M e OLDEN LI E o e se ce d ou g t po d ce ce by o mo t s po m t cosu e

X L [1,†] L [1,†] S obo We [1] Yu o [1] Ho cu Pe [1] Rud e [1] Zefu Lu [1]
Pe W [2] We b Z ou [1,*]

1 Ins e of Crop Sc ences, Ch nese Ac emy of A r cu ar Sc ences, Be j ng 100081, Ch n
2 CAS Cen er for Excellence n Mo ecu r P n Sc ences, Ins e of P n Phys o o y nd Eco o y, Ch nese Ac emy of Sc ences, Sh ng h
200032, Ch n

*Au or for correspon ence: hou enb @c .c n
†These au ors con bu ed equ y.
The au or respons b e for d s bu on of m er s n ed n s e n ccord nce w h the po cy d scr bed n the
Ins uc ons for Au hors h ps:// c em c.oup.com/p phys/p es/Gener Ins uc ons s Wenb n Zhou hou enb @c .c n

Abstract

Drou h h s become one of he mos severe b o c s esses exper enced n r cu ar p oduc on cross he or d. P n
respond o dec ne w s om ovemen n he ea ves, h ch e m n y re u ed by bsc s c d ABA. A prev ous
s udy from our b sho ed he cons u ve expressi on of m e L GOLDEN2-LI E GL d scr p on f c ors n
r ce L c n mprove s om conduc nce nd p n pho synthe c p c y under e d cond ons. In he pre-
sen udy, e uncovered unc on of ZmGL re u on of s om ovemen n r ce dur n drou h s ess. We fou d he
e ev ed drou h o er nce n r ce p n overexpress n or d con erred by r ced ABA-med ed
s om c osure. Comp e ve n ys s of RNA-sequenc n RNA-seq d from r ce ea ves nd DNA f n pur c on
sequenc n DAP-seq d resu s ob ned n v o reve ed he ZmGL s p yed o es n re u n ABA-re ed nd s ess-respon-
sive p ys. Four pre u ed enes c ose y func on n n b o c s ess o er nce s s on b nd n pe s n he DAP-seq
d e ere den fied s pu ed e enes of ZmGL 1 nd ZmG n r ce. These resu s demons ed he un e GL s p y n
mpor n o e n re u n s om ovemen co ord n pho synthes nd s ess o er nce. Th s s v u b e
e for bree n drou h o er n crop p n. hou compro s n pho synthe c p c y.

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de[illegible]c [illegible]or [illegible]e exposed [illegible]o [illegible]er env ronmen[illegible] s[illegible]mul[illegible] e.[illegible] . b[illegible] l[illegible]h[illegible] [illegible] [illegible] h[illegible]um d[illegible] h[illegible] h CO_2 levels, [illegible]d p[illegible]o-[illegible]ens[illegible] s[illegible]om[illegible] [illegible]e r[illegible]p[illegible]dly closed, espec[illegible]lly [illegible] [illegible]osperms (Ser[illegible] e[illegible] [illegible] 2018). Th[illegible]s dyn[illegible]m[illegible]c movemen[illegible] [illegible]s dr[illegible]ven by [illegible]or pressure ch[illegible]n[illegible]es [illegible]n [illegible]e [illegible]u[illegible]rd cells, [illegible] [illegible] resul[illegible] of [illegible]e [illegible] [illegible]on of [illegible]on ch[illegible]nnels [illegible]d [illegible]e [illegible]nh[illegible]b[illegible]on of [illegible]n[illegible]rd-rec[illegible]fy[illegible]n[illegible] $^{+}$ ch[illegible]nnels, [illegible]h[illegible]ch [illegible] encoded by [illegible]

[illegible] [illegible]enes ([illegible] m[illegible] e[illegible] [illegible] 2010). The efflux of [illegible]ons [illegible]d sm[illegible]ll me[illegible]bol[illegible]es, [illegible]nclud[illegible]n[illegible] Cl^-, NO_3^-, [illegible]d m[illegible]l[illegible]c [illegible]uses membr[illegible]ne depol[illegible]r[illegible][illegible]on [illegible] [illegible]e ou[illegible]rd-rec[illegible]fy[illegible]n[illegible] $^{+}$ ch[illegible]nnel [illegible]d [illegible]c[illegible]l[illegible] $^{+}$ efflux, fur[illegible]er reduc[illegible]n[illegible] [illegible]or pressure [illegible]ns[illegible]de [illegible]e [illegible]u[illegible]rd cells [illegible]d le[illegible]d[illegible]n[illegible] [illegible] [illegible]e s[illegible]om[illegible]l closure (P[illegible]ndey e[illegible] [illegible] 2007). Under [illegible]er-def[illegible]c[illegible] cond[illegible]ons, [illegible]e phy[illegible]ohormone [illegible]bsc[illegible]s[illegible]c [illegible]c[illegible]d (ABA) pl[illegible]ys [illegible] [illegible]e pr[illegible]m[illegible]ry re[illegible]ul[illegible]or of s[illegible]om[illegible]l movemen[illegible] [illegible] preven[illegible] [illegible]er loss, [illegible]n [illegible]h[illegible]ch endo[illegible]enous ABA levels [illegible]re con[illegible]rolled by [illegible] prec[illegible]se b[illegible]l[illegible]nce be[illegible]een b[illegible]osyn[illegible]es[illegible]s [illegible]d c[illegible]bol[illegible]sm, [illegible]h[illegible]ch [illegible]lso [illegible]nfluenced by [illegible]r[illegible]nspor[illegible] [illegible]d conju[illegible]on process (Ku[illegible]sh[illegible]ro e[illegible] [illegible] 2004; [illegible]l[illegible] e[illegible] [illegible] 2021). ABA [illegible]s [illegible]n[illegible][illegible]lly syn[illegible]es[illegible]ed from C_{40} c[illegible]ro[illegible]o[illegible]ds [illegible] form [illegible]x[illegible]n[illegible]ophylls e.[illegible] . 9-[illegible]-v[illegible]ol[illegible]x[illegible]n[illegible]n [illegible]d 9-[illegible]-neox[illegible]n[illegible]n), [illegible] C_{15} [illegible]n[illegible]ermed[illegible][illegible]e x[illegible]n[illegible]ox[illegible]n, [illegible]s formed [illegible]n [illegible]e pl[illegible]s[illegible]ds [illegible] [illegible] ox[illegible]d[illegible]ve cle[illegible]v[illegible]e c[illegible]ly[illegible]ed by 9-[illegible]-epoxyc[illegible]ro[illegible]o[illegible]d d[illegible]oxy[illegible]en[illegible]se (NCED). [illegible]n[illegible]ox[illegible]n [illegible]s [illegible]en expor[illegible]ed [illegible] [illegible]e cy[illegible]osol [illegible]d conver[illegible]ed [illegible] ABA [illegible]rou[illegible]h [illegible] 2-s[illegible]p re[illegible]c[illegible]on v[illegible] sh[illegible]or[illegible]ch[illegible]n dehydro[illegible]en[illegible]se/reduc[illegible]se 1 (SDR1/ABA2) [illegible]d Ar[illegible]b[illegible]dops[illegible]s [illegible]ldehyde ox[illegible]d[illegible]se 3 (AAO3) (Seo [illegible]d [illegible]osh[illegible]b[illegible] 2002; [illegible]on[illegible] [illegible]d Zhu 2003).

Tr[illegible]nscr[illegible]p[illegible]on f[illegible]c[illegible]ors (TFs) [illegible]re cruc[illegible]l re[illegible]ul[illegible]ors of m[illegible]ny b[illegible]olo[illegible]c[illegible]l processes, [illegible]nclud[illegible]n[illegible] responses [illegible] env[illegible]ronmen[illegible]l s[illegible]n[illegible]ls [illegible]d hormone re[illegible]ul[illegible]on. These re[illegible]ul[illegible]ory func[illegible]ons [illegible]re [illegible]ccompl[illegible]shed [illegible]rou[illegible]h b[illegible]nd[illegible]n[illegible] [illegible] spec[illegible]f[illegible]c [illegible]-elemen[illegible]s [illegible]n [illegible]e promo[illegible]er re[illegible]ons of [illegible]r[illegible]e[illegible] [illegible]enes (Tod[illegible] e[illegible] [illegible] 2012). Numerous [illegible]b[illegible]o[illegible]c s[illegible]ress-respons[illegible]ve TFs h[illegible]ve been [illegible]den[illegible]f[illegible]ed [illegible]n pl[illegible]n[illegible]s, for [illegible]ns[illegible]nce, WR[illegible]Y, MYB, [illegible]d DREB/CBF TFs h[illegible]ve [illegible]ll been repor[illegible]ed [illegible] [illegible] key re[illegible]ul[illegible]ors of pl[illegible]n[illegible] s[illegible]ress responses (M[illegible]n [illegible] e[illegible] [illegible] 2021). GOLDEN2-LI[illegible]E (GL[illegible]) TFs [illegible]ener[illegible]lly [illegible]c[illegible] [illegible] [illegible]r[illegible]nscr[illegible]p[illegible]on[illegible]l [illegible]c[illegible]ors of chloropl[illegible]s[illegible] developmen[illegible] [illegible]d b[illegible]o[illegible]enes[illegible]s (Ross[illegible]n[illegible] e[illegible] [illegible] 2001; W[illegible]n[illegible] e[illegible] [illegible] 2013) [illegible]d pl[illegible]y [illegible]mpor[illegible]n[illegible] roles [illegible]n re[illegible]ul[illegible]n[illegible] nucle[illegible]r pho[illegible]osyn[illegible]es[illegible]s-rel[illegible]ed [illegible]enes (Chen e[illegible] [illegible] 2016). In m[illegible]e, [illegible] [illegible] [illegible]L[illegible] 2 [illegible] [illegible]enes, [illegible]d [illegible] [illegible] h[illegible]ve sho[illegible]n d[illegible]fferen[illegible]l express[illegible]on p[illegible]erns be[illegible]een mesophyll cells [illegible]d [illegible]e bundle she[illegible]h (H[illegible]ll e[illegible] [illegible] 1998; Ch[illegible]n[illegible] e[illegible] [illegible] 2012). Ec[illegible]op[illegible]c overexpress[illegible]on of m[illegible]e [illegible]enes [illegible]n r[illegible]ce [illegible]nduces chloropl[illegible]s[illegible] developmen[illegible] [illegible]n bundle she[illegible]h cells [illegible]d [illegible]c[illegible] [illegible]s [illegible]n[illegible]ercellul[illegible]r pl[illegible]smodesm[illegible]l connec[illegible]ons, cons[illegible]der[illegible]n[illegible] [illegible]e key s[illegible]ep [illegible]n form[illegible]n[illegible] [illegible]n[illegible]ermed[illegible]e pro[illegible] [illegible]r[illegible] [illegible]n[illegible][illegible]omy [illegible]n [illegible]e [illegible]r[illegible]ns[illegible]on from C_3 [illegible] C_4 pho[illegible]o[illegible]syn[illegible]es[illegible]s (W[illegible]n[illegible] e[illegible] [illegible] 2017). A prev[illegible]ous s[illegible]udy from our l[illegible]b sho[illegible]ed [illegible] [illegible]cons[illegible]u[illegible]ve [illegible] express[illegible]on [illegible]n r[illegible]ce le[illegible]ds [illegible] [illegible]ncre[illegible]sed x[illegible]n[illegible]ophyll con[illegible]en[illegible] [illegible]d fur[illegible]er m[illegible]n[illegible][illegible]s [illegible]e pho[illegible]o[illegible]nh[illegible]b[illegible]on under h[illegible]h-l[illegible]h[illegible] cond[illegible]ons, resul[illegible]n[illegible] [illegible]n [illegible]n[illegible] en[illegible]h[illegible]nced pho[illegible]osyn[illegible]e[illegible]c c[illegible]p[illegible]c[illegible]y [illegible] h[illegible]her s[illegible]om[illegible]l conduc[illegible]nce [illegible]d [illegible]mproved b[illegible]om[illegible]ss [illegible]d [illegible]r[illegible]n y[illegible]eld [illegible]n [illegible]e f[illegible]eld (L[illegible] e[illegible] [illegible] 2020). Moreover, GL[illegible]s [illegible]lso func[illegible]on [illegible]n [illegible]b[illegible]o[illegible]c s[illegible]ress responses (Ah[illegible]m[illegible]d e[illegible] [illegible] 2019) [illegible]d p[illegible]o[illegible]en res[illegible]s[illegible]nce (Murmu e[illegible] [illegible] 2014). For ex[illegible]mple, GL[illegible]s [illegible]ffec[illegible] s[illegible]om[illegible]l movemen[illegible] [illegible]n Ar[illegible]b[illegible]dops[illegible]s [illegible] [illegible]hen exposed [illegible]o ozone (N[illegible] [illegible]sh[illegible] e[illegible] [illegible] 2016).

In [illegible]s s[illegible]udy, [illegible]e uncovered [illegible]e du[illegible]l func[illegible]on of m[illegible]e GL[illegible]s, [illegible]d [illegible] [illegible]c[illegible]op[illegible]c overexpress[illegible]on of [illegible] [illegible]d [illegible] [illegible]n r[illegible]ce conferred [illegible]mproved drou[illegible]h [illegible]ol[illegible]r[illegible]nce by promo[illegible]n[illegible] s[illegible]om[illegible]l closure [illegible]n response [illegible] [illegible]er def[illegible]c[illegible] [illegible]h[illegible]le m[illegible]n[illegible]n[illegible]n[illegible] h[illegible]h s[illegible]om[illegible]l conduc[illegible]nce [illegible] ob[illegible]n eff[illegible]c[illegible]en[illegible] pho[illegible]osyn[illegible]es[illegible]s [illegible]hen suff[illegible]c[illegible]en[illegible] [illegible]er [illegible]s [illegible]v[illegible]l[illegible]ble. We fur[illegible]er sho[illegible]ed [illegible] [illegible]er r[illegible]p[illegible]d s[illegible]om[illegible]l movemen[illegible] [illegible]s med[illegible]ed by ABA-[illegible]nvolved p[illegible] [illegible]y under drou[illegible]h cond[illegible]ons. These resul[illegible]s su[illegible]es[illegible] [illegible] [illegible] [illegible]enes m[illegible]y be prom[illegible]s[illegible]n[illegible] c[illegible]nd[illegible]d[illegible]es for breed[illegible]n[illegible] r[illegible]ce v[illegible]r[illegible]e[illegible]es [illegible] h[illegible]h s[illegible]om[illegible]l flex[illegible]b[illegible]l[illegible]y [illegible]d sus[illegible]n[illegible]ble y[illegible]eld, [illegible]h[illegible]ch [illegible]ould s[illegible]ron[illegible]ly [illegible]mprove [illegible]r[illegible]cul[illegible]ur[illegible]l produc[illegible]on [illegible]d [illegible]ncre[illegible]se food secur[illegible]y [illegible]n [illegible]e con[illegible]ex[illegible] of cl[illegible]m[illegible]e ch[illegible]n[illegible]e.

Results

Zm[illegible]L[illegible]1 [illegible]d Zm[illegible] co[illegible]fe[illegible]ed [illegible]mproved d[illegible]ou[illegible]h[illegible] [illegible]o[illegible]e[illegible]nce [illegible] [illegible]ce

In our prev[illegible]ous s[illegible]udy, f[illegible]eld-[illegible]ro[illegible]n [illegible]r[illegible]ns[illegible]en[illegible]c r[illegible]ce l[illegible]nes cons[illegible]u[illegible]vely express[illegible]n[illegible] [illegible] or [illegible] dr[illegible]ven by [illegible]e m[illegible]e [illegible] promo[illegible]er performed [illegible]mproved pho[illegible]osyn[illegible]es[illegible]s r[illegible]es [illegible]d h[illegible]her s[illegible]om[illegible]l conduc[illegible]nce (L[illegible] e[illegible] [illegible] 2020). We fur[illegible]er explored [illegible]e s[illegible]om[illegible]l responses of [illegible]r[illegible]ns[illegible]en[illegible]c r[illegible]ce pl[illegible]n[illegible]s [illegible] [illegible]er def[illegible]c[illegible] [illegible] [illegible]e po[illegible] exper[illegible]men[illegible] [illegible]n [illegible]e [illegible]ro[illegible] [illegible]e ch[illegible]mber. Surpr[illegible]s[illegible]n[illegible]ly, [illegible]r[illegible]ns[illegible]en[illegible]c r[illegible]ce pl[illegible]n[illegible]s exh[illegible]b[illegible]ed s[illegible]ron[illegible]er drou[illegible]h [illegible]ol[illegible]r[illegible]nce [illegible]n [illegible]ld-[illegible]ype (WT) pl[illegible]n[illegible]s [illegible]er recovery from [illegible] 10-d drou[illegible]h [illegible]re[illegible]men[illegible] (F[illegible] . 1A). Spec[illegible]f[illegible]c[illegible]lly, [illegible]e surv[illegible]v[illegible]l r[illegible]es of [illegible] [illegible] [illegible]d [illegible] [illegible] pl[illegible]n[illegible]s [illegible]ere 53.0% [illegible] 64.0% [illegible]er [illegible]e 6-d recovery per[illegible]od, [illegible]h[illegible]ch [illegible]ere s[illegible]n[illegible]f[illegible]c[illegible]n[illegible]ly h[illegible]her [illegible]n [illegible]e WT (14.3%; F[illegible] . 1B). Moreover, [illegible]e rel[illegible]ve [illegible]er con[illegible]en[illegible] (RWC) [illegible]n [illegible]e le[illegible]ves of WT [illegible]d [illegible]r[illegible]ns[illegible]en[illegible]c pl[illegible]n[illegible]s r[illegible]n[illegible]ed from 94.7% [illegible] 95.3% before drou[illegible]h b[illegible]u[illegible] decre[illegible]sed [illegible] 73.1% [illegible]n [illegible]e WT [illegible]er [illegible] [illegible] [illegible]h[illegible]eld for 7 d. In comp[illegible]r[illegible]son, [illegible] [illegible]d [illegible] pl[illegible]n[illegible]s m[illegible]n[illegible]ned [illegible] rel[illegible]vely h[illegible]h RWC, espec[illegible]lly [illegible], r[illegible]n[illegible]n[illegible] from 86.2% [illegible] 90.9%. Af[illegible]er 10 d of drou[illegible]h s[illegible]ress, [illegible]e RWC v[illegible]lues of WT [illegible]d [illegible] pl[illegible]n[illegible]s decre[illegible]sed [illegible] 11.6% [illegible] 12.9%, [illegible]h[illegible]ch [illegible]ere s[illegible]n[illegible]f[illegible]c[illegible]n[illegible]ly lo[illegible]er [illegible]n [illegible]ose of [illegible] pl[illegible]n[illegible]s (14.5% [illegible] 18.6%; F[illegible] . 1C). These resul[illegible]s [illegible]nd[illegible]c[illegible]ed [illegible] ZmGL[illegible]1 [illegible]d ZmG[illegible]2 bo[illegible]h conferred h[illegible]her c[illegible]p[illegible]c[illegible]es for [illegible]er conserv[illegible]on [illegible]d [illegible]us drou[illegible]h [illegible]ol[illegible]r[illegible]nce.

We nex[illegible] [illegible]es[illegible]ed [illegible]e [illegible]ro[illegible]h perform[illegible]nce of WT, [illegible], [illegible]d [illegible] r[illegible]ce pl[illegible]n[illegible]s [illegible] PEG-[illegible]nduced osmo[illegible]c s[illegible]ress [illegible] [illegible] drou[illegible]h s[illegible]mul[illegible]on. Af[illegible]er [illegible]ro[illegible]h [illegible]n 20% PEG 6000 for 10 d, [illegible] [illegible]d [illegible] r[illegible]ce pl[illegible]n[illegible]s sho[illegible]ed less [illegible]l[illegible]n[illegible] [illegible]d chloros[illegible]s comp[illegible]red [illegible] [illegible]e WT (Supplemen[illegible]ry F[illegible] . S1A). The m[illegible]x[illegible]mum qu[illegible]n[illegible]um eff[illegible]c[illegible]ency of pho[illegible]osys[illegible]em II (PSII; F_v/F_m) [illegible]s me[illegible]sured [illegible] [illegible]n [illegible]mpor[illegible]n[illegible] [illegible]nd[illegible]c[illegible]or of pl[illegible]n[illegible] phys[illegible]olo[illegible]c[illegible]l s[illegible]us under s[illegible]ress cond[illegible]ons, [illegible]d [illegible]e [illegible]

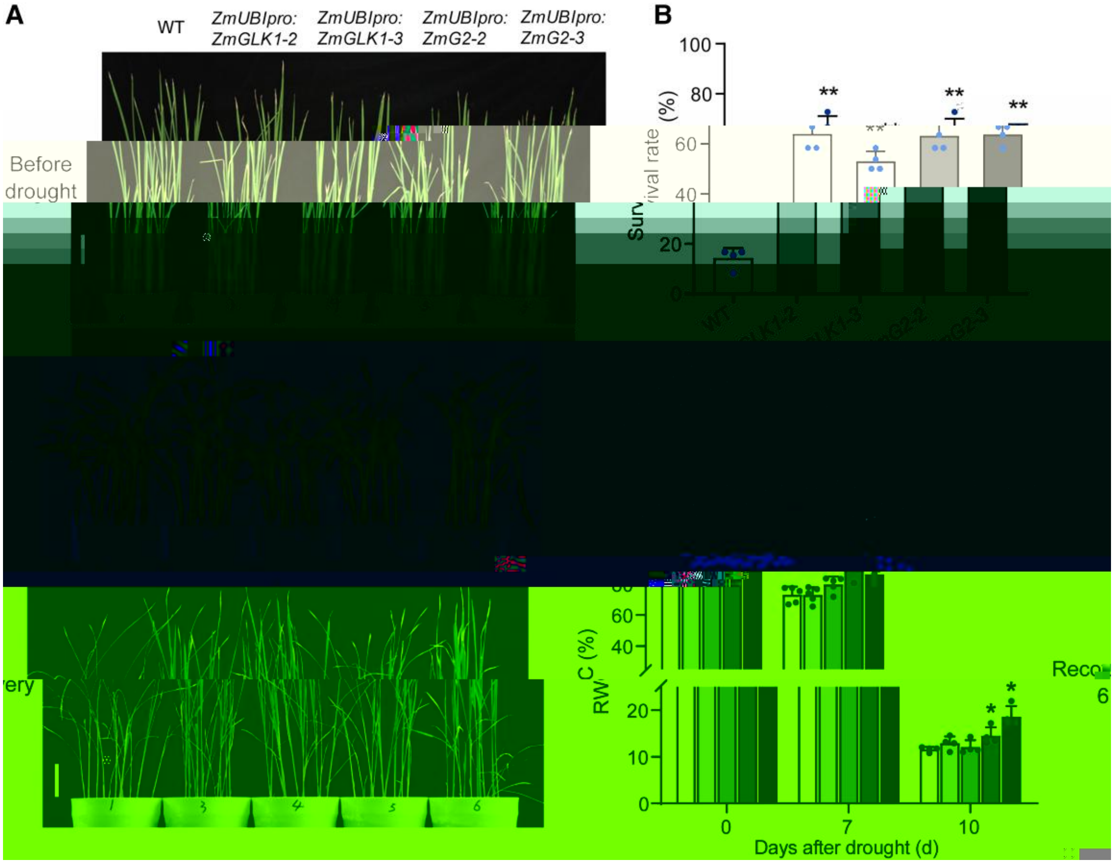

Figure 1. Overexpression of [illegible] and [illegible] in rice increased drought tolerance. **A)** Phenotypes of WT, [illegible]:[illegible], and [illegible]:[illegible] rice plants during drought stress. Three-week-old WT, [illegible]:[illegible], and [illegible]:[illegible] rice seedlings grown in 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.[illegible]% to 12.1% and 29.5% to 29.7% higher in [illegible]:[illegible] and [illegible]:[illegible] rice plants, respectively, compared with the WT (Supplemental Fig. S1C). These results together indicate that overexpression of [illegible] and [illegible] in rice significantly improve the tolerance to drought and osmotic stress.

ZmGLK1 and ZmG2 [illegible] stomatal closure [illegible] drought-stressed rice [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 stomatal status of rice seedlings grown in the pot in the growth chamber, since stomata are the main channels for gas exchange and water transpiration in plants, serving as the dominant limitation to photosynthesis under drought. We therefore first measured stomatal conductance and photosynthesis-related parameters under control conditions using a LI-COR-6400XT portable photosynthesis system. The results revealed significantly higher stomatal conductance in [illegible]:[illegible] and [illegible]:[illegible] rice seedlings (0.118–0.139 and 0.126–0.131, 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. S2), 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 (0.062–0.073 and 0.05[illegible]–0.059, respectively), whereas that of WT remained relatively stable under drought conditions (0.087; Supplemental Fig. S2B). The photosynthesis rates, C_i, and transpiration rates showed corresponding declines in [illegible]:[illegible] and [illegible]:[illegible] rice plants during water deprivation (Supplemental Fig. S2, A, C, and D).

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

drou h deepened, o f h ch : nd
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r nd he s m l conduc nce comp red o he WT
F . 3, D nd E . These resul s e her cle rly nd c nd

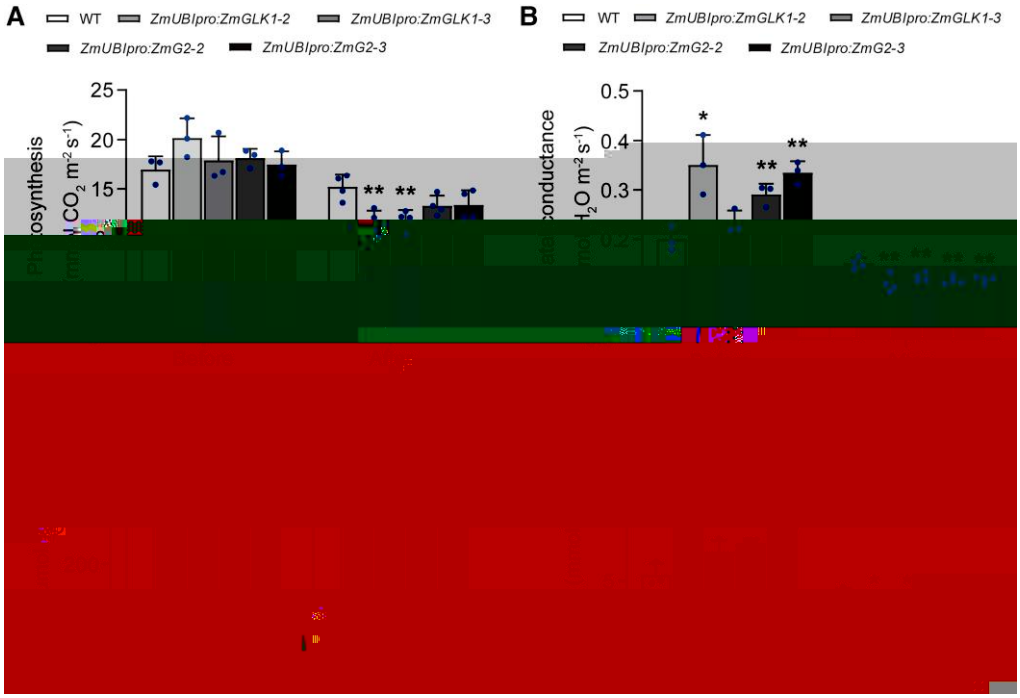

Figure 4. Exo enous ABA pplic on reduced the pho osyn hesis r e nd s om l conduc nce n rice pl n overexpress n or comp red o the WT. **A)** Pho osyn hesis r es, **B)** s om l conduc nce, **C)** C , nd **D)** nsp r on r es of 3- -old WT, : , nd : rice pl n s ro n solu before or 2.5 h fter ABA re tmen D e re sho n s the me n ± sd from 3 bio ic l repli es. * < 0.05, ** < 0.01 Stu en s -test.

the WT nd n s en c pl n m m c ed the resul obt ined from the dro ht-s ess re tmen s, h ch ind c ted the re u l on of r pid s om l closure in response to ter-de c s ess con erred by ZmGL 1 nd ZmG s ABA me ted.

Zm L 1 nd Zm e gu ted s ow t e ed ge es p omoted ou t e ce

To fur her unders nd the molecul r mech ni sms re u l ted by ZmGL s under dro ht-s ess, e nex comp red the ex- press on levels of sever l genes ssoci ted with s om l movemen in WT, : , nd : rice pl n under con ol nd dro ht-s ess cond ions. Under con ol cond ions, sever l key genes were hi hly ex- pressed in the n s en c pl n comp red to the WT bu pro oundly do nre u l ted in response to dro ht s ess. These comprised 4 enes encod ng pro ins ssoci ted with n ocl recti ying sh ker-l ke po ssium ch nnels 3 s nd 1 gene), 1 -ATP se), nd sever l s ess-respons ve genes nclud ng , nd ; Fig. 5 . These esul demons ted the ZmGL 1 nd ZmG mproved dro ht oler nce by do nre u l ting genes nvolved in s om l movemen when suffering from ter de c .

A genome- de n scr p om c n lys s l so con- ducted in WT, : , nd : rice pl n s 3 h fter ABA re tmen to nves ga e the bb l effec s of ZmGL 1 nd ZmG n duced by ABA, espec lly on s om l movemen WT pl n cle rly sho ed s nific n expression p erns comp red to : nd : pl n s demons ted by the cle sep ra- on in the pr nc p l component n lys s PCA; Fig. 6A . Spec fic lly, ter ABA re tmen 70 nd 775 genes ere s g- n fic n y up re u l ted in : nd : pl n respec ve y, comp red to the WT, of h ch 8 genes ere up re u l ted in bo n s en c lines Fig. 6B . Gene On olo y GO) enr ch men n lys s reve led that the up re u l ted d ffer en lly expressed genes DEGs) in : nd : pl n func oned in mu ple bio ic l processes bu pr m ri ly in the ABA nd ter de pr va on p hw ys Fig. 6, C nd D . Next e per formed DNA ffin pu fic on sequen ng DAP-seq) n lys s to den fy genes d rec y re u l ted by the ZmGL TFs. Th s n lys s reve led 6,601 nd 6,565 pu ve b nd ng s s of ZmGL 1 nd ZmG n the rice ge nome, respec ve y, in more th n h lf of the den fied s s be ng bound by bo ZmGL nd ZmG Supplemen l Fig. S4A . Of the 3,835 b nd ng s s sh red by ZmGL 1 nd ZmG , 17.4% ere loc l ed to promo s, 8.59% to exons, nd 5.6% to n ge en c re ons Supplemen l Fig. S4B . Mo f n lys s de- mons ted the mos enr ched core mo fs found in the ZmGL 1- nd ZmG -b nd ng re ons ere GCCTCT nd AGATTCT Supplemen l Fig. S4C nd D . F rthe ge nes den fied from the DAP-seq d ta s po en al ge es of ZmGL 1 nd ZmG n rice ere lso den fied from the RNA-sequen ng RNA-seq) d ta s d ffer en lly expressed

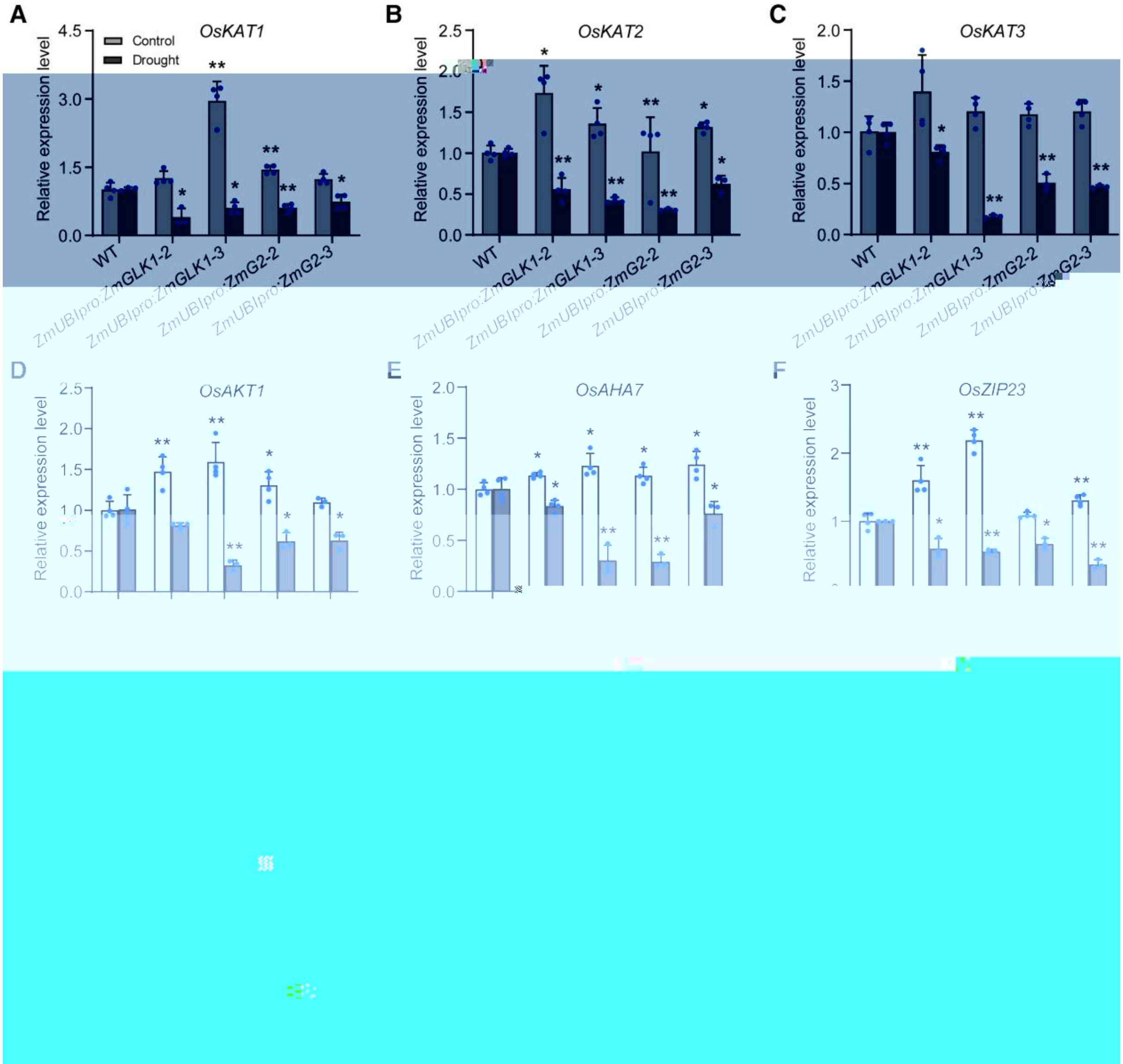

Figure 5. Rel ve express on levels o genes nvolved n s om movemen nd s om l per ure n WT, : , nd : r ce under norm l cond ons nd er 7 d o drou h s ess. Express on levels o **A)** , **B)** , **C)** , **D)** , **E)** , **F)** , **G)** , **H)** , nd **I)** . Gene express on levels ere me sured RT-qPCR n e le ves o 3- -old r ce pl n s ro n n so l under norm l cond ons or drou h s ess or 7 d. D re presen ed s e me n ± SD rom 3 b olo c l repl c es. * < 0.05, ** < 0.01 S uden es .

n pl n s overexpress n or F . 6B; Supplemen l T ble S1). We no ed upre ul ed DEGs ere nno ed o b o c s ess oler nce nd sho ed s ron b nd n pe s n e DAP-seq n lys s s mul neously. Therefore, ese enes ere den ed s pu ve r e enes o ZmGL 1 nd ZmG2 n r ce, nclud n r ce enes ; F . 7, A o D). The ene express on rom RNA-seq d o ese enes s prom nen ly h her n : nd : r ce pl n s (F . 7, E o H). Fur er reverse r nscr p on qu n ve PCR (RT-qPCR) n lys s ver ed ese enes ere h hly nduced n : nd : r ce under drou h s ess cond ons (F . 7, I o L). These pu ve r e enes m y con bu e o enh nced drou h oler nce by en bl n r p d s om l movemen hen su er n rom er de c .

Discussion

GL TFs h ve lon been re rded s some o e mos m- por n re ul ors o chloropl s b o enes s nd pho osyn- e c or nelle orm on; ey h ve been den ed n Arb dops s, om o L) nd m e

Figure 6. Tr nscr pom c n lysis of WT, , nd : r ce pl nts h r ABA tre tmen **A)** PCA of ene expression in WT, : , nd : r ce pl nts b sed on RNA-seq d t **B)** Unique nd overl pping DEGs upre ul ted in : nd : r ce pl nts comp red to the WT nd unique nd overl pping ene enes dentified from DAP-seq d t DEGs ere dentified b sed on |log fold ch nge| > 1 nd < 0.05 by DESeq" R p ck ge. **C, D)** GO f nction l c tegories for DEGs upre ul ted in : **C)** nd : **D)** r ce pl nts comp red to the WT. Bubble s indic tes the number of DEG counts in the correspond ng GO c tegory; bubble colors corresponds to the −log 10 f lse discovery r te [FDR] v lue, nd the - xis indic tes the r tio of DEGs in e ch GO c tegory to ll ene in the c tegory.

Ross n et l. 2001; W ters et l. 2009; Po ell et l. 2012). In r ce, ectop c expression of m ize ene enes nd promoters pro oke r pid stom t l closure, ncre sing chlorophyll nd m ch ondri l development in r ce ve scul r she th cells (W ng et l. 2017). A prev ous study by our l b h s reve led th t r ce pl nts overexpressing m ize ene enes h ve ncre sed biom ss nd y ld s results of mproved photosynthetic c p city nd reduced photoinh bition under h gh- nd fluctu ting-l ght conditions (Li et l. 2020).

In the present study, e uncovered th t overexpression of m ize ene enes nd n r ce enh nced drought tolerance by promoting stom t l closure. Specific lly, hen pl nts ere gro n under s ndard, well- ered conditions, e observed sm ller stom t l se but h gher stom t l density nd stom t l per ture in r ce pl nts overexpressing or comp red to WT pl nts (Fig. 2, B nd E). These results ere cons stent th t e rlier studies sho ng th t nd overexpression led to ncre sed stom t l conductance in r ce le ves (Li et l. 2020), reenhouse gro n r ce (Yeh et l. 2012), nd Ar bidopsis (Nie sh et l. 2016). In contr st, under drought stress, the stom t l of - or -overexpressing r ce pl nts rem ned lmost closed (Fig. 3B nd 3E), mproving drought tolerance by preventing ter loss. Prev ous studies in r ce h ve reported th t sm ll, h gh-density stom t l close quickly, thus promoting resilience to drought stress (Caine et l. 2019; Caine et l. 2023). These pr or results ere cons stent th those of the present study. Notably, differences in stom t l ph notypes between control nd drought-stressed pl nts re sulted from or overexpression ere rectly c used by re ul tion of enes involved in stom t l movement, su h s ion (+) ch nnels nd ion (H-ATPase) enes (Fig. 5). Upre ul tion of + ch nnel enes by or overexpression under norm l conditions is line with prev ous study in Ar bidopsis sho ing th t GL s positive re ul tors of + ch nnel enes nd stom t l movement (Nie sh et l. 2016). Thus, the rpd

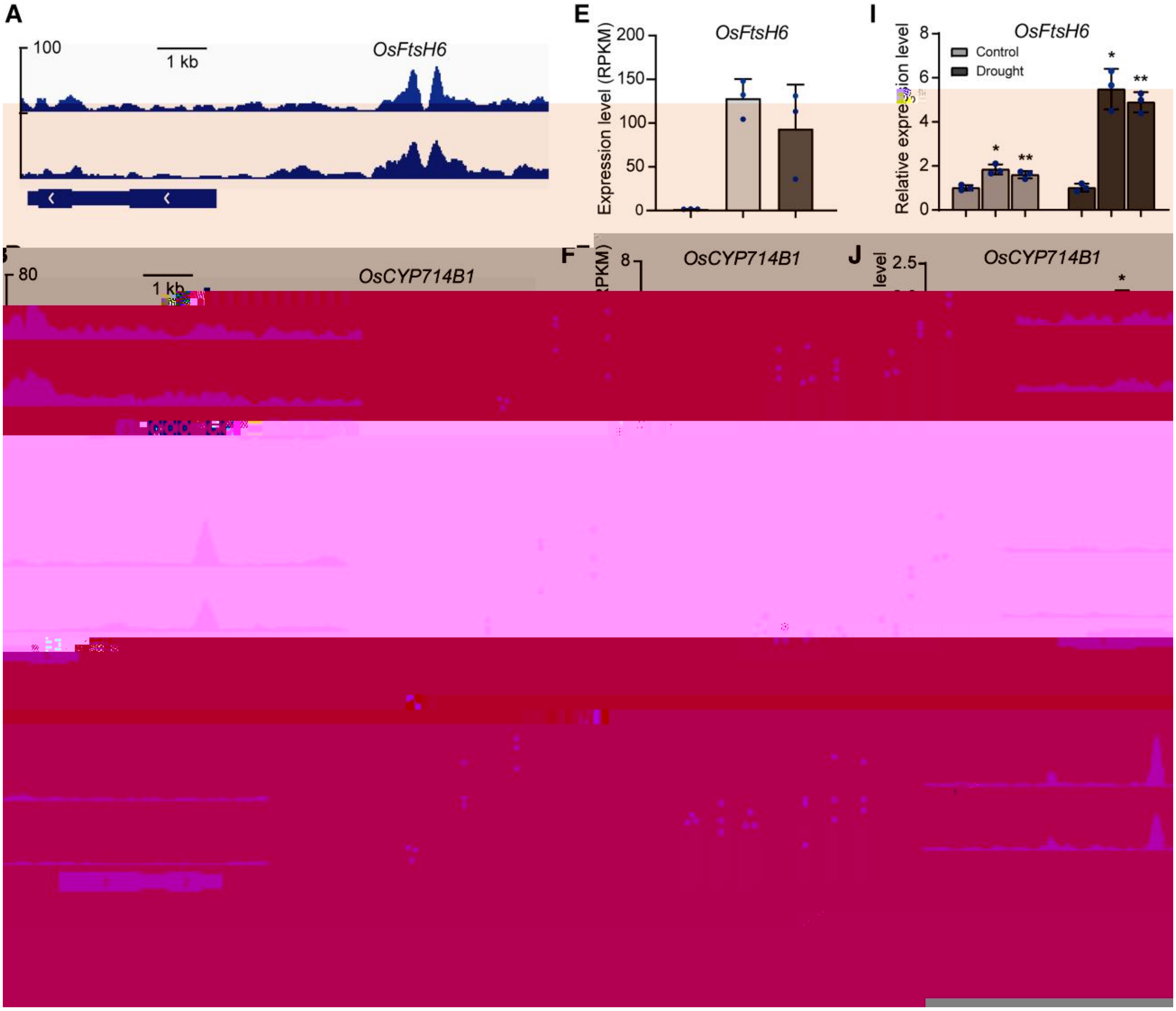

Figure 7. Pu ZmGL 1 ZmG genes n r ce. **A to D)** DAP-seq n c ZmGL 1 ZmG pre eren ly bound pro- mo rs o A), B), C), D). **E to H)** Express on leve s o E), F), G), H) n WT r ce n r ce overexpress n or de rm ned RNA-seq ys s. Gene express on c cul n RP M. **I to L)** Rel e express on leve s o I), J), K), L) n WT, : , : r ce under con ol cond ons 7 d o drou h ss de rm ned RT-qPCR. D re presen me ± SD from 3 b o c l rep c s. * < 0.05, ** < 0.01 S den s .

s m l c osure o n en c r ce p n resul d rec y from s n c n reduc n n express on leve s o ose enes under drou h cond ons.

No bly, e ver ed re ul n o r p d s m l c osure n response c de c ABA me d, sup- por d by exo enous pp c n o ABA nduc n s m l c osure n WT F . 4B), c m m c d e ec o drou h ss. Our nd n s cons s n pre- v ous s dy s es f s m l c osure requ res h h ABA se s C do-Sobr n o e l 2022). Our resul so mp ed ZmGL s m y func n n ABA b osyn- es s p y, s n c d by h her ABA ccumul n Supplemen l F . S5). bn bund n express on o sever l ey enes nvo ved n ABA b osyn es s e . , , , , n response drou h Supplemen l F . S6). ABA b osyn es s s . epox d n o e x n, d s x n ophyll precursor ere ore p ys n mpor ro e n ABA b osyn es s. We pre- v ous y d scovered ZmGL s ncre se leve s o x n ophy ls, nclud n e x n n d lu n L e l 2020), c m y le d mproved ABA b osyn es s n y. Moreover, s dy n Ar b dops s sho ed GL s d rec y c v ex- press on o , d GL -WR Y4 e er ne y re ul s ABA s n n Ah m d e l 2019), su es pos- s b e re ul ry ro e o ZmGL s n ABA s n n p y. We so proposed C l e con erred by ZmGL s s men oned bove m y con bu r p d s m l

closure. This has been demonstrated by models simulations and experimental data that major C4 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 the slower stomatal closure in ferns is associated with reduced responsiveness to ABA and sugars compared to angiosperms (Lima et al., 2019; Cardoso-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).

Stomatal trait measurements with scanning electron microscopy

Rice leaves were detached from control or drought-treated plants and trimmed manually into 3 × 3 mm pieces, excluding the veins. Samples were directly fixed in 2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) and then fixed with 1% osmium tetroxide. After washing twice with 0.1 M phosphate buffer, samples were dehydrated gradually in an ethanol series (30%, 50%, 60%, 70%, 80%, 90%, and 100%) for 15 min each, followed by incubating in tertiary butanol for 35 min. Then, samples were dried using a critical point dryer, placed on the sample stage, and then coated with gold. Stomata were observed and photographed using a SU-8010 scanning electron microscope (Hitachi, Japan). The size, number, and aperture sizes of stomata were calculated using ImageJ software.

Quantification of endogenous ABA content

The uppermost expanded leaves of control and drought-stressed rice seedlings were detached and flash frozen in liquid nitrogen. Ground samples (100 mg each) were extracted with an extraction solution containing an internal standard at [illegible]°C overnight. Samples were centrifuged, and the resulting supernatant was extracted again. The combined extract was purified on a C_{18} solid-phase column and dried with nitrogen gas. After resolving in methanol and passing through a 0.22-μm filter, ABA was quantified on an HPLC–tandem mass spectroscopy (MS/MS) system as described by Liu et al. (2012).

Exogenous ABA treatment

Forty-day-old rice seedlings grown in a pot were sprayed with 100 μM ABA solution containing 0.5% (v/v) Tween-20 as surfactant until the leaves were moist. The volume of ABA solution applied was consistent between seedlings. At 5 h after treatment, gas exchange parameters and stomatal traits were evaluated as described above.

RNA extraction and RT-qPCR

The uppermost fully expanded leaves were harvested from 3-week-old rice seedlings grown in pots under normal conditions or drought stress for 7 d. Samples were flash frozen in liquid nitrogen and ground into powder, and then total RNA was extracted with TRIzol reagent (Invitrogen). RNA purity and quantity were evaluated using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). After DNase treatment, cDNA was synthesized from 1 μg of total RNA per sample using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). RT-qPCR was performed using KOD SYBR Green mix (TOYOBO) on an ABI QuantStudio 6 Flex instrument (Applied Biosystems, USA). Relative transcript levels were calculated with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001), with 3 biological replicates for each treatment, using [illegible] as the internal control. Primers are listed in Supplemental Table S2.

RNA-seq analysis

At 3 h after exogenous ABA treatment, leaves were collected from 4-week-old rice seedlings grown in pots. Total RNA was extracted with TRIzol reagent and then RNA integrity was assessed with the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). RNA-seq libraries were constructed from WT, [illegible], and [illegible] rice plants using the TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, USA) with 3 biological replicates per line. The resulting 9 libraries were sequenced on the Illumina HiSeq X Ten sequencing platform. After removing adapter sequences, low-quality reads, clean reads were mapped to the [illegible] cv. Nipponbare reference genome using HISAT (Kim et al. 2015) and Bowtie (Langmead et al. 2009). Gene expression levels were calculated in reads per kilobase of transcript per million mapped reads (RPKM) using Cufflinks. DEGs were identified with the "DESeq" R package. The thresholds for classification as DEG in the transgenic lines compared with the WT were $P < 0.05$ and $|\log_2 \text{fold change}| > 1$.

DAP-seq and data analysis

The full-length coding sequences of [illegible] and [illegible] were amplified from cDNA of the maize accession B73. Each sequence was recombined into the pIX-HALO vector using LR Clonase II (Invitrogen). The HALO-ZmGLK1 and HALO-ZmG2 proteins were generated using 500 ng each of the pIX-HALO-ZmGLK1 and pIX-HALO-ZmG2 [illegible]

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Acknowledgments

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Author contributions

W.Z. ... ...Y.L. conce...ved ... ...des...ned ... exper...en... Y.L., J.L., S.W., Y.G., ... R.G. per...formed mos... ...f ... exper...en... Z.L. ... H.P. per...formed ... DAP-seq exper...en... P.W. cr... ...ll...y comment... ...ed ... ed ... ...m...nuscr...p... The ...m...nuscr...p... ...s prep...red by ...Y.L., J.L., ... W.Z. All ...thors d...scussed ... comment... on ... ...m...nuscr...p...

Supplemental data

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Supplemental Figure S1. Enh...nced ...sen...t...ve ...ce ...f ... ... : ... ... : r...ce pl...nts ... drou...h-...ress ...duced by ...0% PEG 6000.

Supplemental Figure S2. Overexpress...on ...f ... ...r... ...n r...ce...l...d ... decre...sed ...m...l conduc...nce ... ...d pho...synthe...c p...r...meters ...n response ... drou...h.

Supplemental Figure S3. Dyn...mic ch...ges ...f so...l ...ter con... ...n ... ...ur...g ... drou...h ...ress ...n ... ...reenh...se exper...en...

Supplemental Figure S4. Genome- ...de summ...ry ...f ... re...l...tory ne...r...k ...do...nsse...n ...f ZmGL1 ... ZmG... b...sed on DAP-seq d...

Supplemental Figure S5. Ch...ges ...n endo...enous ABA con... ...n ... WT, ... : r...ce le...es under norm...l cond...ons ... ...r 7 d ...f drou...h ...ress.

Supplemental Figure S6. Rel...ve express...on levels ...f ABA b...synthes... ...enes ...n ... le...es ...f WT, ... : ... , ... : r...ce pl...nts under norm...l cond...ons ... ...r 7 d ...f drou...h ...ress.

Supplemental Table S1. Rel...ve ch...ge ...f ...ene express...on le...el ...f 59 over...pped ...enes ...from RNA-seq ... DAP-seq ...lyses.

Supplemental Table S2. Pr...mers used ...or RT-qPCR.

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Data availability

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