

New Phytologist Supporting Information

Article title: Abscisic acid promotes jasmonic acid biosynthesis *via* a "SAPK10-bZIP72-*AOC*" pathway to synergistically inhibit seed germination in rice (*Oryza sativa* L.) Authors: Yifeng Wang, Yuxuan Hou, Jiehua Qiu, Huimei Wang, Shuang Wang¹, Liqun Tang, Xiaohong Tong, Jian Zhang Article acceptance date: 15 June 2020

The following Supporting Information is available for this article:

			Ser175 Thr176																	
Ê.		120 *		140 *		÷	160 *			190		* 200		*		220	*	*		
CADE C		TONACREERE	DEFECOT	Tecvev	OU MOUC	UDDTVIEN	TTDCOD		TODECVOR	OUTUO	DZOW	UCHDAVIAD		TENDOR	ADUMCCCU	ZZU	VDEED	PP		222
Copy2 1	:	TOWAGKISEDI	DARTETUQI	TOCUTY	CHANQVC	UDDIVIEN	TTTTCC	APALA	TOPECVOR		PAST	VGIPALIAP		REIDGRU	ADVWSCGV	TLIVILVG	VDEEDE	ND		205
SHRKZ.I	1	TOTAGKISLA	CARIFICQI	TOGVLI	CHSLQIC	HEDTETEN	TLLDGSP	APLIN	TOPPOUGA	SILHS	RPAST	VGTPAILAP	EVISE	TEIDGNA	ADVWSCGV	TLIVMLVG	VERELE	NE		105
SNRKZ.Z	:	TONAGRESED	TARFFFQQL	ISGVSI	CHAMQIC	HRULKLEN	TLLDGSP	APRIK	ICDEGISKS	SVLHS	PKST	VGTPAILAP	ELLER	EIDGRE	ADVWSCGV	TLIVMLVG	AIPFEDE	QE.	: 4	124
SnRK2.3	÷	IONAGRESED	CARFFFQQI	ISGVSY	CHSMQIC	HRDLKLEN	TLLDGS P.	APRLK	ICDFGYSKS	SVLHS	QPKST	VGTPAYIAP	EVILLR	EYDGKI	ADVWSCGV	TLYVMLVG	AYPFEDE	EE	: 4	23
SnRK2.4	:	IOSAGRESEDI	ÐARYFFQQI	ISGVSY	CHAMQIC	CHRDLKLEN	TLLDGS P.	APRIK	ICDFGYSKS	SLLHS	RPKST	VGTPAYIAP	EVISE	REYDGKM	ADVWSCGV	TLYVMLVG	AYPFEDC	ED	: 2	205
SnRK2.5	:	I CNAGRESEA	EARYFFQQI	ICGVDY	CHSLQIC	HRDLKLEN	TLLDGSP	APILK	ICDFGYSKS	SLLHS	RPKST	VGTPAYIAP	EVISRI	REYDGKH	ADVWSCGV	TLYVMLVG	YPEEDI	DD	: 2	205
SnRK2.7	÷	ICNAGRESED	EGRYYFKQI	ISGVSY	CHAMQIC	CHRDLKLEN	TLLDGSP	SSHLK	ICDFGYSKS	SVLHS	QPKST	VGTPAYVAP	EVISRI	KEYNGKI	ADVWSCGV	TLYVMLVG	AYPFEDI	ED	: 2	205
SnRK2.8	:	ICSAGRESEDI	EARFFFQQI	ISGVNY	CHSLQIC	HRDLKLEN	TLLDGSE	APRVK	ICDFGYSKS	GVLHS	QPKTT	VGTPAYIAP	EVIST	KEYDGKI	ADVWSCGV	TLYVMLVG	AYPFEDE	SD	: 2	205
SnRK2.9	:	ISSVGRESEA	EARYFFOQI	ICGVHY	LHALQIC	HRDLKLEN	TLLDGS P.	APR <mark>IK</mark>	ICDFGYSKS	SVLHS	NPKST	VGTPAYIAP	EVFCR	SEYDGKS	VDVWSCGV	ALYVMLVG	AYPFEDE	KD	: 2	205
SnRK2.10	:	ICSAGRESED	EARYFFQQI	ISGVSY	CHAMQIC	HRDLKLEN	TLLDGSP	APRIK	ICDFGYSKS	SLLHS	MPKST	VGTPAYIAP	EVISR	EYDGKM	ADVWSCGV	TLYVMLVG	YPFED	ED	: 2	205
SAPK1	:	IONAGRESEDI	EARFFFQQI	ISGVSY	CHSMQVC	HRDLKLEN	TLLDGSV	TPRLK	ICDFGYSKS	SVLHS	PKS T	VGTPAYIAP	EVLSR	EYDGKV	ADVWSCGV	TLYVMLVG	YPFEDE	DD	: 2	205
SAPK2	:	ICSAGRESED	ARFFFOOL	ISGVSY	CHSMQIC	HRDLKLEN	TLLDGSI	APRIK	ICDFGYSKS	SLLHS	OPKST	VGTPAYIAP	EVLAR	KEYDGKV	ADVWSCGV	TLYVMLVG	AYPFEDE	DE	: 2	205
SAPK3		ICTAGRESED	EARYFFOOL	ISGVSY	CHSLEIC	HRDLKLEN	TLLDGSP	TPRVK	ICDFGYSKS	ALLHS	KPKST	VGTPAYIAP	EVISR	KEYDGKU	ADVWSCGV	TLYVMLVG	YPFEDE	GD	: 2	206
SAPK4	:	IODRGRESED	DARYFFOOI	ICGVSY	CHHMOIC	HRDLKLEN	VLLDGSP	APRIK	ICDFGYSKS	SVLHS	RPKSA	VGTPAYIAP	EVISR	REYDGKI	ADVWSCGV	TLYVMLVG	YPFEDO	DD	: 2	205
SAPK5		TOPACREHED	PARYFEOOT	VCGVSY	CHAMOTO	HRDLKLEN	TLLDGSP	APRTK	TCDEGYSKS	STTHS	RPKST	VGTPAYTAP	EVISRI	FYDGKT	ADVWSCGV	TLYVMLVG	YPEED	KD		205
SADKE	÷	TOSACRESED	SRVEFOOT	TOCVEY	CHEMOTO	HRDIKLEN	TLLDCSD	ADRIK	TCDECVSK	STTHS	KDKST	VCTDAVIAD	FULSE	FYDCKM	ADVWSCCV	T.VVMLVG	VDEEDE	DD		205
CADK7	:	TONACREED	DADVEROOT	Tecvev	CUMMOTO	UDDT KT EN	TLDCCD	ADDTK	TOPECVER	ettue	K DK CW	UCTERITIE	DUTION	EVDCK	ADVWCCCV	TT VUMTUC	VDFFD	DD		205
CA DVO	Ċ	TOKNUPROPRI	DARTEROOT	TACUCU	CHEMQIC	UPDT VI PN		APALA	TODECVOR	OUT UC	ODVOD	VOIPAILAP		EIDGNI	ADVWSCGV	TTT VUMUUC	VEREDE	DD	: -	100
OAPKO	•	TOOLORDERD		TOCUCU	CHSHQVC	UNDER A DA	TTTTCSP	APRLA	TODECVOR	SVLHS	PAST	VGIPAIIAP		TUDGNI	ADVWSCGV	THIVMV G	VDDDDD	DD		104
SAPKS	•	TOSAGKESELI	THREFFUQI	ISGVSY	CHSMQVC	HRULKLEN	TLUGST	AFRLK	ICDFGYSKS	SVLHS	PASI	VGTPAYIAP	EVILLA	LIDGKI	ADVWSCGV	TLIVMLVG	IPEEDE	EL	. 4	223
SAPK10	:	TONAGRESED	PARFFFQQI	TREAS	CHSMQVC	HRDIKLEN	TLLDGST	APRLK	ICDFGYSKS	SATHS	QPKST	VGTPAYIAP	EVILLK	REYDGKI	ADVWSCGV	TTAAWTAC	RAFEDE	DE	: 4	.24

Fig. S1 Sequence alignment of T- loop region of 10 *Arabidopsis* and 10 Rice SnRK2 family members. Arrows indicate the residues of SnRK2.6 (OST1) phosphorylation sites. The putative T- loop predicted for SnRK2.6 is underlined in red.



Fig. S2 Molecular characterization of *sapk10* mutant and seed germination in response to ABA. (A) Schematic presentation of the gene structure of *SAPK10* and CRISPR-cas9 editing site. Grey boxes: untranslated regions; Black boxes: exons; black line: intron. PAM: protospacer adjacent motif. (B) Sanger sequencing chromatograph of the CRISPR-cas9 target site in homozygous mutants of *crsapk10*. The letter in red represented the mutant sites. The letter in blue represented the PAM sequence. (C) Germination time courses of WT and *crsapk10* lines grown on half-strength MS medium containing different concentration of ABA (0, 2, 5 μM), respectively. (D) The relative germination of the WT and *crsapk10*



seeds under ABA treatments were determined after 4 days and expressed as a percentage of those grown on Mock condition. (E) Germination phenotypes of WT and *crsapk10* treated with 0, 2, 5 μ M ABA, respectively. Photographs were taken on day 4. Bar=1cm. (F) Seedling heights of WT and *crsapk10* in accordance to (E). Error bars indicate SD with biological triplicates (n=3, each replicates containing 50 seeds) in (C) and 50 biological replicates (n=50) in (F).



Fig. S3 qRT-PCR analysis for transcript accumulation of nine SnRK2 family members (*SAPK1-SAPK9*) in the seeds of WT or *crsapk10* lines, respectively. Seeds were grown on mock or 10 μ M ABA treated half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t-test* analysis: ** P<0.01.





Fig. S4 Molecular characterization of *SAPK10, SAPK10^{S177A}* over-expressing transgenic lines. (A) qRT-PCR analysis for transcript accumulation of *SAK10* in the seeds of *OxSAPK10* or *OxSAPK10^{S177A}* transgenic lines, respectively. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t-test* analysis: ** P<0.01. (B) Sanger sequencing chromatograph of the mutated *SAPK10* cDNA in *OxSAPK10^{S177A}* transgenic lines. The letter in red represented the mutant sites.



Fig. S5 Expression pattern of SAPK10 and bZIP72. (A) qRT-PCR analysis of *SAPK10* and *bZIP72* transcription level in flower, leaf, stem, root and seed, respectively. (B) qRT-PCR

analysis of *SAPK10* and *bZIP72* during seeds germination grown on half-strength MS medium treated with 10 μ M ABA. (C) GUS staining of the primary root (a), lateral roots (b), root hairs (c), crown root (d), leaf (e), seed (f) and plumule (g) of the transgenic plants harboring the *proSAPK10:GUS* fused gene. Bar =1 cm in (a). (D and E) Subcellular localization of SAPK10 in (D) and bZIP72 in (E). *35S:SAPK10-GFP* or *35S:bZIP72-GFP* was co-transformed with a nuclear marker *35S:D53-mKate* (Zhou *et al.*, 2016) into protoplast. Bar=10 μ m. Error bars indicate SD with biological triplicates (n=3) in (A and B). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t-test* analysis: * P<0.05, ** P<0.01.



Fig. S6 Molecular characterization of *crbzip72*, *crtrab1* and *crbzip72/crtrab1* mutant. (A) Schematic presentation of the gene structure of *bZIP72* and CRISPR-cas9 editing site. PAM: protospacer adjacent motif. Grey boxes: untranslated regions; Black boxes: exons; black line: intron. (B) Sanger sequencing chromatograph of the CRISPR-cas9 target site in homozygous mutants of *crbzip72*. The letter in red represented the mutant sites. The letter in blue represented the PAM sequence. (C) Schematic presentation of the gene structure of *bZIP72* and *TRAB1*. Both genes shared the same CRISPR-cas9 editing PAM sequence. (D)

Sanger sequencing chromatograph of the CRISPR-cas9 target site in homozygous mutants of *crbzip72/crtrab1*.



Fig. S7 qRT-PCR analysis for transcript accumulation of *TRAB1* in the seeds of *crbzip72* transgenic line. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t-test* analysis: ** P<0.01.



Fig. S8 Molecular characterization of *bZIP72* and *bZIP72^{S71A}* over-expressing transgenic lines. (A) qRT-PCR analysis for transcript accumulation of *bZIP72* in the seeds of *OxbZIP72* and *OxbZIP72^{S71A}* transgenic lines, respectively. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t-test* analysis: ** P<0.01. (B) Sanger sequencing chromatograph of the mutated *bZIP72* cDNA in *OxbZIP72^{S71A}* transgenic lines. The letter in red represented the mutant sites.



Fig. S9 qRT-PCR analysis for transcript accumulation of JA and GA pathway genes in the seeds of the WT, *OxbZIP72 and OxbZIP72^{S71A}* transgenic lines. Seeds were grown on half-strength MS medium for 6 hours before harvest for total RNA isolation. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of differences between the WT and transgenic lines as determined by Student's *t-test* analysis: *P<0.05, ** P<0.01.



Fig. S10 EMSA of bZIP72 on JA pathway genes *AOC*, *AOS1* and *LOX1* promoter regions and GA pathway genes *GA3ox1*, *GA2ox5* and *GA20ox2* promoter regions.



Fig. S11 qRT-PCR analysis for transcript accumulation of ABA and JA pathway genes in the seeds of the WT. Seeds were grown on half-strength MS medium treated with 0 or 10 μ M ABA for 3 or 6 hours before harvest for total RNA isolation, respectively. Error bars indicate SD with biological triplicates (n=3). Asterisks indicate the significance of

differences between the mock-treated and ABA-treated seeds as determined by Student's *t*-*test* analysis: *P<0.05, ** P<0.01.



Fig. S12 IBU relieved the inhibition of ABA on post-germination growth. (A) Growth phenotypes of the WT after grown in nutrient solution containing 0, 10 μ M ABA, 10 μ M ABA + 50 μ M IBU or 10 μ M ABA + 100 μ M IBU for 7 days, respectively. Bar= 2.5 cm. (B) Seedling heights of the WT in accordance to (A). IBU: Ibuprofen. Error bars indicate SD with 50 biological replicates (n=50). Asterisks indicate the significance of differences between the ABA-treated seedlings and ABA+ IBU treated seedlings as determined by Student's *t-test* analysis: *P<0.05, ** P<0.01.



Fig. S13 SAPK10-mediated retarded seed germination was relieved by exogenous IBU applied. (A) Germination time courses of the WT and *OxSAPK10* grown on half-strength MS medium under mock or 100 μ M IBU treatment, respectively. (B) The relative germination of the WT and *OxSAPK10* seeds under 100 μ M IBU treatment was determined after 4 days and expressed as a percentage of those grown on Mock condition. (C) Germination phenotypes of the WT and *OxSAPK10* treated with 0 or 100 μ M IBU, respectively. Photographs were taken on day 4. Bar=1cm. (D) Seedling heights of the WT and *OxSAPK10* in accordance to (C). Error bars indicate SD with biological triplicates (n=3, each replicates containing 50 seeds) in (A) and 50 biological replicates (n=50) in (D). Asterisks indicate the significance of differences between the mock and IBU treatment in (B and D) (Student's *t*-*test* analysis; ** P<0.01).