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1445 - 4408

10.1071/FP05161\_AC

Functional Plant Biology 2005, 33, 43–57

## **Supplementary material**

# TaNAC69 from the NAC superfamily of transcription factors is up-regulated by abiotic stresses in wheat and recognises two consensus DNA-binding sequences

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TaNAC69-1 TaNAC69-2 TaNAC69-3 TaNAC69-1	MPMG-SSAAMPALPPGFRFHPTDEELIVHYLRRQAASMPSPVPIIAEVNIYKCNPWDLPG MPMGSSSAAMPALPPGFRFHPTDEELIVHYLGRQAASMPSPVPIIAEVNIYKCNPWDLPG MPMGSSSAAMPALPPGFRFHPTDEELIVHYLGRQAASMPSPVPIIAEVNIYKCNPWDLPG **** ********************************
TaNAC69-2	KALFGENEWYFFSPRDRKYPNGARPNRAAGSGYWKATGTDKAILSTPANESIGVKKALVF
TaNAC69-3	KALFGENEWYFFSPRDRKYPNGARPNRAAGSGYWKATGTDKAILSTPANESIGVKKALVF
TaNAC69-1	YRGKPPKGVKTDWIMHEYRLTAADNRTTKRRGSSMRLDDWVLCRIHKKCGNLPNFSSSDQ
TaNAC69-2	YRGKPPKGVKTDWIMHEYRLTAADNRTTKRRGSSMRLDDWVLCRIHKKCGNLPNFSSSDQ
TaNAC69-3	YRGKPPKGVKTDWIMHEYRLTAADNRTTKRRGSSMRLDDWVLCRIHKKCNNLHNFSSSDQ
	***************************************
TaNAC69-1	EQEHEQESS-TVEDSQNNHTVSSPKSEAFDGDGDDHLQLQQFRPMAIAKSCSLTDLLNTV
TaNAC69-2	EQEHEQESS-TVEDSQNNHTVSSPKSEAFDGDGNDHLQLQQFRPMAIAKSCSLTDLLNTV
TaNAC69-3	EQEHEQESSTTVEDSHNNHTVSSPKSEAFDGDGDDQLQLQQFRPMAIAKSCSLTDLLNTV
	********* *****************************
TaNAC69-1	DYAALSHLLLDGAGASSSDAGADYQLPPENPLIYSQPPWQQTLHYNNNN-GYVNNETI
TaNAC69-2	DYAALSHLLLDGAGASSSDAGADYQLPPENPLIYSQPPWQQTLHYNNNN-GYVNNETI
TaNAC69-3	DYAALSHLLLDGAGAGASSSDAGADYQLPPENPLIYSQPPWQQTLHYNNNNNGYVNIDTI
TaNAC69-4	AGADYQLPPENPLIYSQPPWQQTVHYNNNN-GYVNNDTI
	***************************************
TaNAC69-1	DVPQLPEARVDDYGMNGDKYNGMKRKRSSGSLYCSQLQLPADQYSGMLIHPFLSQQLHM
TaNAC69-2	DVPQLPEAGVDDYGMNGDKYNGMKRKRSSGSLYCSQLQLPADQYSGMLTHPFLSQQLHM
TaNAC69-3	DVPQIPEARVDDYGMNGDRYNGMKRKRSSGSLYCSQLQLPADQYSGMLIHPFLSQQLHM
TaNAC69-4	DVPQLPEAHVDDYGMNGDRYNGMKRKRSSGSLYCSQLQLPADQYSGMLIHPFLSQQLHM
	****:*** ********:*********************

Supplementary Fig. 1. Sequence alignment of three TaNAC69 proteins and TaNAC69-4 from a wheat EST TaNAC69-1 (AY625682), TaNAC69-2 (DQ022842), TaNAC69-3 (DQ022843), TaNAC69-4 (wheat EST ID: 203331236). TaNAC69-4 is an EST clone in the wheat EST database, identified by homology searching using the TaNAC69-1 sequence.

### Publisher: CSIRO; Journal: FP:Functional Plant Biology Article Type: accessory-material; Volume: 33; Issue: 1; Article ID: FP05161\_AC DOI: 10.1071/FP05161\_AC; TOC Head:

TaNAC69-1	LPPGFRFHPTDEELIVHYLRRQAASMPSPVPIIAEVNIYKCNPWDLPGK	ALFGENEWY	FF
Hv	LPPGFRFHPTDEELIVHYLGRQAASMPSPVPIIAEVNIYKCNPWDLPGK	ALFGENEWY	FF
Os-I	LPPGFRFHPTDEELIVHYLMNQAASVKCPVPIIAEVNIYKCNPWDLPGK	ALFGENEWY	FF
Os-J	LPPGFRFHPTDEELIVHYLMNQAASIKCPVPIIAEVNIYKCNPWDLPGK	ALFGENEWY	FF
Sb	LPAGFRFHPTDEELIVHYLMNQAASIPCPVPIIAEVNIYQCNPWDLPAK	ALFGESEWY	FF
Mt	LPPGFRFHPTDEELIVHYLCNQATSKPCPASIIPEVDIYKFDPWELPDK	SEFEENEWY	FF
At	LPPGFRFHPTDEELIVYYLRNQTMSKPCPVSIIPEVDIYKFDPWQLPEK	TEFGENEWY	FF
St	LPPGFRFHPTDEELIMYYLRYQATSRPCPVSIIPEIDVYKFDPWELPEK	AEFGENEWY	FF
Sd	LPPGFRFHPTDEELIMYYLRYQATSRPCPVSIIPEIDVYKFDPWELPEK	AEFGENEWY	FF
Le	LPPGFRFHPTDEELIMYYLRYQATSRPCPVSIIPEIDVYKFDPWVLPEK	AEFGDNEWY	FF
Mc	LPPGFRFHPTDEELIIYYLKNQAMSRPCPVSIIPEVDIYKFDPWQLPDK	AEFGENEWY	FF
Cs	LPPGFRFHPTDEELIVHYLRNQATSRPCPVSIIPEVDIYKFDPWQLPEK	AEFGEKEWY	FF
PtxPt	LPPGFRFHPTDEELVVHYLKKKAASVPLPVTIIAEIDLYKLDPWELPSK	ATFGEQEWY	FF
Gm	LPPGFRFHPTDEELILHYLSKKVASIPLPVSIIAEVDIYKLDPWDLPAK	ATFGEKEWY	FF
	** .***********************************	: * : . * * *	* *
TaNAC69-1	SPRDRKYPNGARPNRAAGSGYWKATGTDKAILSTPANESIGVKKAL	VFYRGKPPK	GV
Hv	SPRDRKYPNGARPNRAAGSGYWKATGTDKAILSTPANESIGVKK		
Os-I	SPRDRKYPNGARPNRAAGSGYWKATGTDKSILSTPTSDNIGVKKAL	VFYKGKPPK	GV
0s-J	SPRDRKYPNGARPNRAAGSGYWKATGTDKSILSTPTSDNIGVKKAL	VFYKGKPPK	GV
Sb	SPRDRKYPNGARPNRAAGSGYWKATGTDKAIMSTPTGQNIGVKKAL	VFYGGRPPR	GV
Mt	SPRERKYPNGVRPNRATLSGYWKATGTDKAIKSGSKQIGVKKSL	VFYKGRPPK	GV
At	SPRERKYPNGVRPNRAAVSGYWKATGTDKAIHSGSSNVGVKKAL	VFYKGRPPK	GI
St	TPRDRKYPNGVRPNRAAVSGYWKATGTDKAIYSANKYVGIKKAL	VFYKGKPPK	GV
Sd	TPRDRKYPNGVRPNRAAVSGYWKATGTDKAIYSANKYVGIKKAL	VFYKGKPPK	GV
Le	TPRDRKYPNGVRPNRAAVSGYWKATGTDKAIYSANKYVGIKKAL	VFYKGKPPK	GV
Mc	TPRDRKYPNGIRPNRATVSGYWKATGTDKAIYSGAKYVGIKKAL	VFYKGKPPK	GI
Cs	SPRDRKYPNGTRPNRATVSGYWKATGTDKAIYGGSKYLGVKKAL	VFYKGRPPK	GI
PtxPt	SPRDRKYPNGARPNRAATSGYWKATGTDKPILTSNGAQKVGVKKAL	VFYGGKPPK	GI
Gm	SPRDRKYPNGARPNRAAASGYWKATGTDKTIVTSLOGGAOESVGVKKAL	VFYKGRPPK	GV
	:* <b>*</b> :***** ****************************	*** *:**:	*:
		%Ide	%Sim
TaNAC69-1	KTDWIMHEYRLTAADN-RTTK-RRGSSMRLDDWVLCRIHKK	100	100
Hv		99	99
0s-I	KTDWIMHEYRLTGTSANSTTTTKORRASSMTMRLDDWVLCRIHKK	86	92
0s-J	KTDWIMHEYRI,TGTSANNTTTTKORRASSMTMRLDDWVLCRIHKK	86	91
Sb	KTDWIMHEYRLVTADADGKTTAAKRRGGSMRLDDWULCRIHKK	84	89
Mt	KTDWIMHEYRI, I GSOKOT - SKHIGS MRLDDWVLCRIYKK	74	83
At	KTDWIMHEYRIHDSRKAS-TKRNGSMRLDEWVICRIYKK	75	83
9+		74	86
Sd	KTDWIMHEYRIS	74	86
L.α	KTDWIMHEYRIS	75	87
Mc	KTDWIMHEIRIS DSKOQI SKQSGS MKIDDWVICKIIKK	73	83
MC Co		73	Ω1
		72	01 01
Cm		75	95
Giii	** ******** **************************	15	00

Supplementary Fig. 2. NAC proteins from other plant species that share the highest homology with TaNAC69-1 in the NAC domain among wheat NAC proteins present in the wheat EST database. Only one NAC protein per plant species sharing the highest homology and > 70% identity with the TaNAC69 NAC domain is presented. Hv, *Hordeum vulgare* NAC (gi: 21182621); Os-I, *Oryza sativa* indica NAC (AAAA02030644); Os-J, *Oryza sativa* japonica NAC (gi: 29674907); Sb, *Sorghum bicolour* NAC (gi: 45949087); Mt, *Medicago truncatula* NAC (gi: 11910349); At, *Arabidopsis thaliana* NAP (AJ222713); St, *Solanum tuberosum* NAC (gi: 21920677); Sd, *Solanum demissum* NAC (AC154033); Le, *Lycopersicon esculentum* NAC (gi: 5604792); Mc, *Mesembryanthemum crystallinum* NAC (gi: 26564003); Cs, *Citrus sinensis* NAC (gi: 38051081); PtxPt, *Populus tremula* × *Populus tremuloides* NAC (gi:24106131); Gm, *Glycine max* NAC (the sequence derived from three EST sequences gi:17962878, 9987195, 21677753). Ide, identity; Sim, similarity. Note: to ascertain whether the above non-cereal NAC genes are the most homologous to TaNAC69 among wheat NAC genes, the NAC domain sequences of these non-cereal genes were used to search the wheat EST database that currently contains 561 100 sequences, using the 'tblastn' program. The *NAC* genes from these non-cereal species exhibited the highest homology with a wheat 5'-sequence EST (gi:23401647), which contains the partial sequence of TaNAC69-1.

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Α		<i>a.</i>							-				•	-								
		.C69-s	elect	ed o	ligo	nuc	leot	ides	- D.	NA-	bin	ding	site	e I				RI	<b>5A</b> (	%) ∖_1		
	SOI			gag	gat	CCG	f. L. G	cac	agt	ACO	Jta	act	gti	cac	a			10	)±⊿ 1	2.4		
	S02		to	aag	gtg	gCG	Ta	tgg	tat	ACO	Jta	act	aca	ag				98	1 ±	3.0		
:	SO3		gaco	tga	att	gCC	Ta	tcc	att	ACO	Jta	act	ac					96	± 0.	3.8		
	S04			g	gag	tCC	Tg	tgc	gtc	ACO	Gta	acc	tco	ctg	са			95	3 ±	2.7		
	S05		ct	gag	gag	aCO	Ta	tag	gac	AC	Gtc	act	cga	aa				94	2 ±	2.9		
	S06	g	ggtg	,t <b>g</b>	gag	aCO	Tg	ttt	ctt	AC	Ggc	act	g					89	1 ±	2.5		
	S07	gg	cctg	ja <b>a</b> g	gag	aCO	Ta	cct	ga <b>c</b>	AC	3gc	atc						86	.4 ±	1.8		
1	S08	g	gaat	tga	ata	gCC	Ta	tcg	tat	AC	Gca	acc	t					82	.0 ±	3.4		
	S09			a <b>a</b> a	gaa	gCC	Ta	atg	gat	AC	Gca	acc	cad	cgg	С			79	.3 ±	2.8		
	SO10		agct	g <b>a</b>	aaa	gCC	Ta	tga	tac	AC	Gta	atc	!					77.	5 ±	1.5		
1	S011			gag	gtg	aCO	Ta	tga	ggt	AC	Gta	atc	agg	gcg	С			74	.6 ±	2.3		
	S012			a <b>a</b> g	gat	gCC	Ta	cac	gat	AC	Gta	acc	cag	gtc	С			70	2 ±	4.0		
:	SO13			ga	atg	gCO	Ta	cat	cat	AC	Gta	act	tat	tcg	cct			68	.0 ±	1.9		
:	S014		g	JC <b>a</b> a	att	tCC	Tgi	tgt	gt	AC	Gtc	act	gca	atc				67	4 ±	2.2		
:	SO15			agg	gta	gCC	Tag	gct	cat	AC	Gta	acc	gt	gag	ta			65	.0 ±	1.7		
:	SO16			taa	aag	aCO	Tga	att	acc	AC	Gta	act	CCa	atg	ac			63	2 ±	2.8		
:	SO17			ag	gag	aCO	Tg	gac	atc	ACO	Gtc	ttc	agg	gac	tat			60	9 ±	3.1		
;	S018			gg	gtt	gCG	Ta	agg	tct	AC	Gta	t <b>tc</b>	cta	agt	g			58	8 ±	1.4		
:	SO19		Ca	aga	att	gCC	Ta	atc	aat	AC	Gca	act	С					57	0 ±	1.8		
;	SO20			gga	ata	gCC	Tga	agg	tg <b>t</b>	AC	Gta	att	gta	agt	g			56	2 ±	2.3		
	S021			ga	atg	CCC	Tgi	tct	tat	AC	Gca	agt	gt	gaa	tc			55	7 ±	2.0		
;	SO22		gccc	cgt	ttg	cCC	Ta	cag	tg <b>c</b>	AC	Gtc	atc	gt					54	2 ±	1.6		
;	SO23		acct	g <b>a</b> g	gtg	aCO	TCa	agg	ttc	AC	Gta	act	ggt	t				44	8 ±	2.4		
;	SO24			g	cta	gCC	Ta	tgg	ag <b>c</b>	AC	Gta	act	cta	atg	gtc	aad	ctc	42	7 ±	1.3		
;	SO25			g	cta	gCC	Ta	tgc	at <b>t</b>	AC	Gca	act	aat	tac	gtc	tgo	ctc	41	2 ±	1.8		
;	SO26		ca	cga	aag	gCG	Tga	agg	tac	AC	Gta	atc	ati	tgc				38	6 ±	1.7		
;	S027	gat	cgcc	aa	cat	gCG	Ta	aag	ta <b>t</b>	AC	Gtc	acc	!					38	3 ±	1.9		
1	SO28		ca	aag	gag	cCC	Ta	tgt	gct	AC	Gca	aca	gt	gc				35	± 0.	1.4		
;	SO29			cgg	gat	tCG	Tat	tcg	atg	ACO	Gta	acc	gaa	agc	t			31	8 ±	1.8		
1	SO30		ca	gaa	ata	tCC	Tgi	ttg	ct	200	2 <b>⊢</b> +											
1	SO31									AC	JUL	act	tgg	ga				31	3 ±	1.3		
1			to	rc <b>g</b> t	tta	tCC	Tg	gtt	cat	AC	Gta	act aca	tgg	ga ca				31 24	.3 ± .9 ±	1.3 1.5		
1	SO32		tg	cgi	tta aag	t <b>CC</b>	Tg Ta	gtt tcc	ca <b>t</b> ta <b>c</b>	AC(	Sta Sqa	act aca tct	tgg gto qti	ga ca tcq				31 24 23	.3 ± .9 ± .2 ±	1.3 1.5 1.2		
	SO32 SO33		to t caa	ic <b>g</b> i c <b>g</b> i	tta aag ata	t <b>CC</b> g <b>CC</b>	Tg Ta Ta	gtt tcc agg	ca <b>t</b> ta <b>c</b> cc <b>t</b>	AC( AC( AC(	Gta Gga Gtt	act aca tct atc	tgg gt gt gaa	ga ca tog at				31 24 23 19	.3 ± .9 ± .2 ± .9 ±	1.3 1.5 1.2 0.9		
	SO32 SO33 SO34		tg t caa	icgi cga cga ac	tta aag ata gta	t <b>CC</b> g <b>CC</b> g <b>CC</b> a <b>CC</b>	GTg GTa GTa GTa GTg	gtt tcc agg ccq	ca <b>t</b> ta <b>c</b> cc <b>t</b> ta <b>c</b>	AC( AC( AC( AC(	Gta Gga Gtt Gtc	act aca tct atc ttg	tgg gto gto gaa tao	ga ca tog at cto	tqc	!		31 24 23 19 14	.3 ± .9 ± .2 ± .9 ± .0 ±	1.3 1.5 1.2 0.9 1.2		
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:	SO32 SO33 SO34 SO35 SO36		to t	icga icga ag ga ga	tta aag ata gta acg	t CC gCC gCC aCC aCC aCC	STg STa STa STg STg STa STa	gtt tcc agg ccg cat	cat tac cct tac gat	AC( AC( AC( AC( AC( AC( AC( AC( AC(	Sta Sga Stt Stc Stc	act aca tct atc ttg act	tgg gti gaa tao cgi	ga ca tog at cto ttg	tgc gt	!		31. 24. 23. 19. 14. 13. 12.	.3 ± .9 ± .2 ± .9 ± .0 ± .1 ± .6 ±	1.3 1.5 1.2 0.9 1.2 1.5 1.4		
:	SO32 SO33 SO34 SO35 SO36 SO37		to t caa	cgi cga cga ga ga cga	tta aag ata gta acg ggg ggg	t C0 g C0 g C0 a C0 c C0 a C0 a C0	STg STa STa STg STg STg STg STg	gtt tcc agg ccg cat gca gca	cat tac cct tac gat cgt	:AC( :AC( :AC( :AC( :AC( :AC( :AC(	Gta Gga Gtt Gtc Gga Gta	act aca tct atc act act act	tg gt gt ga ta cg ca	ga ca tog at ctc ttg agc	tgc gt c	ł		31 24 23 19 14 13 12 11	$3 \pm .9 \pm .2 \pm .9 \pm .0 \pm .1 \pm .6 \pm .2 \pm$	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7		
:	SO32 SO33 SO34 SO35 SO36 SO37 SO38		tg t caa a	cgi cgi ga ga ga cgi cgi	tta aag ata gta acg ggg gaa	t CC gCC aCC aCC gCC gCC t CC	STg STa STa STg STg STg STg STg STg STg	gtt tcc agg ccg cat gca gca	cat tac tac gat cgt cca	AC( AC( AC( AC( AC( AC( AC( AC( AC( AC(	Gta Gga Gtt Gtc Gga Gta Gta	act aca tct atc act act act	tg gt ga ga ta cg ca ca	ga tog at ttg agc tag tag	tgc gt c	!		31. 24. 23. 19. 14. 13. 12. 11. 9.1	$3 \pm .9 \pm .2 \pm .9 \pm .0 \pm .1 \pm .2 \pm .2 \pm .2 \pm .2 \pm .2 \pm .2 \pm .2$	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7		
	SO32 SO33 SO34 SO35 SO36 SO37 SO38 Cons	ensu	tg caa cga s	IC 91 IC 92 IC 93 IC 94 IC 94	tta aag ata gta ggg gga gga ata rwd	t CC gCC aCC aCC aCC gCC t CC	GTG GTa GTa GTa GTa GTG GTG GTG GTT GTT	gtt tcc agg ccg cat gca gat gac	cat cct tac gat cga cca cto	AC( AC( AC( AC( AC( AC( AC( AC( AC( AC(	Gta Ggt Gtc Gtc Ggt Gtc Gta Gta	act act atc act act act act	itge igti igaa itao icgi icci icci iago	ga ca tog at ctc ttg tagc tagc	tgc gt c	!		31. 24. 23. 19. 14. 13. 12. 11. 9.1	$3 \pm 9 \pm 2 \pm 9 \pm 0 \pm 1 \pm 6 \pm 2 \pm 0 \pm 1 \pm 0 \pm 1 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0$	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8		
	SO32 SO33 SO34 SO35 SO36 SO37 SO38 Cons	ensu	tg caa cga s		tta aag ata gta acg ggg gaa ata rwd	t C0 gC0 aC0 aC0 gC0 aC0 gC0 t C0 nC0	STag STad STad STgo STgo STgo STgo STgo STgo STto STro	gtt tcc agg ccg cat gca gat gac	cat cct tac gat cgt cca ctc	AC( AC( AC( AC( AC( AC( AC( AC( AC( AC(	Gta Gta Gtt Gtc Gtc Gta Gta Gta	act tct atc act act act act act	tge gti gaa tao cgi caa cci	ga ca tog at ctc tog tag ct	tgc gt c			31. 24. 23. 19. 14. 13. 12. 11. 9.1	$3 \pm 9 \pm 2 \pm 9 \pm 0 \pm 1 \pm 6 \pm 2 \pm 0 \pm 0 \pm 1 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0$	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8		
	S032 S033 S034 S035 S036 S037 S038 Cons	ensu			tta agata gta ggg ggaa rwd	t CC gCC aCC aCC aCC gCC t CC nCC	Hgg Had Had Had Had Had Had Had Had Had Had	gtt tcc agg ccg gcat gca gac nnn	cat tac cct gat cca cca ctc	ACC ACC ACC ACC ACC ACC ACC ACC	Sta Stc Stc Stc Sta Sta Sta	act aca tct act act act act		ga tog tog tot tot tago tago	tgc gt c		C	31. 24. 23. 19. 14. 13. 12. 11. 9.1	$3 \pm 9 \pm 2 \pm 9 \pm 0 \pm 1 \pm 6 \pm 2 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0$	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8		
	s032 s033 s034 s035 s036 s037 s038 Cons <b>r</b>	ensu <b>r w</b>	cga s d	gagi gagi gagi gagi gagi gagi ri n	tta aag ata gta ggg gga ata rwd C		GTGG GTAG GTAG GTAG GTAG GTAG GTAG GTAG	gtt tcc agg ccg gca gca gat gac nnn	cat tac tac gat cgt cca ctc nny	AC( 2AC( 2AC( 2AC( 2AC( 2AC( 2AC( 2AC( 2	Gita Gita Gita Gita Gita Gita Gita Diagonality Gita Gita Gita Gita Gita Gita Gita Gita	act aca tct atc act act act act act act		ya ca tog at ctc tog agc tag ct <b>y</b>	tgc gt c <b>A</b>	C	G	31. 24. 23. 19. 14. 13. 12. 11. 9.1 <b>t</b>	$3 \pm 9 \pm 2 \pm 9 \pm 0 \pm 1 \pm 0 \pm 2 \pm 0$	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8	У	Y
A	s032 s033 s034 s035 s036 s037 s038 Cons <b>r</b> 16	ensu <b>r w</b> 15 16	caa cga s d 5 13	icgi icga icga ga ga icga icaa jri n 9	tta aag ata gta ggg gga ata rwd C 0	t CC gCC gCC aCC gCC ccCC gCC tCC gCC tCC gCC gCC ccCC gCC gCC gCC gCC gCC gCC	3799 374 374 37790 37790 37799 37779 377779 37779 377779 377770 377770 377770 37779 37779 37779 377700 377700 377700 377700 377700000000	gtt tcc agg ccg cat gac gat gac nnn <b>r</b> 22	cat tac cct tac gat cca cca ctc nny 9	ACC ACC ACC ACC ACC ACC ACC ACC	Grand	actattgattattgatta	itgg igto igto igaa itao iccol i iccol i iccol iccol iccol i iccol i i i i i i i i i i i i i i i i i i i	ya ca togyat ctcc ttg agc ttag ct <b>y</b> 1	tgc gt c <b>A</b> 38	<b>C</b> 0	<b>G</b> 0	31. 24. 23. 19 14. 13 12 11. 9.1 <b>t</b> 0	$3 \pm 9 \pm 2 \pm 9 \pm 0 \pm 1 \pm 0 \pm 0 \pm 2 \pm 0$ $4 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0 \pm 0$ <b>a</b> 27	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8 <b>a</b> 34	<b>y</b> 0	<b>Y</b> 2
AC	s032 s033 s034 s035 s036 s037 s038 Cons <b>r</b> 16 0	ensu <b>r w</b> 15 16 3 1	cga s d 5 13 0	icgi icga icga ga icgg icaa n    	tta aag ata ggg ggg gaa ata rwd C 0 38	t CC gCC gCC aCC ccCC gCC tCC nCC G 0 0	FT99 FT1 FT2 FT2 FT2 FT2 FT2 FT2 FT2 FT2 FT2 FT2	gtt tcc agg ccg cat gca gat gac <b>r</b> 22 1	cat tac cct tac gat cca cca cca cca cca nny 9 7	ACC ACC ACC ACC ACC ACC ACC ACC ACC ACC	Grand	actatto acto	itggi igto igto iggaa itao iccaa iccol iccaa iccol iccaa iccol iccaa iccol iccaa iccol i 17	ya ca tcg at ctc tcg agc tag ct <b>y</b> 1	tgc gt c <b>A</b> 38 0	<b>C</b> 0 38	<b>G</b> 0	31. 24 23. 19 14. 13. 12. 11. 9.1 <b>t</b> 0 6	$3 \pm 9 \pm 2 \pm 9 \pm 0 \pm 1 \pm 6 \pm 2 \pm 0 \pm 0 = 27$	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8 <b>a</b> 34 0	<b>Y</b> 0 26	<b>y</b> 2 16
A C G	s032 s033 s034 s035 s036 s037 s038 Cons <b>r</b> 16 0 22	ensu <b>r w</b> 15 16 3 1 18 1	cga s d 13 0	icga icga icga ga ga icga icaa jri n 9 5	tta aag ata gta gggg gga ata rwd C 0 38 0	t CC gCC gCC aCC gCC aCC gCC t CC gCC d c CC gCC d gCC gCC gCC aCC gCC gCC aCC gCC aCC gCC aCC gCC aCC gCC aCC gCC g	3799 3731 3733 3739 3739 37790	gtt tcc agg ccg gca ggat ggac nnn 22 1 14	cat tac cct tac gat cca ctc nny 9 7 6	ACC ACC ACC ACC ACC ACC ACC ACC	Je Constraints of the second s	actattattatta acttogt acttogt acttog acttog n 8 9 9	itagi igta igaa itagi icaa iccol iccol iccol iccol iccol iccol iccol iccol iccol iccol iccol iccol iccol iccol iccol iccol i iccol i iccol i iccol i iccol i i iccol i i i i i i i i i i i i i i i i i i i	ya ca tcg at ctc tcg agc tag ct y 1 15 1	tgc gt c <b>A</b> 38 0 0	<b>C</b> 0 38 0	<b>G</b> 0 38	31. 24 23. 19 14 13. 12. 11. 9.1 <b>t</b> 0 6 4	$3 \pm 9 \pm 2 \pm 9 \pm 0 \pm 1 \pm 6 \pm 2 \pm 0 = 0$ a = 27 $9 = 2 \pm 0$ a = 27 9 = 0	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8 <b>a</b> 34 0 0	<b>y</b> 0 26 1	<b>Y</b> 2 16 1
A C G T	so32 so33 so34 so35 so36 so37 so38 Cons <b>r</b> 16 0 22 0	ensu <b>r w</b> 15 16 3 1 18 1 2 20	caa cga s d 13 0 17 8	icga icga icga ga ga iccaa jru n 9 5 18 6	tta aag ata gta acg ggg aata rwd C 0 38 0 0	t CC gCC aCC aCC gCC aCC gCC d t CC gCC d t CC gCC d gCC d aCC gCC g	5799 574 574 5770 5779 5779 5779 5779 5779 5779 5779	gtt tcc agg ccg gca ggat ggac 122 14 14	cat tac cct cct cct cct cct cct cct nny 9 7 6	ACC ACC ACC ACC ACC ACC ACC ACC	Grand	actattattattattattatta		ya ca tcg at ctc tcg agc ttg agc t 1 15 1 21	tgc gt c <b>A</b> 38 0 0 0	<b>C</b> 0 38 0 0	<b>G</b> 0 38 0	31. 24. 23. 19. 14. 13. 12. 11. 9.1 <b>t</b> 0 6 4 28	$3 \pm 9 \pm 2 \pm 9 \pm 9 \pm 1 \pm 6 \pm 2 \pm 0$ a = 27 = 9 = 27 = 27 a = 27 = 27 = 27 = 27 a = 27 = 27 = 27 = 27 = 27 a = 27 = 27 = 27 = 27 = 27 = 27 = 27 = 2	1.3 1.5 1.2 0.9 1.2 1.5 1.4 0.7 .8 <b>a</b> 34 0 0 4	<b>Y</b> 0 26 1 11	<b>Y</b> 2 16 1 19

B		
TaNAC69-sel	ected oligonucleotides – DNA-binding site II	<b>RBA</b> (%)
SO39	gga <b>ggtgttt</b> aatgttta <b>cACGtctct</b> agt	$93.3 \pm 3.4$
SO40	c <b>ggtgttt</b> tgctattc <b>cACG</b> c <b>ctct</b> ttcat	$54.9 \pm 2.2$
SO41	cat <b>g</b> c <b>tagtt</b> cacctggg <b>cACGt</b> t <b>tct</b> gga	$39.1 \pm 1.8$
SO42	ag <b>a</b> c <b>tggt</b> caaatttag <b>cACGcaact</b> actg	$33.4 \pm 1.5$
SO43	ag <b>agactt</b> aggetttta <b>tACGteact</b> ttag	$31.0 \pm 1.7$
SO44	gaaag <b>agtcg</b> g <b>t</b> tccccatt <b>cACGtaacc</b>	$29.1 \pm 1.6$
SO45	cga <b>ag</b> a <b>ct</b> c <b>t</b> atgctccg <b>tACGtcatt</b> gca	$27.3 \pm 1.2$
SO46	cctt <b>a</b> a <b>t</b> ta <b>t</b> cataggtaa <b>tACGtaatc</b> ct	$17.5 \pm 1.1$
SO47	tc <b>g</b> a <b>tagt</b> ggtgctgtt <b>tACG</b> g <b>cact</b> gaac	$10.7 \pm 1.2$
Consensus	rgtskttnnnnnnnnyACGtmwyy	

Supplementary Fig. 3. Sequence alignment of TaNAC69-selected oligonucleotides and their binding activity. (A) TaNAC69 binding site I. Oligonucleotides were cloned from the fourth and fifth round of binding site selection. The consensus binding sequence is highlighted in bold. The lower-case letters of the consensus sequence indicate the bases which are present in > 50% of TaNAC69-selected oligonucleotides. Relative binding activity (RBA) is measured as fluorescent units and is relative to the binding activity of SO1. Values are means  $\pm$  s.d. of triplicate assays. (B) TaNAC69 binding site II. RBA is relative to the binding activity of SO1. Values are means  $\pm$  s.d. of triplicate assays. d = agt, m = ca, s = gc, k = gt, r = ag, w = at, y = ct. Note: initially, 15 TaNAC69-selected oligonucleotides, cloned from the fifth round of

### Publisher: CSIRO; Journal: FP:Functional Plant Biology Article Type: accessory-material; Volume: 33; Issue: 1; Article ID: FP05161\_AC DOI: 10.1071/FP05161\_AC; TOC Head:

binding site selection, were analysed for TaNAC69-binding activity. Fourteen of them exhibited binding activity and were sequenced. Sequence alignment of these oligonucleotides revealed a binding sequence spanning 23 bp [these oligonucleotide sequences were presented for supporting the methodology paper (Xue, 2005)]. As TaNAC69 binds to a relatively long DNA sequence, many more selected oligonucleotides are required for the identification of consensus binding sequences. Thus, 15 more oligonucleotides cloned from the fifth round selection and 35 from the fourth round selection were analysed for binding activity. Thirty-three oligonucleotides of 50 with significant binding activity were sequenced. The sequence alignment of a total of 47 oligonucleotides revealed the two consensus binding sites above.

TN69L29	LPPGFRFHPTDEELIVHYLRRQAASMPSPVPIIAEVNIYKCNPWDL	PGKALFGEI	NEWYFF
NAP	LPPGFRFHPTDEELIVYYLRNQTMSKPCPVSIIPEVDIYKFDPWQL	PEKTEFGEI	NEWYFF
ANAC025	LPPGFRFHPTDEELVVHYLKKKADSVPLPVSIIAEIDLYKFDPWEL	PSKASFGE	HEWYFF
ANAC056	LPPGFRFHPTDEELVVHYLKRKAASAPLPVAIIAEVDLYKFDPWEL	PAKASFGE	QEWYFF
AtNAM	LPPGFRFHPTDEELVIHYLKRKADSVPLPVAIIADVDLYKFDPWEL	PAKASFGE	QEWYFF
	AB	-	
TN69L29	SPRDRKYPNGARPNRAAGSGYWKATGTDKAILSTPANESIGVKK	ALVFYRGKI	PPKGVK
NAP	SPRERKYPNGVRPNRAAVSGYWKATGTDKAIHSGSSNVGVKK	ALVFYKGRI	PPKGIK
ANAC025	SPRDRKYPNGVRPNRAATSGYWKATGTDKPIFTCNSHKVGVKK	ALVFYGGK	PPKGIK
ANAC056	SPRDRKYPNGARPNRAATSGYWKATGTDKPVLASDGNQKVGVKK	ALVFYSGK	PPKG <mark>V</mark> K
AtNAM	SPRDRKYPNGARPNRAATSGYWKATGTDKPVISTGGGGSKKVGVKK	ALVFYSGK	PPKG <mark>V</mark> K
·	C	D	
		%Ide	%Sim
TaNAC69-1	TDWIMHEYRLTAADNRTTKRRGSSMRLDDWVLCRIHKK	100	100
NAP	TDWIMHEYRLHDSRKASTKRSGS-MRLDEWVLCRIYKK	75	83
ANAC025	TDWIMHEYRLTDGNLSTAAKPPDLTTTRKNSLRLDDWVLCRIYKK	70	81
ANAC056	SDWIMHEYRLIENKPNNRPPGCDFGN-KKNSLRLDDWVLCRIYKK	71	82
AtNAM	SDWIMHEYRLTDNKPTHICDFGN-KKNSLRLDDWVLCRIYKK	71	84

Supplementary Fig. 4. Sequence alignment TaNAC69-1 NAC domain with *Arabidopsis* NAC proteins. Only NAC proteins with amino acid identity at 70% or higher are shown. In terms of sequence homology in five conserved subdomains, the homology level of these four *Arabidopsis* NAC proteins with TaNAC69 is very similar. Relatively lower percentages in the amino acid identity with TaNAC69 in ANAC025, ANAC056 and AtNAM are attributed to linker length. NAP (AJ222713, At1g69490), ANAC025 (At1g61110), ANAC056 (At3g15510) and AtNAM (AF123311, At1g52880). Ide, identity; Sim, similarity.

The sequences underlined are restriction sites for cloning into the DBP-CELD vector

Primer name	Sequence
NAC69S1	5'-GCAGCATTTTTATGCAGTAGCCA
NAC69S2	5'-ATTGATTCCGTTCCCACCTG
TaNAC69F	5'-CCATTTGAACTTGCTGTAGATCGA
TaNAC69R	5'-TTCTGAAATTTGTCCGTTCTTATCG
TaNAC69-1F	5'-TGCCTCCCGAAAACCCA
TaNAC69-1R	5'-TTGTTCACGTAGCCGTTGTTGT
TaNAC69-2F	5'-CAGCTCCCAGCGGATCAGT
TaNAC69-2R	5'-CTTTTCGATCCAAGCTCTTTGG
TaNAC69-3F	5'-AACAATGGCTACGTGAACATCGA
TaNAC69-3R	5'-AAACTGCCGCTGGACCTCTT
TaNAC69-4F	5'-CACTATAACAATAACAACGGGTACGTG
TaNAC69-4R	5'-TCTACATGCGCCTCGGGTA
TaCCFF	5'-GCTCTAACCCACTCGGCCTAA
TaCCFR	5'-TCTACACCCCAGTACACATATGACATAA
C12B07F	5'-GAACTGTCTGGATTGTCCCATCA
C12B07R	5'-ACAGTAGGCCCACACCAATGTAC
ANAC029S	5'-CCAG <u>GCTAGC</u> GAAGTAACTTCCCAATCTACCCT
ANAC029A	5'-CC <u>GGATCC</u> AAACTTAAACATCGCTTGACGATGATGGT

Supplementary Table 2. The final concentrations of components in the association reactions

Hepes 25 mM 25 mM   KCl 100 mM 50 mM   EDTA 0.16 mM 0.25 mM	Component	High stringency	Low stringency
KCl 100 mM 50 mM   EDTA 0.16 mM 0.25 mM   Mach 5 mM 45 mM	Hepes	25 mM	25 mM
EDTA 0.16 mM 0.25 mM	KCl	100 mM	50 mM
$M_{\alpha}$ (1) 5 m)(	EDTA	0.16 mM	0.25 mm
MgCl <sub>2</sub> J IIM 4.J IIM	$MgCl_2$	5 mM	4.5 mM
Bovine serum albumin $1 \text{ mg mL}^{-1}$ $0.9 \text{ mg mL}^{-1}$	Bovine serum albumin	$1 \text{ mg mL}^{-1}$	$0.9 \text{ mg mL}^{-1}$
DTT 1.1 mM 1.2 mM	DTT	1.1 mM	1.2 mM
Triton X-100 0.067% –	Triton X-100	0.067%	_
Nonidet P-40 – 0.02%	Nonidet P-40	_	0.02%
Glycerol – 9%	Glycerol	_	9%