



Supplementary

# The Expression of *CARK1* or *RCAR11* Driven by Synthetic Promoters Increases Drought Tolerance in *Arabidopsis thaliana*

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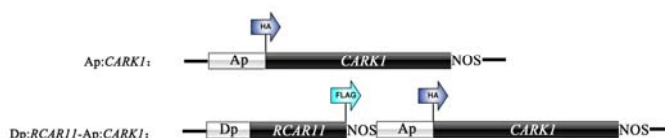


Figure S1. Construction of vectors for transgenic plants. Construction of vectors of Dp:*CARK1* and ANDp:*CARK1* transgenic plants were the same as Ap:*CARK1* transgenic plants.

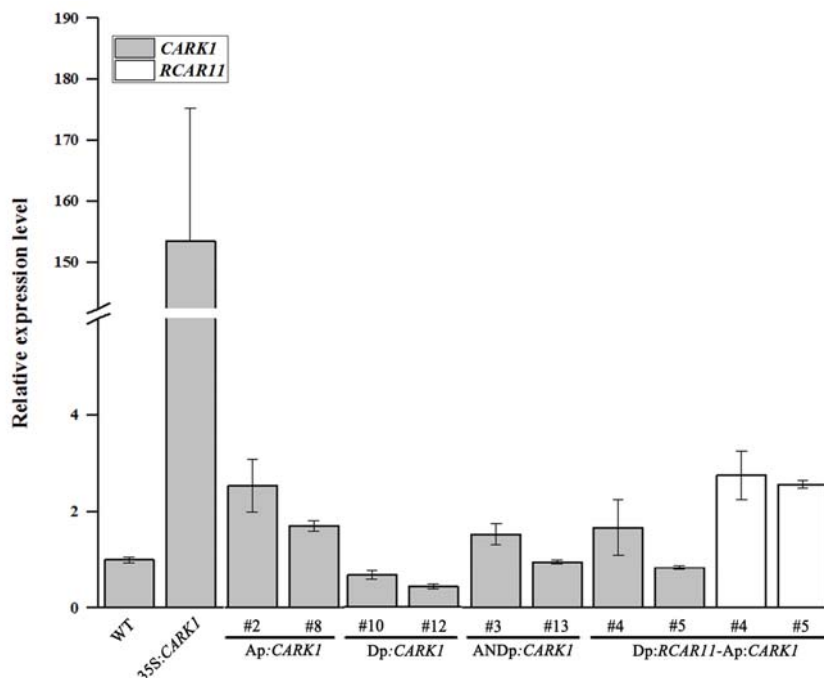


Figure S2. Relative expression levels of effector genes of transgenic plants under normal conditions were determined by qRT-PCR analysis.

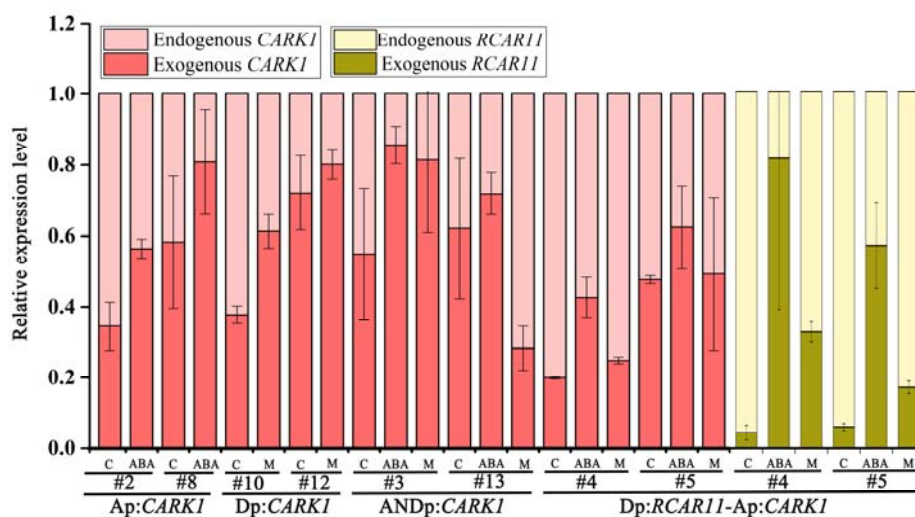


Figure S3. Expression levels of *CARK1* and *RCAR11* under the treatment of ABA or D-mannitol by qRT-PCR analysis. Twelve-day-old seedlings were incubated in MS liquid medium with or without 50  $\mu$ M ABA for 3 h and 200 mM D-mannitol for 2 h. The total *CARK1* and *RCAR11* were amplified by primers F and R, respectively. The exogenous *CARK1* was amplified by primers F(HA) and R. The exogenous *RCAR11* was amplified by primers F and R(FLAG). *ACTIN2/8* was used as an internal control. The expression level of total *CARK1* or *RCAR11* was 1.

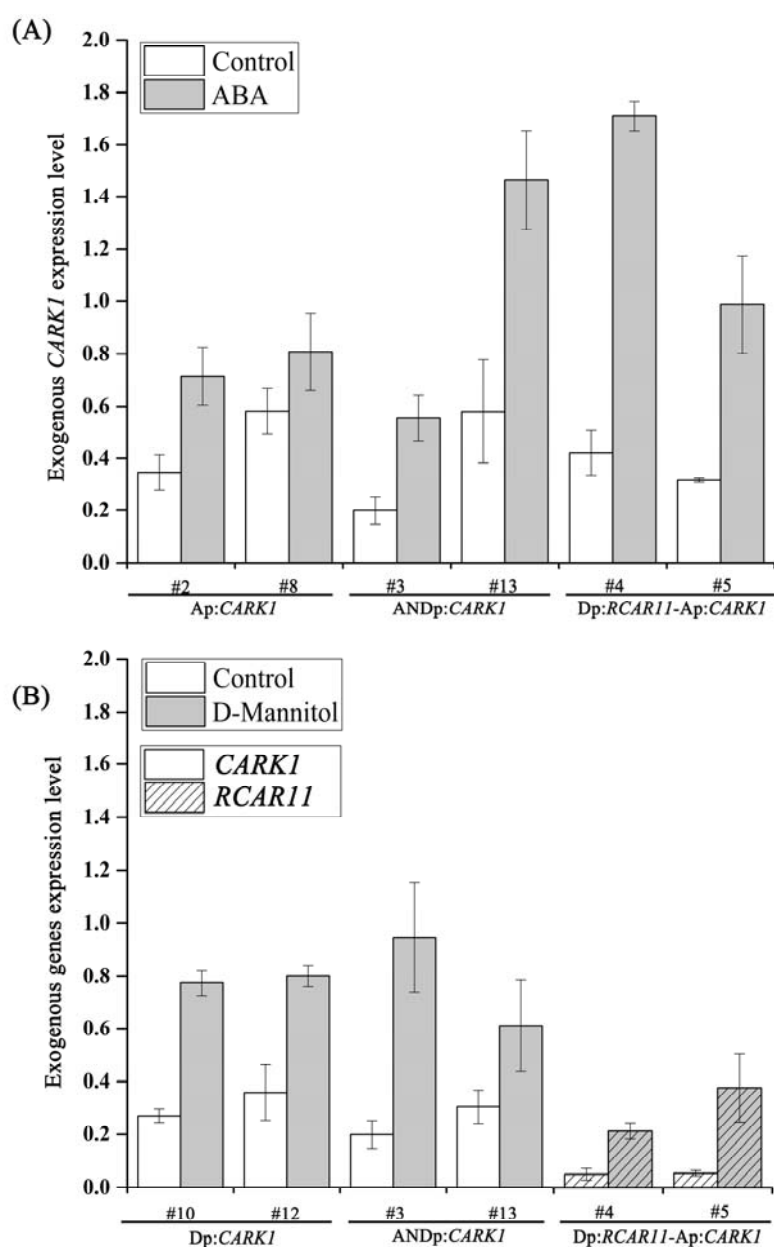


Figure S4. Expression levels of exogenous *CARK1* and *RCAR11* under the treatment of ABA or D-mannitol by qRT-PCR analysis. **(A)** ABA 12-day-old seedlings were incubated in MS liquid medium with or without 50  $\mu$ M ABA for 3 h. **(B)** D-Mannitol 12-day-old seedlings were incubated in MS liquid medium with or without 200 mM D-mannitol for 2 h. The exogenous *CARK1* was amplified by primers F(HA) and R(CARK1), and the exogenous *RCAR11* was amplified by primers F(RCAR11) and R(FLAG).

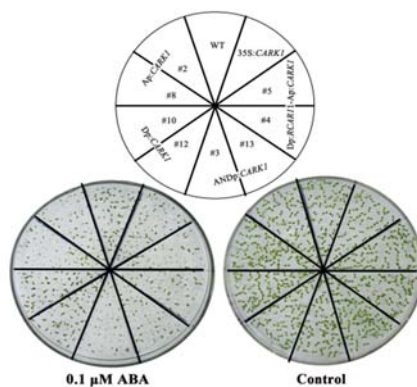


Figure S5. Cotyledon greening assay. Seeds were in MS medium supplemented with or without 0.1 μM ABA, and cotyledon greening rates were recorded after 5 days. The ratio of cotyledon greening was determined for over 70 seeds from three independent experiments.

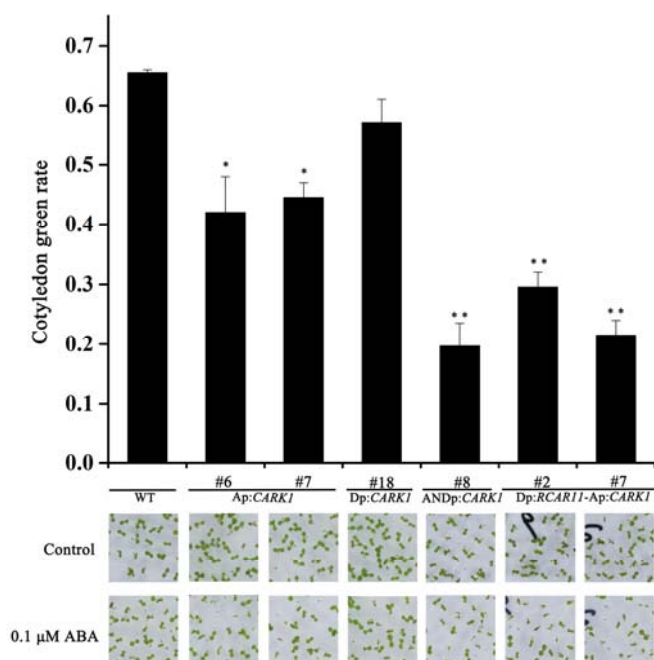


Figure S6. Cotyledon greening assay.

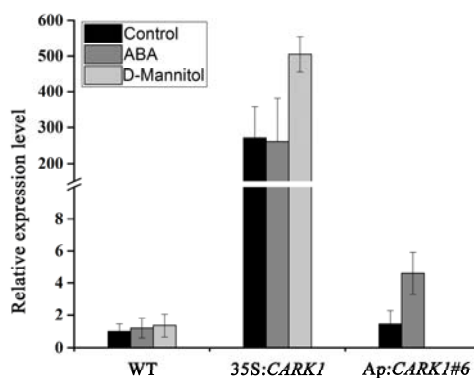


Figure S7. Relative expression levels of total *CARK1* by qRT-PCR analysis. Twelve-day-old seedlings were incubated in MS liquid medium with or without 50  $\mu$ M ABA for 3 h and 200 mM D-mannitol for 2 h.

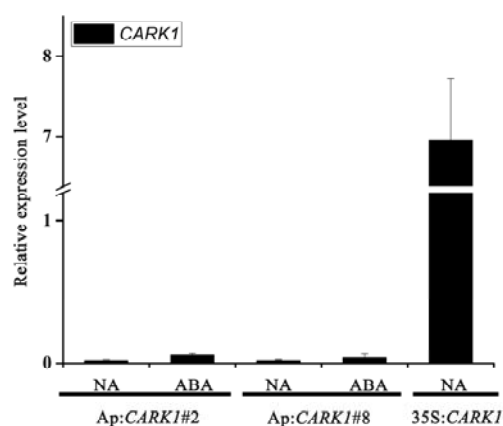


Figure S8. Relative expression levels of exogenous *CARK1* by qRT-PCR analysis. Twelve-day-old seedlings were incubated in MS liquid medium with or without 50  $\mu$ M ABA for 3 h. The primers were F(*CARK1*) and R(*NOS*).

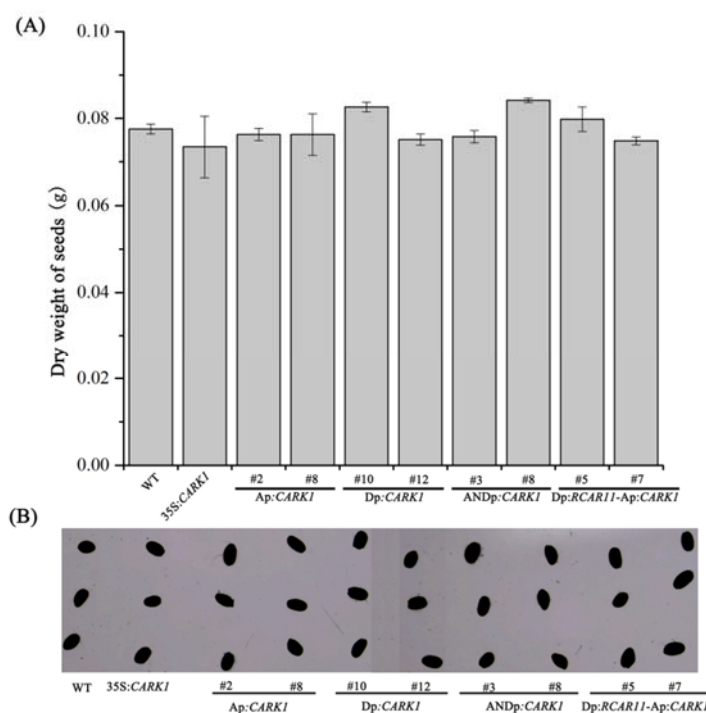


Figure S9. Phenotypic analysis of seeds. (A) Dry weight of seeds. Weighing 0.1 mL volume of dried seeds of all transgenic plants under normal growth conditions. (B) The size of seeds. The image was taken by the microscope.

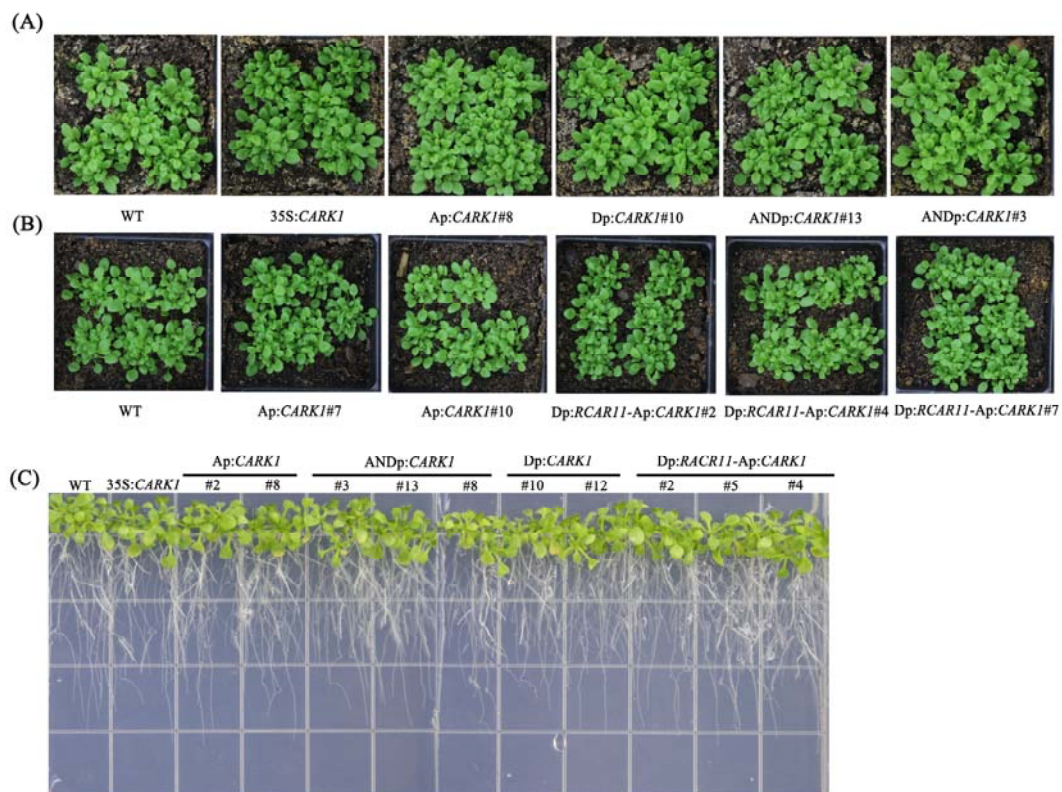


Figure S10. The growth of plants under normal conditions. (A) and (B) Two-week-old *Arabidopsis thaliana* plants in soil. (C) The growth of *Arabidopsis thaliana* plants in MS medium.

Table S1. Survival rate of drought tolerance assay.

	WT	35S:C ARK1	Ap:CARK1					Dp:CARK1				ANDp:CARK1			Dp:RCAR11-Ap:CARK1				
			#2	#6	#7	#8	#10	#10	#12	#14	#18	#3	#8	#13	#1	#4	#5	#7	#10
1	0	40%	-	-	-	-	-	0	51%	-	0	100%	100%	100%	-	-	-	-	-
2	39%	36%	29%	0	18%	100%	32%	100%	17%	-	0	67%	100%	100%	-	-	-	-	-
3	0	0	100%	5%	0	100%	0	100%	0	50%	100%	100%	96%	95%	95%	100%	7%	95%	70%
4	0	6%	0	20%	0	0	5%	2%	0	0	30%	85%	100%	100%	100%	100%	90%	100%	96%

Table S2. Primers for assays in this study.

Assays	Vectors/Genes	Primer sequences (5'-3')
Primers for transgenic plant construction	Dp:CARK1	F( <i>Hind</i> III):GCCTGTAAGCTTGCACACGACGTAAACGT
		R( <i>Xba</i> I):ATCTAGAGTCCCCCGTGTCTCTCCAAATG
	Ap:CARK1	F( <i>Hind</i> III):GCCTGTAAGCTTGCACACGTA GAGAGCAACT
		R( <i>Xba</i> I):ATCTAGAGTCCCCCGTGTCTCTCCAAATG
	ANDp:CARK1	F( <i>Hind</i> III):GCCTGTAAGCTTGCACACGACGTAAACGT
		R( <i>Xba</i> I):ATCTAGAGTCCCCCGTGTCTCTCCAAATG
	Dp:RCAR11-Ap:CARK1	F( <i>Hind</i> III):GACCATGATTACGCCAAGCTTGCCACACGACGTAAA
		R( <i>Hind</i> III):TGCTCTCTACGTGGCAAGCTTGATCTAGTAA CATAG
Primers for qRT-PCR analysis	Total CARK1	F:ACCTTCCAGTCATT CAGA
		R:ACCATAGTTATCCGTTATATCC
	Exogenous CARK1	F(HA):CCATACGACGTCCCAGACTACG
		R(CARK1):TCGCAATCTCGAAACCATTGATACCT
	Total RCAR11	F:ATCGTCATCAGTGGATTA
		R:TAATTCGTCAGCCTATGT
	Exogenous RCAR11	F(RCAR11):ATCGTCATCAGTGGATTA
		R(FLAG):CTTATCGTCGTCATCCTTGTA
	RD29A	F:TCAACACACACCAGCAGCAC
		R:ATCGGAAGACACGACAGGAA
	RD29B	F:ATCGGAAGACACGACAGGAA
		R:TCTCTTTTCGCTTCCCAGT
	ACTIN2/8	F:GGTAACATTGTGCTCAGTGGTGG
		R:AACGACCTTAATCTTCATGCTGC



Table S3. Primers for assays in this study.

Assays	Vectors/Genes	Primer sequences (5'-3')
Primers for synthetic promoter analysis	Dp:eGFP	F( <i>Hind</i> III):GCCTGTAAGCTTGCACACGACGTAAACGT
		R( <i>Xba</i> I):ATCTAGAGTCCCCCGTGTCTCTCCAAATG
	Ap:eGFP	F( <i>Hind</i> III):GCCTGTAAGCTTGCCACGTAGAGAGCAACT
		R( <i>Xba</i> I):ATCTAGAGTCCCCCGTGTCTCTCCAAATG
	ANDp:eGFP	F( <i>Hind</i> III):GCCTGTAAGCTTGCACACGACGTAAACGT
		R( <i>Xba</i> I):ATCTAGAGTCCCCCGTGTCTCTCCAAATG
	Dp:FLUC	F( <i>Hind</i> III):GTCGACGGTATCGATAAGCTTGCCACACGACGTAATA
		R( <i>Bam</i> HI):CGCTCTAGAAGTGGATCCGTCCTCCGTGTTCTC
	Ap:FLUC	F( <i>Hind</i> III):GTCGACGGTATCGATAAGCTTGCCACGTAGAGAGCA
		R( <i>Bam</i> HI):CGCTCTAGAAGTGGATCCGTCCTCCGTGTTCTC
	ANDp:FLUC	F( <i>Hind</i> III):GTCGACGGTATCGATAAGCTTGCCACACGACGTAATA
		R( <i>Bam</i> HI):CGCTCTAGAAGTGGATCCGTCCTCCGTGTTCTC
	35S:DREB2A	F( <i>Xba</i> I):GAGAACACGGGGGACTCTAGAATGGCAGTTTATGATCAGAGTGGAGATA
		R( <i>Sac</i> I):CGATCGGGGAAATTCGAGCTCTTAGTTCTCAGATC

Table S4. Dry weight of seeds of all transgenic plants under normal growth conditions.

Lines	Weight (g)
WT	0.0775 ± 0.0011
35S:CARK1	0.0735 ± 0.0070
Ap:CARK1 #2	0.0763 ± 0.0014
Ap:CARK1 #8	0.0763 ± 0.0049
Dp:CARK1 #10	0.0826 ± 0.0011
Dp:CARK1 #12	0.0751 ± 0.0013
ANDp:CARK1 #3	0.0758 ± 0.0014
ANDp:CARK1 #8	0.0841±0.0005
Dp:RCAR11-Ap:CARK1 #5	0.0798±0.0028
Dp:RCAR11-Ap:CARK1 #7	0.0748 ± 0.0009