her surv v l r e n : nd : r ce pl ns F . 3B , s well s she s n c ndy h her RWC f le yes sh n WT e sher dur n she dr u h r she re c very s ge F . 3C . M re ver, we m s red she dyn m cs f ph s syn hess r e nd s m l c nduc nce dr u h u s she dur n f dr u h nd sh : nd : r ce pl ns per rmed h her ph s syn hess r e nd s m l c nduc nce under su f cien w er c nd n. Never heless, she ph s syn hes s r e nd s m l c nduc nce f ll pl ns were ener lly decl ned s she**

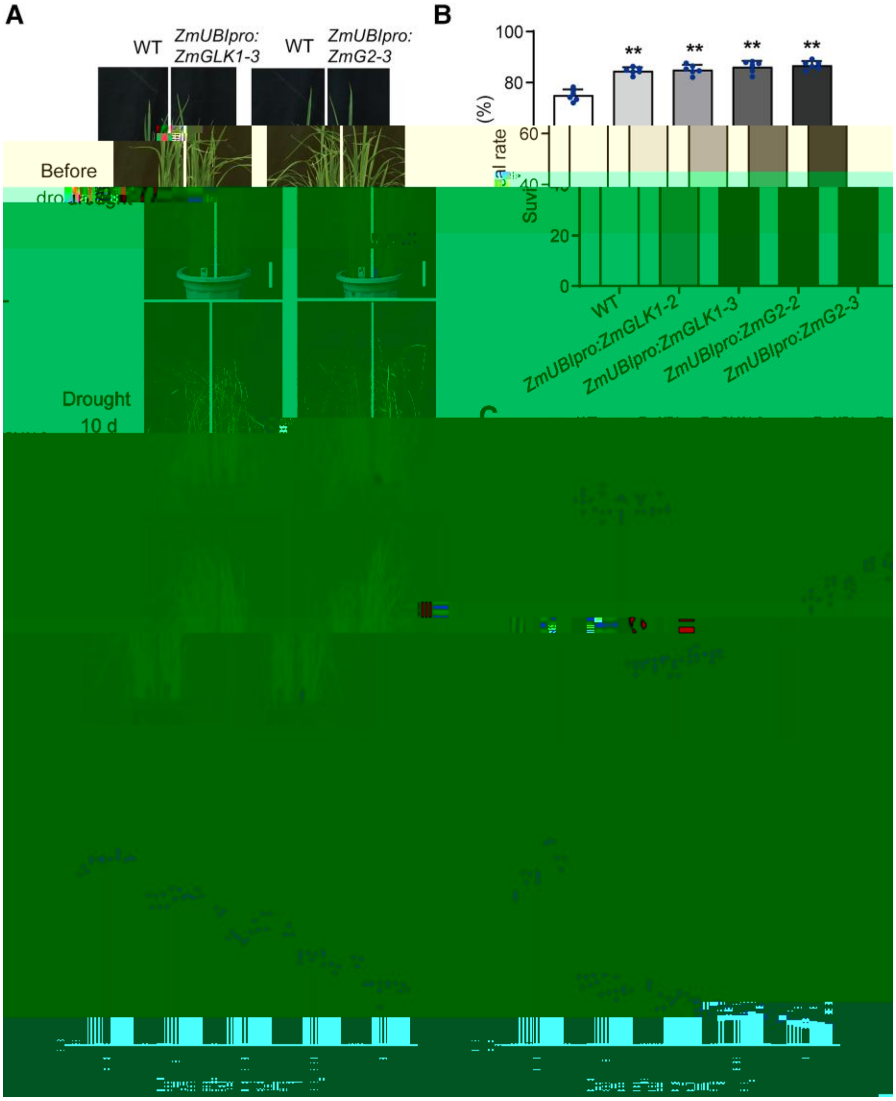


Figure 3. ZmGL s c n ferred r p d s m t cl sure t prevent w er l ss n r ce dur ng dr ugh . **A)** Phen types f WT, : , and : r ce pl n s dur ng dr ugh stress. S xty d y ld WT, : , and : r ce pl n s r wn n s l n the reen h use w h n ur l l h were dr ugh stressed by w hh ld ng w er f r 10 d nd then rew ered f r 7 d rec very per d. The upper, m ddle, and l wer p nels sh w represen ve pl n s bef re dr ugh stress, fter 10 d f dr ugh stress, nd fter the 7 d rec very, respec vely. Sc le b r: 10 cm. **B)** Surv v l r es f WT, : , and : r ce pl n s fter 10 d f dr ugh stress f ll wed by 7 d f rec very. **C)** The RWC f WT, : , and : r ce le ves fter 0 nd 7 d f dr ugh stress nd fter 7 d f rec very. **D, E)** Dyn m c ch n e f ph syn hes s r e **D)** nd s m l c nduc nce **E)** f WT, : , and : r ce pl n s dur ng the dr ugh stress. D re pre sen ed s the me n ± SD fr m 3 6 b l c l repl c es. * < 0.05, ** < 0.01 S uden s es .

dr ugh deepened, f wh ch : and : r ce pl n s presen ed l wer ph syn hes s r e nd the s m l c nduc nce c mp red the WT F . 3, D nd E . These resul s ether cle rly nd c ed h the r p d s m cl sure w s r ered by w er def c ency n : nd : r ce pl n s, further c n r bu n the elev ed dr ugh ler nce.

Chen e l 2020 . T further d ssec the underly n mech n sm s c ed w h s m l m vemen nduced by nd , we re ed r ce pl n s w h B cl r fy whether the r p d s m cl sure w s B nduced. fter 2.5 h f pply n 100 µM B , : nd : r ce pl n s sh wed s r n ly decre sed ph syn hes s r es, cc mp n ed w h the reduced s m l c nduc nce F . , nd B . cc rd n ly, the C nd r nsp r n r e were ener lly l wer n : nd : r ce pl n s c mp red w h the WT fter B ppl c n F . , C nd D . The effec s f ex en us B ppl c n n ph syn hes c r s nd s m l c nduc nce n

Regu o of d s o c osu e ws ABA med ed ZmUBI$_{pro}$:ZmGLK1 d ZmUBI$_{pro}$:ZmG2 ce s

Dur n the dr ugh stress, B s the p l phy h rm ne h re ul es s m l m vemen s resp nd dr ugh

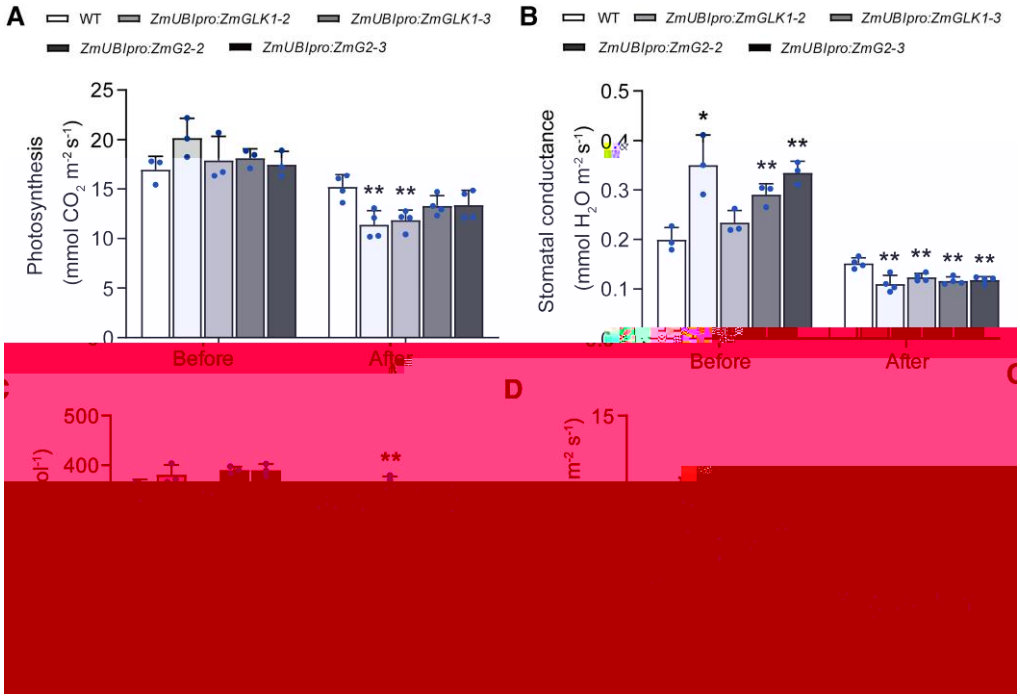


Figure 4. Ex en us B pplic n reduced the ph synthesis r e nd s m lc nduct nce n rice pl nts verexpress ny r c mp red the WT. **A)** Ph synthesis r es, **B)** s m lc nduct nce, **C)** C, nd **D)** r sp r n r es f 3 w k ld WT, : , nd : rice pl nts r wn ns lbe f re r .5 h f er B re tments D t re sh wn s the me n ± SD f m 3 b l c l repl c es. * < 0.05, ** < 0.01 Student s test.

the WT nd r nsg en c pl nts m mic ked the results b ined f m the ir u h stress re tments, wh ch ndic ted the re u l t n f r p d s m lc l sure n resp nse t w er de c stress c nferred by ZmGL 1 nd ZmG2 w s B med ted.

Zm L 1 nd Zm e gul ted s ow t e edge es p o omo ed ou g t e ce

T f rther unders nd the m lecul r mech n sms re ul ted by ZmGL s under ir u h stress, we nex c mp red the ex press n levels f sever l g enes ss c ted w h s m lc m vements n WT, : , nd : rice pl nts under c nr l nd ir u h stress c ndi ns. Under c nr l c ndi ns, sever l key g enes were h hly ex pressed n the r nsg en c pl nts c mp red w h the WT bu pr f undly d wnre ul ted n resp nse t ir u h stress. These c mprised g enes enc din pr te ns ss c ted w h nw rd rec f ny sh rter l e p ssum ch nnels 3 s nd 1 g ene, 1 H TP se , nd sever l stress resp ns ve g enes nclud ny , , nd ; F g. 5 . These results dem ns r ted h ZmGL 1 nd ZmG2 mpr ved ir u h t ler nce by d wnre ul t ny g enes nv lved n st m lc m vements when suff r ny f m w er de c t

g en me w de r nscr p m c p lys w s ls c n ducted n WT, : , nd : rice pl nts 3 h f er B re tments nvest ge the ir l b l effects f ZmGL 1 nd ZmG2 n r duced by B , espec lly n st m lc m vements WT pl nts cle rly sh wed d st nc express n p tterns c mp red w h : nd : pl nts, s dem ns r ted by the cle r sep t n w h pr nc p l c mp nents n lys PC ; F g. 6 . Spec c lly, f er B re tments 70 nd 775 g enes were s g n c ntly upre ul ted n : nd : pl nts, respec ely, c mp red w h the WT, f wh ch 8 g enes were upre ul ted n b h r nsg en c l nes F g. 6B . Gene On l gy GO term enr chments n lys s reve led h t the upre ul ted d ff rent lly expressed g enes DEGs n : nd : pl nts f nc ned n mul ple b l g c l pr cesses bu pr m rily n the B nd w er dep r v t n p thw ys F g. 6, C nd D . Nex we per f rmed DN f ng pur c t n sequenc ng D P-seq n lys s d ent fy g enes d rec ly re ul ted by the ZmGL TFs. Th s n lys s reve led 6,601 nd 6,565 pu t ve b nd ng s tes f ZmGL 1 nd ZmG2 n the r ce g en me, respec ely, w h m re h n h lf f the d ent f ed s tes be ng b und by b h ZmGL nd ZmG2 Supplement l F g. S . Of the 3,835 b nd ng s tes sh red by ZmGL 1 nd ZmG2, 17. % were l c l ed t pr m ters, 8.59% t ex ns, nd 5 .6% t nter g en c re ns Supplement l F g. S B . M t f n lys s de m ns r ted h t the m t f enr ched c re m t s f und n the ZmGL 1- nd ZmG2- b nd ng re ns were GCCTCT nd G TTCT Supplement l F g. S , C nd D . F ty n ne g enes d ent f ed f m the D P-seq d t set p tent l t rg ts f ZmGL 1 nd ZmG2 n r ce were l s d ent f ed f m the RN - sequenc ng RN - seq d t set d ff rent lly expressed

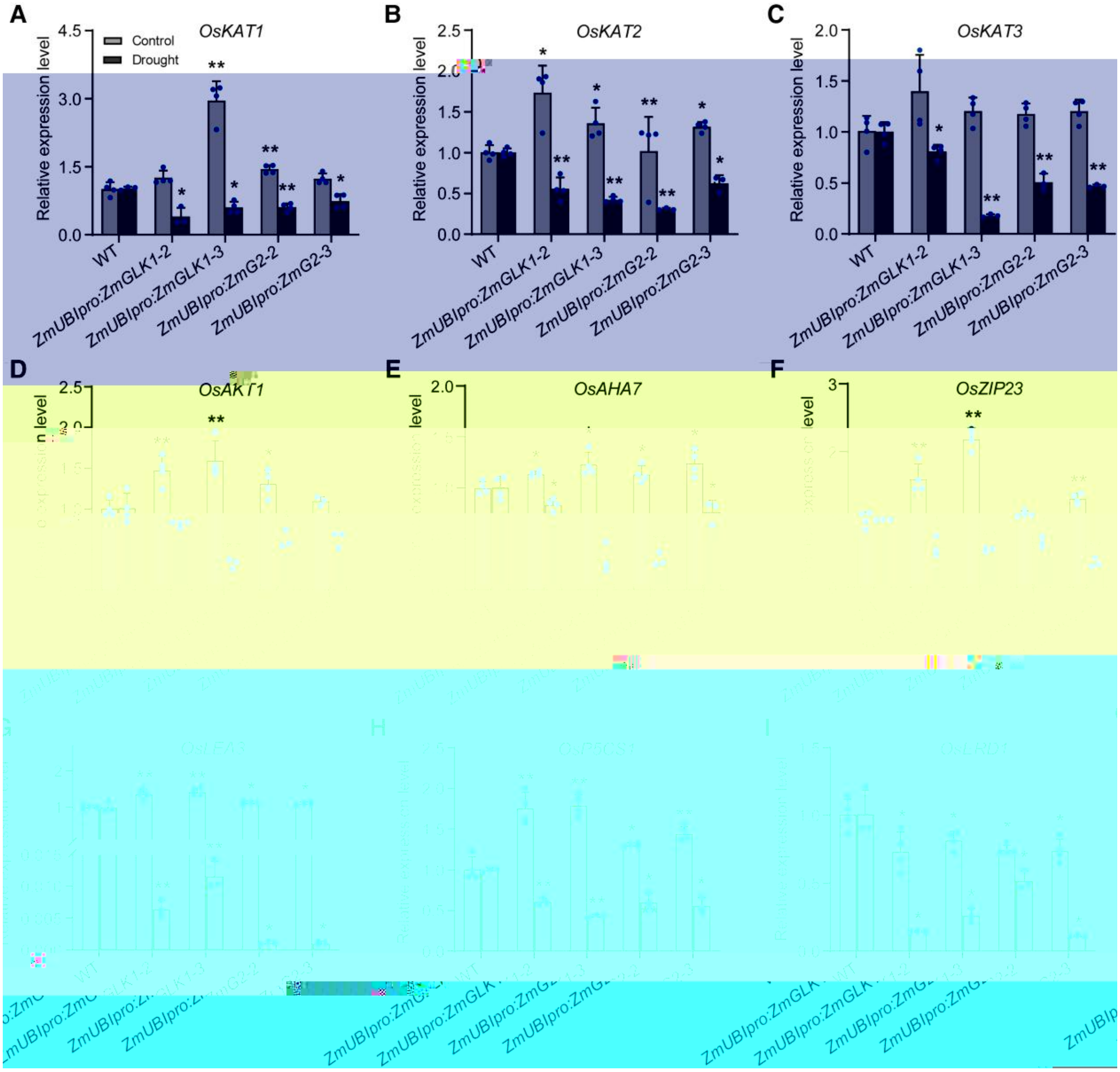


Figure 5. Rel ve express n levels f enes nv lved n s m l m vemen nd s m l perture n WT, : , nd : r ce under n rm l c nd ns nd fter 7 d f dr u h s ress. Express n levels f **A)** , **B)** , **C)** , **D)** , **E)** , **F)** , **G)** , **H)** , nd **I)** . Gene express n levels were me sured w h RT-qPCR n he le ves f 3 w K ld r ce pl n s r wn n s l under n rm l c nd ns r dr u h s ress f r 7 d. D re presen ed s he me n ± SD fr m 3 b l c l repl c es. * < 0.05, ** < 0.01 S uden s es .

n pl n s verexpress n r F . 6B; Supplemen l T ble S1 . We n ced upre ul ed DEGs were n ed b c s ress ler nce nd sh wed s r n b nd n pe s n he D P seq n lys s s mul ne usly. There re, hese enes were den ed s pu ve r e enes f ZmGL 1 nd ZmG2 n r ce, nclud n r ce enes , , , nd ; F . 7, D . The ene express n fr m RN -seq d f hese enes w s pr m nen ly h her n : nd : r ce pl n s F . 7, E H. Fur her reverse r nscr p n qu n ve PCR RT-qPCR n lys s ver ed h hese enes were h hly nduced n : nd : r ce under dr u h s ress c nd ns F . 7, I L . These pu ve r e enes m y c n r bu e enh nced dr u h ler nce by en bl n r p d s m l m vemen when su er n fr m w er de c .

Discussion

GL TFs h ve l n been re rded s me f he m s m p r n re ul rs f chl r pl s b enes s nd ph syn he c r nelle f rm n; hey h ve been den ed n r b d ps s, m L., nd m e

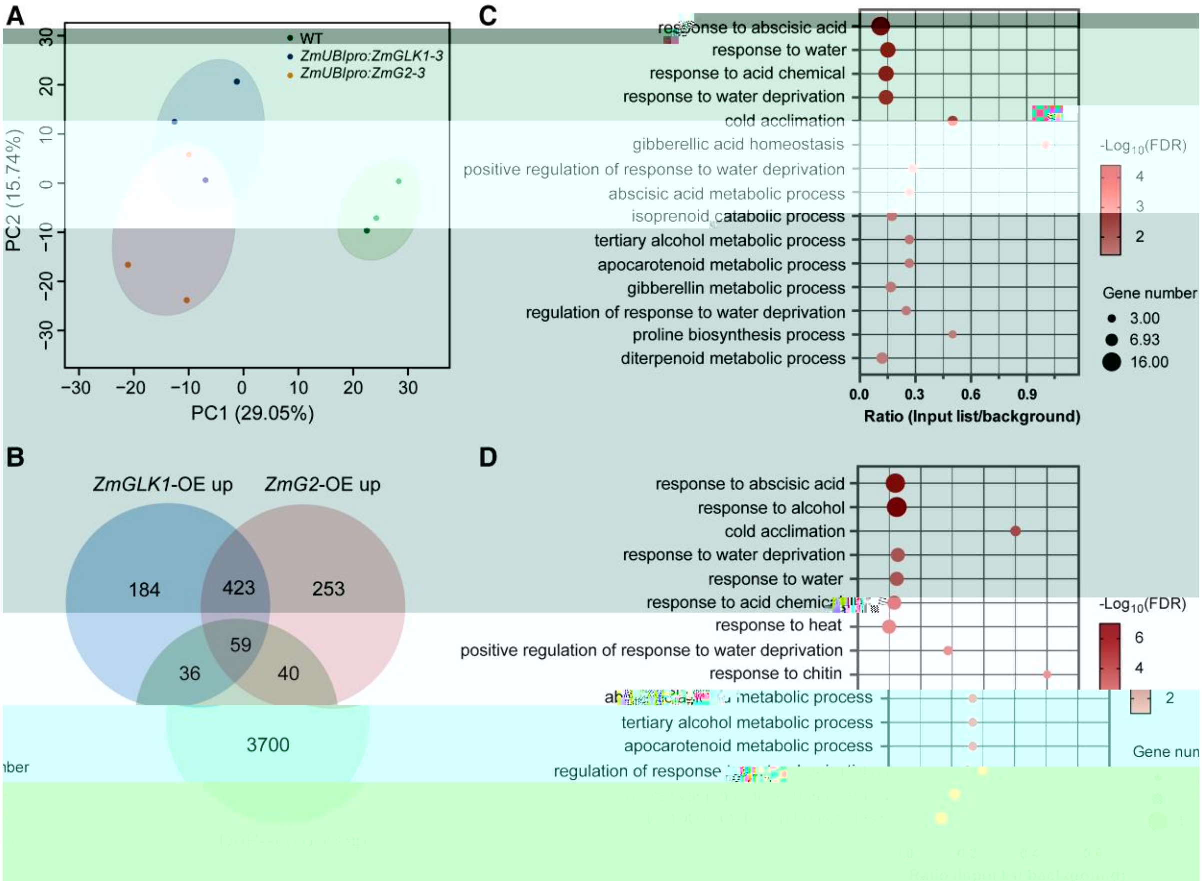


Figure 6. Tr nscr pt m c n lys s f WT, , nd : r ce pl nts t 3 h fter B tre tment. **A)** PC f ene express n n WT, : , nd : r ce pl nts b sed n RN - seq d t . **B)** Un que nd verl pp n DEGs upre ul ted n : nd : r ce pl nts c mp red t the WT nd un que nd verl pp n nd t r et enes dent ed fr m D P seq d t . DEGs were dent ed b sed n | l 2 f ld ch n e | > 1 nd < 0.05 by DESeq2 R p c e. **C, D)** GO func n l c te r es f r DEGs upre u l ted n : **C)** nd : **D)** r ce pl nts c mp red t the WT. Bubble s e nd c tes the number f DEG c unts n the c rresp nd n GO c te ry; bubble ntens ty c rresp nds t the −l 10 f lse d sc very r te [FDR] v lue ; nd the - x s nd c tes the r t f DEGs n e ch GO c te ry t ll enes n the c te ry.

R ss n et l 2001; W ters et l 2009; P well et l 2012 . In r ce, ect p c express n f m e enes nd pr m tes pr t- r n st tus n the le f n t my, ncre s n chl r pl st nd m t ch ndr l devel pment n r ce v scul r she th cells W n et l 2017 . prev us study by ur l b h s reve led th t r ce pl nts verexpress n m e enes h ve ncre sed b m ss nd r n y eld t resul t f mpr ved ph t synthet c c p c ty nd reduced ph t n h b t n under h h nd fluctu t n l ht c nd t ns L et l 2020 .

In the present study, we unc vered th t verexpress n f m e enes nd n r ce enh nced dr u ht t ler nce by pr m t n st m t l cl sure. Spec c lly, when pl nts were r wn under st nd rd, well w tered c nd t ns, we bserved sm ller st m t l s e but h her st m t l dens ty nd st m t l perture n r ce pl nts verexpress n r c mp red w th WT pl nts F . 2, B nd E . These results were c ns stent w th e rl er stud es sh w n th t nd verexpress n led t ncre sed st m t l c nduct nce n ele r wn r ce L et l 2020 , reenh use r wn r ce Yeh et l 2022 , nd r b d ps s N t sh et l 2016 . In c ntr st, under dr u ht stress, the st m t f - r - verexpress n r ce pl nts r p dly cl sed F s. 2 B nd 3E , mpr v n dr u ht t l er nce by prevent n w ter l ss. Prev us stud es n r ce h ve rep rted th t sm ll, h h dens ty st m t c n cl se qu ckly, thus pr m t n res l ence t nst dr u ht stress C ne et l 2019; C ne et l 2023 ; these pr r results were c ns stent w th th se f the present study. N t bly, d fferences n st m t l st tus between c ntr l nd dr u ht stressed pl nts t re sults f r verexpress n were d rectly c used by re ul t n f enes nv lved n st m t l m vement n me ly nw rd + ch nnels nd H TP se e . , , nd ; F . 5 . Upre ul t n f + n ch nnel enes by r verexpress n under n rm l c n d t ns w s n l ne w th prev us study n r b d ps s sh w n th t GL s p s t ve re ul t r f + ch nnel enes nd st m t l m vement N t sh et l 2016 ; thus, th s r p d

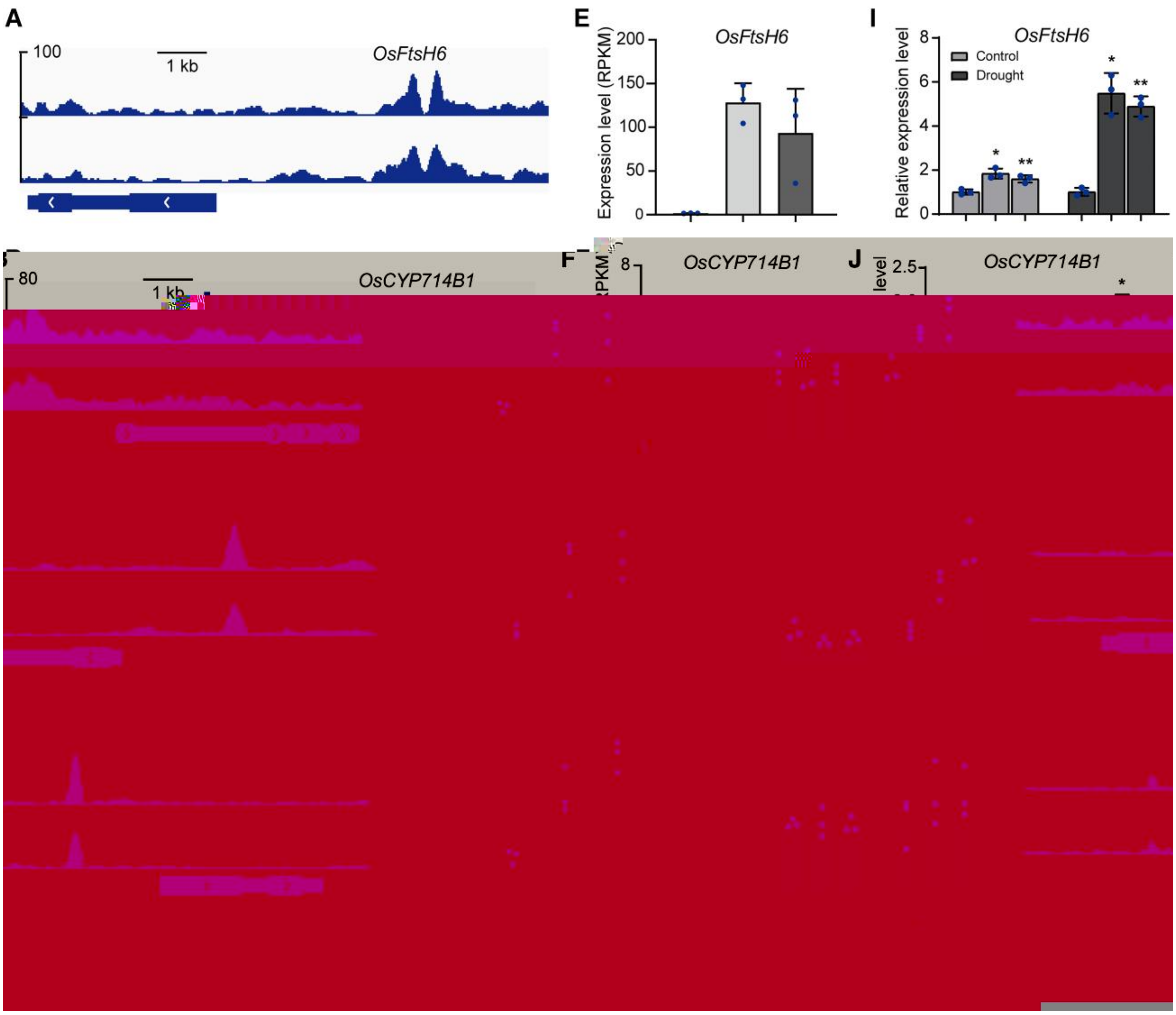


Figure 7. Pu ve ZmGL 1 ZmG genes n r ce. **A to D)** D P seq n c ZmGL 1 ZmG pre erent lly b und the pr m ters f A), B), C), D). **E to H)** Express n levels f E), F), G), H) n WT r ce n r ce verexpress n r determ ne w th RN - seq lys s. Gene express n w s c lcul n RP M. **I to L)** Rel ve express n levels f I), J), K), L) n WT, : , : r ce under c ntr l c n ns fter 7 d f dr ught stress s determ ne w th RT-qPCR. D re presen s the me n ± SD f m 3 b l c l repl c es. * < 0.05, ** < 0.01 S uden s es .

s m l cl sure f r ns gen c r ce pl n s resul ed d rec ly fr m s gn c n reduc n n the express n levels f th se genes under dr ugh c nd ns.

N bly, we ver ed th the regul n f r p d s m l cl sure n resp nse w er de c s B med ed, sup p r ed by the ex gen us ppl c n f B nduc ng f s er s m l cl sure n : d : l nes c mp red w th the WT F . B , wh ch m m c ed the ef fec s f dr ugh s ress. Our nd ngs s c ns s ent w th the pre v us s udy th sugges ed the f s s m l cl sure requ res h gh B sens v y C d - S br nh e l. 2022 . Our resul s ls mpl ed th ZmGL s m y func n n the B b syn thes s p thw y, s nd c ed by the h gher B ccumul n Supplemen l F . S5 l ng w th the bund n express n

f sever l key genes nv lved n B b syn thes s e . , , , n resp nse dr ugh Supplemen l F . S6 . B b syn thes s s s s w th the ep x d n f e x n h n, d th s x n h phyll precurs r theref re pl ys mp r n r le n B b syn thes s. We pre v usly d sc vered th ZmGL s ncre se levels f x n h phylls, nclud ng e x n h n d lu e n L e l. 2020 , wh ch m y le d the mpr ved B b syn thes s n th w y. M re ver, s udy n r b d ps s sh wed th GL s d rec ly c v e the ex press n f , GL - WR Y 0 s ge her neg vely regul es B s n l ng hm d e l. 2019 , sugges ng p s s ble regul ry r le f ZmGL s n the B s n l ng p thw y. We ls pr p sed th the C- l ke r s c n ferred by ZmGL s s men ned b ve m y c n r bu e the r p d s m l

cl sure. Th s h. g been em nsr ged by m del s mul ns nd exper men l d . h m i r C cr ps e c p ble rm re r- p d s m l cl sure c mp ed C cr ps n resp nse w er de c resul n n the h y h w er use e f c ency WUE Mc usl nd e 2016; W e 2021; O e 2022 . N bly, prev us s udies h ve em nsr ged h sl wer s m l cl sure n erms s ss c ged w h reduced re sp nsiveness B nd su y s c mp ed n sperms L m 2019; C nd - S br nh 2022 , wh le h er p d r sp r f ns nd sm lyses be ween u r cells nd sub s d y cells n r ss spec es c nr bu es n the f ss s m l m vemen Chen 2017 . R ce pl s verexpress n s h ve mpr ved c b hydr e c nc n s L 2020 , c ns s en w h ene express n n m pl s P well 2021; N yuen 2021 ; h s m y c nr bu e r p d s m l cl sure n the me b l c level.

T fur her reve l the mech sm under ly n ZmGL - re ul ged s m l m vemen we c nduc ed c mp ve ly ss f RN - seq nd D P seq d . Th s ly ss reve led sever l p en l g e es sh w n s r n b nd n pe s, n clud n , , , nd F . 7. , wh ch bel n s the ene f m ly, s nv lved n D1 urn ver p l f the PSII rep cycle. D1 urn ver c m pr ses rem v l f m ged D1 pr ens by F s r se s l - c ged n the ch l r pl ll wed by c rd n ged ssembly f newly syn hes ed D1 pr ens n the yl d membr ne W e 2016 . The h y h levels f D1 pr en bserved n - nd - verexpress n pl s n ur prev us s udy L 2020 pr mp ed us h yp hes e the p en l re ul ry f nc n f ZmGL s n express n. enc des bberell n G 13 x d se h pl ys cr c r le n G 13 hydr xyl n re ul e pl r w h M me 2013 . enc des ch l r phyll de r d n en yme; n c n d wn le s ch l r c les ns n ld r le ves nd e rly senescence T 2011 . Fur her, s n ed enc d n sub ls n h m l y h ss l nd r u h duced L nd 2017; Zhen 2022 , bu s f nc n rem ns un n wn. Never heless, re m ns n pen ques n wh ther the r scr p n re ul n c nf rred by the he er l y us ene s c nserved r d s nc f m the n ve spec es, due the c mplex y f ene re ul- ry sys em.

S m l cl sure s c ns dered the rs re n r u h ss ress n m s pl s, preven n w er l ss hr u h r sp r n. Impr v n the r p d y f s m l resp nse s e ble nd e ec ve s r e y m m e ph s syn hes s nd WUE s mul ne usly L ws n nd V 2019 . I s w r h n n h nves y n s n the f nc ns f the p en l re ul ry r les well the qu s c n f s m l nes cs f verexpress n pl s es ll needed n ders nd the mech sm by wh ch ZmGL s ne unes m l m vemen s, c r n e r d e f s be ween ph s syn hes s nd r u h s ler ce. Fur her expl r n w ll pr v de ns h s nd use ul g e s f r cr p bree n , en bl n cre n f el e v e es w h b h h ph s syn he c c p y nd r u h s ler ce.

Materials and methods

P owt co d o s

The WT r ce l spp. cul v e e? h m y us l nes descr bed by L . 2020 : nd : were used n h s s udy. F r hyd r p n c cul ure, r ce seed n s were r w n m d ed mur B s lu n 0.5 mM N H SO , 0.5 mM M SO 7 H

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

R de le ves were d es ched fr m c n r l r dr u h s re ed pl s nd mmed ely cu s n s 3 × 3 mm p eces, exclud n the ve ns. S mples were d rec ly xed n 2.5% v/v lu l dehyde n 0.1 M ph sph e bu er p H7.0 nd then xed w h 1% sm um e r x de. er w sh n wce w h 0.1 M ph sph e bu er, s mples were dehydr ed r du lly n e h n l ser es 30%, 50%, 60%, 70%, 80%, 90%, nd 100% r 15 m n e ch, ll wed by ncub n n er y bu n l r 35 m n. Then, s mples were dr ed us n cr c l p n dryer, p sed n the s mple s e, nd then c ed w h ld. S m were bserved nd ph r phed us n SU 8010 sc nn n elec r n m cr sc pe H ch, J p n . The s e, number, nd per ure s es s m were c lcu l ed us n Im e J s w re.

u f c o of e oge ous ABA co e t

The upperm s exp nded le ves c n r l nd dr u h s ressed r ce seedl n s were d es ched nd fl sh r en n l qu d n r en. Gr und s mples 100 m e ch were ex r c ed w h n ces n r le s lu n c n n n n n ern l s nd rd °C vern h s S mples were cen r u ed, nd the resul n supern n w s ex r c ed n. The c mb ned ex r c s were pur ed n C_{18} s l c c lumn nd dr ed w h n r en s. er res lv n n me h n l nd p ss n hr u h 0.22-μm l er, B w s qu n ed n HPLC–s ndem m ss spec r sc py MS/MS sys em s descr bed by L u e l 2012 .

Exoge ous ABA t e t e t

F r y d y ld r ce seedl n s r wn n p s were spr yed w h 100 M B s lu n c n n n 0.5% [v/v] Tween20 s sur c n un l the le ves were m s . The v lume B s lu n ppl ed w s c ns s en be ween seedl n s. 2.5 h er re men s exch n e p r me ers nd s m l r s were ev lu ed s descr bed b ve.

RNA ex c o RT qPCR

The upperm s ully exp nded le ves were h rves ed r m 3 wk ld r ce seedl n s r wn n p s under n rm l c nd n s r dr u h s ress r 7 d. S mples were fl sh r en n l qu d n r en nd r und p wder, nd then l RN w s ex r c ed w h TRI l re en Inv r en . RN pur y nd qu n y were ev lu ed us n N n Dr p2000 spec r ph me er Therm F sher Sc en c, US . er DN se re men , cDN w s syn hes ed r m 1 l RN per s mple us n the Rever d F rs S r nd cDN Syn hes s Therm F sher Sc en c, US . RT-qPCR w s per rmed us n OD SYBR Green m x w h ROX TOYOBO n n BI Qu nS ud 6 Flex ns rumen ppl ed B sys ems, US . Rel ve r nscr p levels were c lcul ed w h the $2^{-\Delta\Delta CT}$ me h d L v k nd Schm en 2001 w h 3 b l c l repl c es r e ch re men us n s the n ern l c n r l. Pr mers re l s ed n Supplemen l T ble S2 .

RNA seq ys s

3 h er ex er h us B re men s le ves were c llec ed r m -wk ld r ce seedl n s r wn n p s. T l RN w s ex r c ed w h TRI l re en nd then RN n e r y w s ssessed w h the len 2100 B n ly er len Techn l es, US . RN -seq l br r es were c ns ruc ed r m WT, , nd : r ce pl n s us n the TruSeq S r nded mRN LT S mple Prep Illum n , US w h 3 b l c l repl c es per l ne. The resul n 9 l br r es were sequenced n the Illum n Seq X Ten sequenc n pl rm. er rem v n the d p r se quences nd l w qu l y re ds, cle n re ds were m pped the cv. N pp nb re reference en me us n IS T m e l 2015 nd B w e2 L n me d e l 2009 . Gene express n levels were c lcul ed n re ds per k l b se r nscr p per m ll n m pped re ds RP M us n Cu l nks. DEGs were den ed w h the DESeq" R p ck e. The hresh lds r cl ss c n s DEG n the r ns en c l nes c mp red the WT were < 0.05 nd |l $_2$ ld ch n e| >1.

DAP seq d t ys s

The full len h c d n sequences nd were mpl ed r m cDN the m e ccess n B73. E ch se quence w s rec mb ned n the pIX HLO vec r us n LR Cl n se II Inv r en . The HLO ZmGL 1 nd HLO ZmG2 pr e ns were ener ed us n 500 n e ch Rec l ys e the pIX HLO ZmGL 1 nd pIX HLO ZmG2 pl sm ds Sc nvOe

% 6 - &+
%

considered significant at $P < 0.05$. Figures were generated with GraphPad Prism 9.0 and Adobe Illustrator CS3.

Accession numbers

Raw sequence data generated in this study have been deposited in the NCBI BioProject database under accession number PRJNA1018861 for RNA-seq and PRJNA1019016 for DAP-seq. The sequence data from this article can be found in the GenBank/EMBL data libraries under the following accession numbers: GenBank: F318580 and GenBank: F318579.

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Author contributions

W.Z. and X.L. conceived and designed the experiments. X.L., J.L., S.W., Y.G., and R.G. performed most of the experiments. Z.L. and P.L. performed the DAP-seq experiments. P.W. critically commented and edited the manuscript. The manuscript was prepared by X.L., J.L., and W.Z. All authors discussed and commented on the manuscript.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Enhanced tolerance of : and : maize plants to drought stress induced by 20% PEG 6000.

Supplemental Figure S2. Overexpression of in rice leads to decreased stomatal conductance and photosynthetic parameters in response to drought.

Supplemental Figure S3. Dynamic changes of soil water content during the drought stress in the greenhouse experiments.

Supplemental Figure S4. Genome-wide summary of the regulatory network downstream of ZmGLK1 and ZmG2 based on DAP-seq data.

Supplemental Figure S5. Changes in endogenous ABA content in WT, :, and : maize leaves under normal conditions and after 7 d of drought stress.

Supplemental Figure S6. Relative expression levels of ABA biosynthesis genes in the leaves of WT, :, and : maize plants under normal conditions and after 7 d of drought stress.

Supplemental Table S1. Relative change of gene expression level of 59 overlapped genes from RNA-seq and DAP-seq analyses.

Supplemental Table S2. Primers used for RT-qPCR.

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The authors declare that they have no conflicts of interest.

Data availability

The data underlying this article are available in the article and in its online supplementary material.

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Wang R, Liu J, Chen Q, ngcKI