

Supplemental Material

Genome-wide discovery of miRNAs with differential expression patterns in responses to salinity in the two contrasting wheat cultivars

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Table S1. Description, expression and putative target genes of known and novel miRNAs in roots whose expression were significantly down-regulated or up-regulated in of both Suntop (ST), and Sunmate (SM) treated with 100 mM NaCl for 1 day.

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
MIR156	tae-miR156	UGACAGAAGAGAGUGAGCACACA	21	-1.28	-0.73	TraesCS2A01G350100.1	Nitrate transporter 1.2
						TraesCS2B01G368600.1	
						TraesCS2B01G368600.2	
						TraesCS2D01G348500.1	protein NRT1/ PTR FAMILY 4.6-like isoform X1
						TraesCS2D01G348500.2	
						TraesCS2D01G348500.3	
						TraesCS6A01G110100.2	squamosa promoter-binding-like
						TraesCS6B01G138400.1	protein 3
						TraesCS6D01G098500.1	
MIR159	tae-miR159a, tae-miR159b	UUUGGAUUGAAGGGAGCUCUG	21	-0.39	1.93	TraesCS6D01G145200.1	squamosa promoter-binding-like protein 4
						TraesCS3A01G131700.1	protein SPEAR2
						TraesCS3A01G131700.3	
						TraesCS3D01G147900.1	
						TraesCS3D01G147900.2	transcription factor GAMYB
						TraesCS3A01G336500.1	
						TraesCS3D01G329400.1	
						TraesCS3D01G329400.2	R2R3-MYB protein
						TraesCS3D01G329400.3	
MIR160	tae-miR160	UGCCUGGCUCCCUGUAUGCCA	21	-1.58	1.42	TraesCS3B01G367500.1	
						TraesCS3B01G367500.2	
						TraesCS2A01G380300.1	auxin response factor 8-like

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
MIR167	tae-miR167a	UGAAGCUGCCAGCAUGAUCUA	21	-0.82	1.62	TraesCS2B01G397300.1	protein FIZZY-RELATED 2-like
						TraesCS4B01G346200.1	
						TraesCS4B01G346200.2	
						TraesCS4D01G341200.1	
MIR171	tae-miR171a	UGAAGCUGCCAGCAUGAUCUGC	21	-2.46	1.46	TraesCS3B01G253400.1	hypothetical protein TRIUR3_14845
						TraesCS3D01G275100.2	
						TraesCS1A01G209400.1	
						TraesCS1B01G223400.1	
	tae-miR171b	UUGAGCCGUGCCAAUAUCACG	21	-1.03	-0.89	TraesCS1D01G212800.1	scarecrow-like protein 6
						TraesCS6A01G247200.1	
						TraesCS6B01G277400.1	
						TraesCS6D01G229400.1	
	tae-miR319	UUGGACUGAAGGGAGCUCCCU	21	-0.21	-2.97	TraesCS2A01G352600.1	6,7-dimethyl-8-ribityllumazine synthase, chloroplastic
						TraesCS2B01G371700.1	
						TraesCS2D01G351600.1	
						TraesCS3D01G146900.1	
MIR395	tae-miR395a	GUGAAGUGUUUGGGGGAACUC	21	-0.45	3.49	TraesCS3A01G140100.1	transcription factor PCF5-like
						TraesCS7A01G176600.1	
						TraesCS7B01G081600.2	
						TraesCS7D01G177900.1	
	tae-miR395b	UGAAGUGUUUGGGGGAACUC	20	-1.29	-0.16	TraesCS5B01G387300.1	ATP sulfurylase 4, chloroplastic-like
						TraesCS5D01G392300.1	
						TraesCS5A01G382900.1	ATP sulfurylase

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
MIR397	tae-miR397-5p	UCACCGGCGCUGCACACAAUG	21	1.76	-0.69	TraesCS7A01G047900.1	probable protein phosphatase 2C 37
MIR398	tae-miR398	UGUGUUCUCAGGUCGCCCCCG	21	-1.8	-1.2		
undef	tae-miR1117	UAGUACCGGUUCGUGGCACGAACC	24	-0.32	-1.47		
undef	tae-miR1125	AACCAACGAGACCAACUGCGGCGG	24	0.28	-2.41		
MIR818	tae-miR1130b-3p	UCUUAUAUUAUGGGACGGAGG	21	-0.22	1.09	TraesCS2D01G248200.6	probable inactive leucine rich repeat receptor-like protein kinase At3g03770 isoform X2
	tae-miR1137a	UAGUACAAAGUUGAGUCAUC	20	0.25	-1.53	TraesCS6A01G107500.1 TraesCS6B01G136100.1 TraesCS6B01G136200.1 TraesCS2A01G366400.1 TraesCS2A01G366400.2	unnamed protein product hypothetical protein OsI_25001 hypothetical protein F775_04208
Undef	tae-miR5048-5p	UUUGCAGGUUUUAGGUCUAAGU	22	-0.72	1.24	TraesCS3A01G521100.1 TraesCS3A01G521100.2 TraesCS4B01G313900.2 TraesCS4B01G313900.3 TraesCS1B01G119300.3 TraesCS7D01G487800.1	uncharacterized protein LOC109783670 starch synthase III GDSL esterase/lipase
MIR5062	tae-miR5062-5p	UGAACCUUAGGGAACAGCCGCAU	23	-0.77	1.34		
MIR5067	tae-miR5175-5p	UUCCAAUUAUCUCGUCGUGGU	21	-0.32	1.4	TraesCS2A01G485700.2	receptor-like serine/ threonine - protein kinase SD1-8
MIR5200	tae-miR5200	UGUAGAUACUCCCUAAGGCUU	21	-0.09	1.2	TraesCS5D01G072900.1	hypothetical protein F775_12042
MIR7757	tae-miR7757-5p	AUAAAACCUUCAGCUAUCCAUC	22	-1.1	1.78	TraesCS2A01G023400.1 TraesCS2B01G033500.1	putative disease resistance protein At1g50180

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
MIR9652	tae-miR9652-3p	AAGCUUAAUGAGAACAUUGUG	20	1.24	0	TraesCS2D01G024500.1	protein argonaute MEL1
						TraesCS2D01G024500.2	
	tae-miR9652-5p	CCUGUUUGUCAUUAAGUUUCUU	22	-0.54	1.41	TraesCS3B01G287600.2	
						TraesCS5B01G451400.1	
Undef	tae-miR9653b	UGGCCAAGGUCUCUUGAGGCU	21	-1.07	1.8	TraesCS5B01G452500.1	probable protein phosphatase 2C 48
						TraesCS1A01G228200.1	
	tae-miR9654b-3p	UCCGAAAGGCUUGAAGCGAAU	22	-0.9	2.18	TraesCS1A01G228200.2	
Undef	tae-miR9656-3p	CUUCGAGACUCUGAACAGCGG	21	-0.17	1.72		
MIR9657	tae-miR9657a-3p	UGUGCUUCCUCGUCGAACGGU	21	-0.31	1.27	TraesCS3B01G280400.1	WD-40 repeat-containing protein
						TraesCS3D01G251300.1	MSI4 isoforms
						TraesCS3A01G250900.2	
						TraesCS7B01G144300.1	MYB-related protein
						TraesCS7B01G006500.1	putative serpin-Z5
						TraesCS7D01G103400.1	
	tae-miR9657c-3p, tae-miR9657b-3p	CGUGCUUCCUCGUCGAACGGU	21	-0.38	1.16	TraesCS3A01G250900.3	FVE [Triticum aestivum]
						TraesCS3A01G250900.1	
						TraesCS3B01G280400.1	WD-40 repeat-containing protein
						TraesCS3D01G251300.1	MSI4 isoforms
						TraesCS3A01G250900.2	
						TraesCS7B01G006500.1	putative serpin-Z5
						TraesCS7D01G103400.1	
						TraesCS7B01G144300.1	MYB-related protein

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
						TraesCS3A01G250900.1	FVE [Triticum aestivum]
						TraesCS3A01G250900.3	
undef	tae-miR9658-3p	AUCGUUCUGGGUGAAUAGGCC	21	-0.25	-2.03		
MIR9662	tae-miR9662a-3p	UUGAACAUCCCAGAGCCACCG	21	1.12	1.19	TraesCS4B01G290700.1	transcription termination factor MTERF9, chloroplastic-like
						TraesCS5D01G030400.1	transcription termination factor MTEF18, mitochondrial-like
						TraesCS6A01G015200.1	transcription termination factor
						TraesCS6D01G036200.1	MTERF15, mitochondrial-like
						TraesCS6B01G025600.1	transcription termination factor MTERF4, chloroplastic-like
						TraesCS6B01G059500.1	transcription termination factor
						TraesCS6B01G059600.1	MTERF8, chloroplastic-like
						TraesCS7B01G292800.1	GDSL esterase/lipase At3g09930-like
	tae-miR9662b-3p	UGAACAUCCCAGAGCCACCGG	21	-0.09	1.45	TraesCS6B01G021800.1	Uncharacterized protein
						TraesCS6B01G043100.1	LOC109765037
						TraesCS6D01G035100.1	cytosolic sulfotransferase 5-like
undef	tae-miR9664-3p	UUGCAGUCCUCGAUGUCGUAG	21	-0.64	1.04	TraesCS3A01G247700.1	cyclin dependent protein kinase
						TraesCS7D01G096000.1	disease resistance protein
						TraesCS5D01G405600.1	
						TraesCS2D01G541000.1	
						TraesCS2D01G025700.1	
						TraesCS2D01G025700.2	

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
						TraesCS2D01G025700.3	
MIR9666	tae-miR9666a-3p	CGGUAGGGCUGUAUGAUGGCGA	22	-1.71	-0.69	TraesCS5B01G438300.1	BTB/POZ and MATH domain-containing protein 3
	tae-miR9666b-3p	CGGUUGGGCUGUAUGAUGGCGA		-2.18	-1.13		
	tae-miR9666c-5p,	GCCAUCAUACGUCCAACCGUG	21	-2.43	-0.69	TraesCS2B01G562600.1	exocyst complex component
	tae-miR9666b-5p						EXO70A1-like
undef	tae-miR9668-5p	CCAAUGACAAGUAUUUUCGGA	21	-0.28	1.75	TraesCS6B01G070200.1	disease resistance protein RGA2-like
undef	tae-miR9669-5p	UACUGUGGGCACUUAUUUGAC	21	-0.72	2.13		
MIR9672	tae-miR9672a-3p	CCACGACUGUCAUUAAGCAUC	21	0.65	-1.02		
	tae-miR9672b	UACCACGACUGUCAUUAAGCA	21	-1.12	-0.57	TraesCS7D01G383200.1	replication factor C subunit 1
MIR9674	tae-miR9674b-5p	AUAGCAUCAUCCAUCCUACCC	21	-0.08	1.48	TraesCS2A01G531000.1	protein Rf1, mitochondrial-like
						TraesCS6A01G014800.1	
						TraesCS6B01G021600.1	
						TraesCS6D01G017700.1	
undef	tae-miR9675-3p	UUUAUGAUCACUCUCGUUUUG	21	-0.21	2.48	TraesCS3A01G074300.1	premnaspirodiene oxygenase like
MIR9772	tae-miR9772	UGAGAUGAGAUUACCCCAUAC	21	-0.58	1.7	TraesCS6A01G063700.1	F-box protein At5g03970 like
						TraesCS6B01G085300.1	
						TraesCS6B01G085300.2	
						TraesCS6D01G061100.1	
						TraesCS7D01G005400.1	F-box/kelch-repeat protein
						TraesCS6A01G058900.1	At3g61590-like
undef	tae-miR9773	UUUGUUUUUAUGUUAUUUUGUGA	23	-0.39	2.63		

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
Undef	tae-miR9774	CAAGAUAUUGGGUAAUUCUGUC	22	-1.09	-2.13		
Undef	tae-miR9775	UGUGCGCAAUAAGAUUUUGCUA	22	0.46	1.97	TraesCS7D01G005400.1	F-box/kelch-repeat protein SKIP11
Undef	tae-miR9778	UGCAUCAUCUCGAACUCGUCG	21	-0.62	-2	TraesCS1A01G021700.1	Disease resistance protein RPM1
						TraesCS1A01G021800.1	
						TraesCS1A01G021800.2	
						TraesCS1B01G025500.1	
						TraesCS1B01G027000.1	
						TraesCS1B01G027500.1	
						TraesCS1B01G027500.2	
	novel_mir3	AUUGUAGUCUGGAGAGGCGUCCUCA GCGAC	30	1.26	1.54		
	novel_mir8	UCCCGGCCCCGAACCUGUCGGCU	23	-0.37	1.17	TraesCS6A01G114800.1	premnaspirodien oxygenase-like
						TraesCS6B01G142400.1	
	novel_mir9	UUUGAAGACUAGUUUAUUAUUAU	22	-0.36	-2		
	novel_mir11	ACGCAUCAUUCAAAUUUCUGCCCUA UCA	28	-0.23	-1.42		
	novel_mir13	CCAUGGCCAAGGUCUCUUGAGGC	23	-2	-1.34		
	novel_mir14	AGAGAUCCCGCGCGCUACUCCGUCGA	26	-1.53	-0.49		
	novel_mir16	CUUGAAGACUUUGGCCACGUCCAU	24	-1.69	-0.68		
	novel_mir17	ACCUUUGAGACUUUGGCCAUGGCCA	25	-1.83	-0.94		
	novel_mir18	UUGAAGACUUUGGCCAUGUCCAUGG UG	27	-1.34	-0.99		
	novel_mir19	UCCACAGUGCAAAUUUAAAUUUUA UUAGA	30	-0.45	-1.01		

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
	novel_mir23	AGCACCGUUGGCAUCAUCGUCGGCG UUGUC	30	-0.57	-1.16		
	novel_mir24	AUGGGCUACGUGUGUUAAC	20	-2.34	-0.69		
	novel_mir25	UUGGGUUCGAGCCCCAAGGUGGG	23	1.69	0.98		
	novel_mir26	AGCACCGUUGGCAUCAUUGUCGGCGUU GUU		-0.47	-1.25		
	novel_mir27	CGCCUUGGGGCCCCGAACCCAAGACCA	26	-1.91	-2.22		
	novel_mir28	ACUGGUUGGAUCAUGCUUCUGUUUA UGA	28	-0.87	1.38	TraesCS1D01G090600.1	putative disease resistance RPP13-like protein 3
						TraesCS6B01G387200.1	Disease resistance protein RPM1
	novel_mir29	AGUUCGAGUCGGAGGCCACGGUGCU GGG	28	-0.33	-1.47		
	novel_mir30	UUGUUUGGCCAUCAAGAACAAGU AG	27	-0.36	-1.32		
	novel_mir31	AUCGAGAAAUUGGAGCUCGGUGCAG GCAUA	30	-0.62	-1.39		
	novel_mir32	UGUUUGGUGCGGACUCUGGACCU	23	-1.23	-0.96		
	novel_mir33	AAGAACAUCUAAGGGGCUGAGUUG	24	-1.56	-0.23		
	novel_mir34	AAAUGAUGUGUUGUGGAAAGCA	23	-1.91	1.84		
	novel_mir36	UUGGCGAUAGCGAAUGCAGUUCUC	24	-2.31	-0.16		
	novel_mir37	AAGUUGCGUAGUGGAUCGCUUGGGG CCUA	29	-0.78	-1.18		
	novel_mir39	GACGGAAGGAUUUGGCGGGACCG	23	-4.09	-3.07		

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
	novel_mir40	GUGCACCCGUGUUCACCUUUGUAUU U	26	-0.28	1.93		
	novel_mir42	UUGAACCUUGGGGAAAAGCCGCAUA	25	-0.89	1.42		
	novel_mir45	UGAUUAACGGCCAGGAUUUCCCUG	24	-0.13	1.12		
	novel_mir46	GCGGCGACGGGGGCGGCUU	19	3.21	-1.39		
	novel_mir47	GCGAGAGCGGGUCGCCGCGUGCCG	24	-1.35	-1.75	TraesCS4B01G303700.1	hypoth protein TRIUR306439
	novel_mir48	AUGGAUCUGACGGCUGUAGAGGA	23	-0.43	1.16		
	novel_mir49	GUGAUUAACGGCCAGGAUUUCCCUG	26	-2.18	1.69		
	novel_mir51	GAUGAUAUUAUAUUAUCACUCUGA GGGA	29	-1.21	-1.58		
	novel_mir52	CCCGUGGAACUUUCUUUUUGAAAA	24	-9.75	-8.5		
	novel_mir53	CCGGAUGUGUUGUCUUCCUCGACCA UGGUG	30	-0.01	-1.3		
	novel_mir54	UUUGGGGGAUCGAAGAUGAUUAAA	24	-2.91	-2.77		
	novel_mir55	GGUGGUUGCUGGCGCGCGCGU	21	-4.71	-4.94	TraesCS1A01G049600.2	ABC transporter G family member 5-like
						TraesCS1A01G276200.1	Glucanendo-1,3-beta-glucosidase 14
	novel_mir56	GGCCGCUGCACUCCUUGGCCGCUUGG GC	28	-2.1	-0.85		
	novel_mir57	UGGGCCUCACGGUCCAUAU	20	-0.17	-1.12	TraesCS2D01G386500.1	Formin-like protein 3
						TraesCS6B01G142400.1	
						TraesCS5B01G183100.1	
						TraesCS5D01G190100.1	UDP-glycosyltransferase 90A1-like

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
	novel_mir58	CGAAGCGGCGCUCGGCCCCCGG	21	-0.56	-1.81	TraesCS4B01G062900.1 TraesCS4D01G061900.1 TraesCS6A01G026800.1	polyol transporter 5-like 3-ketoacyl-CoA synthase 12-like
	novel_mir59	AUAUUUGCAGGUUUUAGGUCUAAGU GA	27	-0.27	1.54	TraesCS6B01G465600.1 TraesCS6B01G465600.2	CBL-interacting serine/threonine- protein kinase 7-like
	novel_mir60	GUGCUAGAUCACGCCCGCCGUUCAG UGUUG	30	-2.32	-0.65		
	novel_mir61	AACGGCCAGGAUUCGUCUGAU	21	-1.29	0.13	TraesCS4A01G480100.1	putative disease resistance RPP13- like protein 1
	novel_mir63	GUCGAUUAGUUUUAGCUUCAAGCCA UGCUA	30	-2.31	-2.24		
	novel_mir64	ACACUACGCGUGGAUGAGCA	20	-3.24	-0.91		
	novel_mir65	CAGAGCAGCGACAACUGCCCGCUUCCA		-1.6	-2.05		
	novel_mir66	UCUAGAGGAUGCAGUCUUUCCUCAA GG	27	-0.79	-2.2		
	novel_mir68	GACCGUACCCCAAACCGACA	20	0.34	-1.53		
	novel_mir69	CCCAGUAAACGGCGGCAGAGU	21	0	5.36	TraesCS7B01G138500.1	RNA pseudouridine synthase 3, mitochondrial
	novel_mir71	UGUGGCUUGUGGCUAAAACUAGUCU G	26	-1.64	-1.2		
	novel_mir75	CCAUCGUCGGCACGGCCGGUACCCAC GCG	29	1.19	-4.75	TraesCS5A01G555600.1 TraesCS7D01G119200.1 TraesCS5D01G023700.1	hypothetical protein MTR_0021s0160

Family name	miRNA name	Sequence	Length (nt)	Fold change ^[1]		Target gene	Annotation
				ST	SM		
	novel_mir78	GGCGAGAGCGGGUCGCCGCGUGCCG GCC	28	-2.68	-2.04		

Table S2. Evolutionary conservation of known miRNA families in other plant species
“+” sign indicates the existence of miRNA in species, and “-” sign shows no homology in plant species

miRNA family	<i>Solanum lycopersicum</i>	<i>Solanum tuberosum</i>	<i>Arabidopsis thaliana</i>	<i>Pinus taeda</i>	<i>Physcomitrella patens</i>	<i>Medicago truncatula</i>	<i>Glycine max</i>	<i>Gossypium hirsutum</i>	<i>Populus trichocarpa</i>	<i>Brassica napus</i>	<i>Vitis vinifera</i>	<i>Citrus sinensis</i>	<i>Malus domestica</i>	<i>Cucumis melo</i>	<i>Zea mays</i>	<i>Oryza sativa</i>	<i>Sorghum bicolor</i>	<i>Triticum aestivum</i>	<i>Aegilops tauschii</i>	<i>Triticum turgidum</i>	<i>Ricinus communis</i>	<i>Hordeum vulgare</i>	<i>Saccharum officinarum</i>	<i>Vigna unguiculata</i>	<i>Digitalis purpurea</i>	<i>Linum usitatissimum</i>	<i>Manihot esculenta</i>	<i>Prunus persica</i>	<i>Carica papaya</i>	<i>Cynara cardunculus</i>	<i>Theobroma cacao</i>	<i>Brachypodium distachyon</i>	<i>Salvia sclarea</i>	<i>Selaginella moellendorffii</i>	<i>Aquilegia caerulea</i>	<i>Festuca arundinacea</i>	<i>Hevea brasiliensis</i>	Total	
MIR156	+	+	—	—	—	—	+	—	—	+	—	+	+	+	—	—	—	+	+	—	+	—	—	—	—	+	+	+	+	—	—	+	+	—	—	—	—	17	
MIR159	+	—	+	+	—	+	+	—	+	+	+	+	+	+	—	+	+	+	—	—	+	+	—	—	—	+	+	+	+	—	—	+	—	+	+	+	+	27	
MIR160	+	+	—	—	+	+	+	—	+	+	+	—	+	+	+	+	+	+	+	—	+	—	+	+	—	+	+	+	+	+	+	+	—	+	+	+	—	28	
MIR164	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	+	—	—	+	+	+	+	+	+	+	+	—	—	+	—	28	
MIR167	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—	—	+	+	+	+	+	+	—	+	+	—	—	+	—	26	
MIR169	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—	+	—	+	+	+	+	+	+	+	+	+	—	+	+	—	30
MIR171	+	+	—	—	—	+	+	—	+	—	+	+	+	+	+	+	+	+	+	—	+	—	—	—	+	+	+	+	+	+	—	+	+	—	—	+	+	—	24
MIR395	—	+	—	—	—	—	—	—	—	—	—	+	+	+	—	+	+	+	+	—	—	—	—	—	—	—	+	+	+	+	—	—	+	—	—	—	—	12	
MIR397	+	+	+	—	—	+	+	—	+	+	+	+	—	+	+	+	+	+	—	—	+	—	—	—	+	+	+	+	+	—	—	—	+	+	—	—	—	—	21
MIR398	+	+	+	+	—	+	+	+	+	—	+	+	+	+	+	+	+	+	+	—	+	—	—	—	—	+	+	+	+	+	+	+	+	+	—	+	—	+	28
MIR399	+	+	+	—	—	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—	—	—	+	+	+	+	+	—	+	+	+	+	—	—	+	—	27
MIR408	+	+	+	+	+	+	+	—	+	—	+	+	+	+	+	+	+	+	+	—	+	—	+	+	+	+	+	—	+	+	+	+	—	+	+	+	—	+	30
MIR444	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	5	
MIR1119	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	2	
MIR1120	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
MIR1122	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	+	—	3	
MIR1139	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	2	
MIR5050	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2
MIR5062	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	—	3	
MIR5067	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	+	—	—	—	—	1	

Table S3. Description, expression and putative target genes of known miRNAs in roots whose expression were unchanged in Suntop (ST) but up-regulated in Sunmate (SM), or down-regulated in ST but up-regulated or unchanged in SM treated with 100 mM NaCl for 1 day (100 mM NaCl vs control).

Family name	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
				ST	SM		
MIR156	Tae-miR156	UGACAGAAGAGAGUGAGCACA	21	-1.28	-0.73	TraesCS2A01G350100.1	Nitrate transporter 1.2
						TraesCS2B01G368600.1	
						TraesCS2B01G368600.2	
						TraesCS2D01G348500.1	protein NRT1/ PTR FAMILY 4.6-like isoform X1
						TraesCS2D01G348500.2	
						TraesCS2D01G348500.3	
						TraesCS6A01G110100.2	squamosa promoter-binding-like protein 3
						TraesCS6B01G138400.1	
						TraesCS6D01G098500.1	
MIR159	tae-miR159a, tae-miR159b	UUUGGAUUGAAGGGAGCUCUG	21	-0.39	1.92	TraesCS6D01G145200.1	squamosa promoter-binding-like protein 4
						TraesCS6D01G145200.1	
						TraesCS3A01G131700.1	protein SPEAR2
						TraesCS3A01G131700.3	
						TraesCS3D01G147900.1	
						TraesCS3D01G147900.2	transcription factor GAMYB
						TraesCS3A01G336500.1	
						TraesCS3D01G329400.1	
						TraesCS3D01G329400.2	

Family name	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
				ST	SM		
						TraesCS3D01G329400.3	
						TraesCS3B01G367500.1	R2R3-MYB protein
						TraesCS3B01G367500.2	
MIR160	tae-miR160	UGCCUGGCUCCCUGUAUGCCA	21	-1.42	1.58	TraesCS2A01G380300.1	auxin response factor 8-like
						TraesCS2B01G397300.1	
						TraesCS4B01G346200.1	protein FIZZY-RELATED 2-like
						TraesCS4B01G346200.2	
						TraesCS4D01G341200.1	
MIR171	tae-miR171a	UGAUUGAGCCGUGCCAAUAUC	21	-2.46	1.46	TraesCS1A01G209400.1	Scarecrow-like protein 6
						TraesCS1B01G223400.1	
						TraesCS1D01G212800.1	
	tae-miR171b	UUGAGCCGUGCCAAUAUCACG	21	-1.03	-0.89	TraesCS6A01G247200.1	Scarecrow protein 6
						TraesCS6B01G277400.1	
						TraesCS6D01G229400.1	
						TraesCS2A01G352600.1	6,7-dimethyl-8-ribyllumazine synthase, chloroplastic
						TraesCS2B01G371700.1	
						TraesCS2D01G351600.1	
MIR395	tae-miR395b	UGAAGUGUUUGGGGGAACUC	20	-1.29	-0.16	TraesCS5A01G382900.1	ATP sulfurylase
	tae-miR395a	GUGAAGUGUUUGGGGGAACUC	21	-0.45	3.49	TraesCS5B01G387300.1	ATP sulfurylase 4,
						TraesCS5D01G392300.1	chloroplastic-like

Family name	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation	
				ST	SM			
MIR818	tae-miR1130b-3p	UCUUAUAUUAUGGGACGGAGG	21	-0.22	1.09	TraesCS2D01G248200.6	Inactive leucine-rich repeat receptor-like kinase	protein
						TraesCS2A01G366400.1	hypothetical OsI_25001	protein
						TraesCS2A01G366400.2	hypothetical F775_04208	protein
MIR5048	tae-miR5048-5p	UUUGCAGGUUUUAGGUCUAAGU	22	-0.72	1.24	TraesCS3A01G521100.1	uncharacterized LOC109783670	protein
						TraesCS3A01G521100.2		
						TraesCS4B01G313900.2		
						TraesCS4B01G313900.3		
						TraesCS1B01G119300.3	starch synthase III	
						TraesCS7D01G487800.1	GDSL esterase/lipase	
MIR5062	tae-miR5062-5p	UGAACCUUAGGGAACAGCCGCAU	23	-0.77	1.34			
MIR5067	tae-miR5175-5p	UUCCAAUUACUCGUCGUGGU	21	-0.32	1.4	TraesCS2A01G485700.2	receptor-like serine/threonine-protein kinase SD1-8	
MIR5200	tae-miR5200	UGUAGAUACUCCCUAAGGCUU	21	-0.09	1.20	TraesCS5D01G072900.1	hypothetical F775_12042	protein
MIR7757	tae-miR7757-5p	AUAAAACCUUCAGCUAUCCAUC	22	-1.10	1.78	TraesCS2A01G023400.1	putative disease resistance protein At1g50180	
						TraesCS2B01G033500.1		
						TraesCS2D01G024500.1		
						TraesCS2D01G024500.2		

Family name	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation	
				ST	SM			
MIR9652	tae-miR9652-5p	CCUGUUUGUCAUUAAGUUUCUU	22	-0.54	1.41	TraesCS3B01G287600.2 TraesCS5B01G451400.1 TraesCS5B01G452500.1	protein MEL1	argonaute
MIR9653	tae-miR9653b	UGGCCAAGGUCUCUUGAGGCU	21	-1.07	1.80	TraesCS1A01G228200.1 TraesCS1A01G228200.2	probable phosphatase 2C	protein 48
MIR9654	tae-miR9654b-3p	UUCCGAAAGGCUUGAAGCGAAU	22	-0.9	2.18	-	-	
MIR9656	tae-miR9656-3p	CUUCGAGACUCUGAACAGCGG	21	-0.17	1.72	-	-	
MIR9657	tae-miR9657a-3p	UGUGCUUCCUCGUCGAACGGU	21	-0.31	1.27	TraesCS3B01G280400.1 TraesCS3D01G251300.1 TraesCS3A01G250900.2 TraesCS7B01G144300.1 TraesCS7B01G006500.1 TraesCS7D01G103400.1 TraesCS3A01G250900.3 TraesCS3A01G250900.1	WD-40 repeat-containing protein MSI4 isoforms	
	tae-miR9657c-3p, tae-miR9657b-3p	CGUGCUUCCUCGUCGAACGGU	21	-0.38	1.16	TraesCS3B01G280400.1 TraesCS3D01G251300.1 TraesCS3A01G250900.2 TraesCS7B01G006500.1 TraesCS7D01G103400.1	WD-40 repeat-containing protein MSI4 isoforms	putative serpin-Z5

Family name	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
				ST	SM		
	tae-miR9662b-3p	UGAACAUCCCAGAGCCACCGG	21	-0.09	1.45	TraesCS7B01G144300.1	MYB-related protein
						TraesCS3A01G250900.1	FVE [<i>Triticum aestivum</i>]
						TraesCS3A01G250900.3	
						TraesCS6B01G021800.1	uncharacterized protein LOC109765037
						TraesCS6B01G043100.1	
MIR9664	tae-miR9664-3p	UUGCAGUCCUCGAUGUCGUAG	21	-0.64	1.04	TraesCS6D01G035100.1	cytosolic sulfotransferase 5-like
						TraesCS3A01G247700.1	cyclin dependent protein kinase
						TraesCS7D01G096000.1	disease resistance protein
						TraesCS5D01G405600.1	
MIR9666	tae-miR9666a-3p	CGGUAGGGCUGUAUGAUGGCGA	22	-1.71	-0.69	TraesCS2D01G541000.1	
						TraesCS5B01G438300.1	BTB/POZ and MATH domain-containing protein 3
	tae-miR9666c-5p,	GCCAUCAUACGUCCAACCGUG	21	-2.43	-0.69	TraesCS2B01G562600.1	exocyst complex
	tae-miR9666b-5p						component EXO70A1-like
MIR9668	tae-miR9668-5p	CCAAUGACAAGUAUUUUCGGA	21	-0.28	1.75	TraesCS6B01G070200.1	disease resistance protein RGA2-like
MIR9669	tae-miR9669-5p	UACUGUGGGCACUUAUUUGAC	21	-0.72	2.13	-	-
MIR9672	tae-miR9672b	UACCACGACUGUCAUUAAGCA	21	-1.12	-0.57	TraesCS7D01G383200.1	replication factor C subunit 1

Family name	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation	
				ST	SM			
MIR9674	tae-miR9674b-5p	AUAGCAUCAUCCAUCCUACCC	21	-0.08	1.48			
						TraesCS2A01G531000.1	protein	Rf1,
						TraesCS6A01G014800.1	mitochondrial-like	
						TraesCS6B01G021600.1		
						TraesCS6D01G017700.1		
MIR9675	tae-miR9675-3p	UUUAUGAUCACUCUCGUUUUG	21	-0.21	2.48	TraesCS3A01G074300.1	premnaspirodiene oxygenase-like	
MIR9772	tae-miR9772	UGAGAUGAGAUUACCCCAUAC	21	-0.58	1.7	TraesCS6A01G063700.1	F-box protein	At5g03970-like
						TraesCS6B01G085300.1		
						TraesCS6B01G085300.2		
						TraesCS7D01G005400.1	F-box/kelch-repeat protein	At3g61590-like
						TraesCS6A01G058900.1		
MIR9773	tae-miR9773	UUUGUUUUUAUGUUAUUUUGUGAA	24	-0.39	2.63	-	-	
MIR9775	tae-miR9775	UGUGCGCAAUAAGAUUUUGCUA	22	0.46	1.97	TraesCS7D01G005400.1	F-box/kelch-repeat protein	SKIP11

^[1] Fold change (NaCl vs control) is $\log_2 N$, $\log_2 N \geq 1$ are up-regulated, between $0 < |\log_2 N| < 1$ are unchanged and $\log_2 N \leq -1$ are down-regulated, P -values 0.01 and Q -value ≤ 0.001 .

Table S4. Description, expression and putative target genes of known miRNAs in roots whose expression were unchanged in Suntop (ST) but down-regulated in Sunmate (SM), or up-regulated in ST but down-regulated or unchanged in SM treated with 100 mM NaCl for 1 day.

miRNA family	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation	
				ST	SM			
MIR319	tae-miR319	UUGGACUGAAGGGAGCUCCCU	21	-0.21	-2.97	TraesCS3D01G146900.1	transcription	factor
						TraesCS3A01G140100.1	PCF5-like	

miRNA family	miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
				ST	SM		
MIR397	tae-miR397-5p	UCACCGGCGCUGCACACAAUG	21	1.76	-0.69	TraesCS7A01G047900.1	probable protein phosphatase 2C 37
MIR1117	tae-miR1117	UAGUACCGGUUCGUGGCACGA	24	-0.32	-1.47		
MIR1125	tae-miR1125	ACC	24	0.28	-2.41		
	tae-miR1137a	AACCAACGAGACCAACUGCGG CGG	20	0.25	-1.53	TraesCS6A01G107500.1 TraesCS6B01G136100.1 TraesCS6B01G136200.1	unnamed protein product
MIR9652	tae-miR9652-3p	AAGCUUAAUGAGAACAUGUG	20	1.24	0		
MIR9658	tae-miR9658-3p	AUCGUUCUGGGUGAAUAGGCC	21	-0.25	-2.03		
MIR9672	tae-miR9672a-3p	CCACGACUGUCAUUAAGCAUC	21	0.65	-1.02		
MIR9778	tae-miR9778	UGCAUCAUCUGAACUCGUCG	21	-0.62	-2	TraesCS1A01G021700.1 TraesCS1A01G021800.1 TraesCS1A01G021800.2 TraesCS1B01G025500.1 TraesCS1B01G027000.1 TraesCS1B01G027500.1 TraesCS1B01G027500.2	Disease resistance protein RPM1

^[1] Fold change (NaCl vs control) is $\log_2 N$, $\log_2 N \geq 1$ are up-regulated, between $0 < |\log_2 N| < 1$ are unchanged and $\log_2 N \leq -1$ are down-regulated, P -values 0.01 and Q -value ≤ 0.001 .

Table S5. Description, expression and putative target genes of novel miRNAs in roots whose expression were unchanged in Suntop (ST) but up-regulated in Sunmate (SM), or down-regulated in ST but up-regulated (-) or unchanged in SM treated with 100 mM NaCl for 1 day

miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
			ST	SM		
novel_mir8	UCCCGGCCCGAACCUGUCGGCU		- 0.37	1.17	TraesCS6A01G114800.1 TraesCS6B01G142400.1	premnaspirodiene oxygenase-like
novel_mir14	AGAGAUCCCGCGCGCUACUCCGUCG A	26	- 1.53	-0.49		
novel_mir16	CUUGAAGACUUUGGCCACGUCCAU	24	- 1.69	-0.68		
novel_mir17	ACCUUUGAGACUUUGGCCAUGGCC A	25	- 1.83	-0.94		

miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
			ST	SM		
novel_mir18	UUGAAGACUUUGGCCAUGUCCAUGGUG	27	-1.34	-0.99		
novel_mir24	AUGGGCUACGUGUGUUAAC	20	-2.3	-0.69		
			2.34			
novel_mir28	ACUGGUUGGAUCAUGCUUCUGUUUAUGA	26	-0.87	1.38	TraesCS1D01G090600.1	putative disease resistance RPP13-like protein 3
					TraesCS6B01G387200.1	Disease resistance protein RPM1
novel_mir32	UGUUUGGUGCGGACUCUGGACCU	23	-1.23	-0.96		
novel_mir33	AAGAACAUCUAAGGGGCUGAGUUG	24	-1.56	-0.23		
novel_mir34	AAAAUGAUGUGUUGUGGAAAGCA	23	-1.91	1.87		
novel_mir36	UUGGCGAUAGCGAAUGCAGUUCUC	24	-2.31	-0.16		
novel_mir40	GUGCACCCGUGUUCACCUUUGUAUUU	26	-0.28	1.93		
novel_mir45	UGAUUAACGGCCAGGAUUUCCCUG	24	-0.13	1.12		
novel_mir48	AUGGAUCUGACGGCUGUAGAGGA	23	-0.43	1.16		
novel_mir49	GUGAUUAACGGCCAGGAUUUCCCU G	25	-2.18	1.67		
novel_mir56	GGCCGCUGCACUCCUUGGCCGCUUGGCG	28	-2.19	-0.85		
novel_mir59	AUAUUUGCAGGUUUUAGGUCUAAGUGA	27	-0.27	1.54	TraesCS6B01G465600.1	CBL-interacting serine/threonine-
					TraesCS6B01G465600.2	protein kinase 7-like
novel_mir60	GUGCUAGAUAUCCACGCCCGGUUCAGUGUUG	30	-2.32	-0.65		
novel_mir61	AACGGCCAGGAUUCGUCUGAU	21	-1.29	0.13	TraesCS4A01G480100.1	putative disease resistance RPP13-like protein 1

miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
			ST	SM		
novel_mir64	ACACUACGCGUGGAUGAGCA	20	-	-0.91		
			3.24			

^[1] Fold change (NaCl vs control) is $\log_2 N$, $\log_2 N \geq 1$ are up-regulated, between $0 < |\log_2 N| < 1$ are unchanged and $\log_2 N \leq -1$ are down-regulated, P -values 0.01 and Q -value ≤ 0.001 .

Table S6. Description, expression and putative target genes of novel miRNAs in roots whose expression were unchanged in Suntop (ST) but down-regulated (-) in Sunmate (SM), or up-regulated in ST but down-regulated or unchanged in SM treated with 100 mM NaCl for 1 day

miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
			ST	SM		
novel_mir9	UUUGAAGACUAGUUU AUUAU AU	22	-0.36	-2		
novel_mir11	ACGCAUCAUUCAA AUUCUGCCCUAUC A	28	-0.23	-1.42		
novel_mir19	UCCACAGUGCAA AUUUAAA AUUU AUUAGA	30	-0.45	-1.01		
novel_mir23	AGCACCGUUGGCAUCAUCGUCGGCGUUGUC	30	-0.57	-1.16		
novel_mir25	UUGGGUUCGAGCCCCAAGGUGGG	23	1.69	0.98		
novel_mir29	AGUUCGAGUCGGAGGCCACGGUGCUGGG	28	-0.33	-1.47		
novel_mir30	UUGUUUGGCCAUCAAGAACA AUAGUAG	27	-0.36	-1.32		
novel_mir31	AUCGAGAA AUUGGAGCUCGGUGCAGGCAUA	30	-0.62	-1.39		
novel_mir37	AAGUUGCGUAGUGGAUCGCUUGGGGCCUA	29	-0.78	-1.18		
novel_mir46	GCGGCGACGGGGGCGGCUU	19	3.21	-1.39		
novel_mir53	CCGGAUGUGUUGUCUUCUCGACCAUGGUG	30	-0.01	-1.3		
novel_mir57	UGGGCCUCACGGUCCAUA AU	20	-0.17	-1.12	TraesCS2D01G386500.1 TraesCS6B01G142400.1 TraesCS5B01G183100.1 TraesCS5D01G190100.1	Formin-like protein 3 UDP-glycosyltransferase 90A1-like
novel_mir58	CGAAGCGGCGCUCGGCCCCGG	21	-0.56	-1.81	TraesCS4B01G062900.1 TraesCS4D01G061900.1	polyol transporter 5-like
novel_mir66	UCUAGAGGAUGCAGUCU UCCUCAAGG	27	-0.79	-2.2		
novel_mir68	GACCGUACCCCAAACCGACA	20	0.34	-1.53		

miRNA name	Sequence	Length (nt)	Fold change [1]		Target gene	Annotation
			ST	SM		
novel_mir75	CCAUCGUCGGCACGGCCGGUACCCACGCG	29	1.19	-4.75	TraesCS5A01G555600.1 TraesCS7D01G119200.1 TraesCS5D01G023700.1	hypothetical MTR_0021s0160 protein

^[1] Fold change (NaCl vs control) is log₂N, log₂N ≥ 1 are up-regulated, between 0 < |log₂N| < 1 are unchanged and log₂N ≤ -1 are down-regulated, P-values 0.01 and Q-value ≤ 0.001.

Table S7. List of gene specific primers used in qRT PCR analysis

miRNA	Target gene	Primers	Amplicon size
tae-mir160	TraesCS2B01G397300.1	F-GGATCTCCACCTCAGCAACC R-CGTGATGTTGTCGTCTTCGC	154
tae-miR319	TraesCS3A01G140100.1	F-GAAGGACCGGCACAGTAAG R-TCCTTGGCGTTCTTGATGAG	159
tae-miR397-5p	TraesCS7A01G047900.1	F-GAACAGATGCACACCATCTTG R-ATGGGCCATCCCAATTCC	149
tae-mir9653b	TraesCS1A01G228200.1	F-GTCAAGGACTACGGCGTGAT R-AATCTGTCGCTTACCCCGTC	104
tae-miR9657a-3p	TraesCS7B01G144300.1	F-CGATCATTGTTTGCCGGAAG R-ACCCTTGTTGGTCCTCATAC	161
tae-miR9772	TraesCS6A01G058900.1	F-GGCTTTCTTGCGGACATTTG R-CCACCACCAATGCTTGTTTC	141
tae-miR9778	TraesCS1D01G021300.1	F-GTGTGGCAAAGCAGATGAAG R-ACACCGCAATCACCTAAG	168
Novel_mir8	TraesCS6A01G142400.1	F-CATCGACACAAGGGCTGGA R-TCTTGGTGGTGCCTGTTTCTT	75
	Actin	F-CACTGGAATGGTCAAGGCTG R-CTCCATGTCATCCCAGTTG	

Table S8. List of miRNA primers used in qRT PCR analysis

miRNA	Primer sequence	Length
Tae_miR156	UGACAGAAGAGAGUGAGCACA	21
Tae_miR171a	UGAUUGAGCCGUGCCAAUAUC	21
Tae_miR319	UUGGACUGAAGGGAGCUCCCU	21
Tae_miR395b	UGAAGUGUUUGGGGGAACUC	20
Tae_miR397-5p	UCACCGGCGCUGCACACAAUG	21
Novel_miR59	AUAUUUGCAGGUUUUAGGUCUAAGUGA	27
Novel_miR 75	CCAUCGUCGGCACGGCCGGUACCCACGCG	29

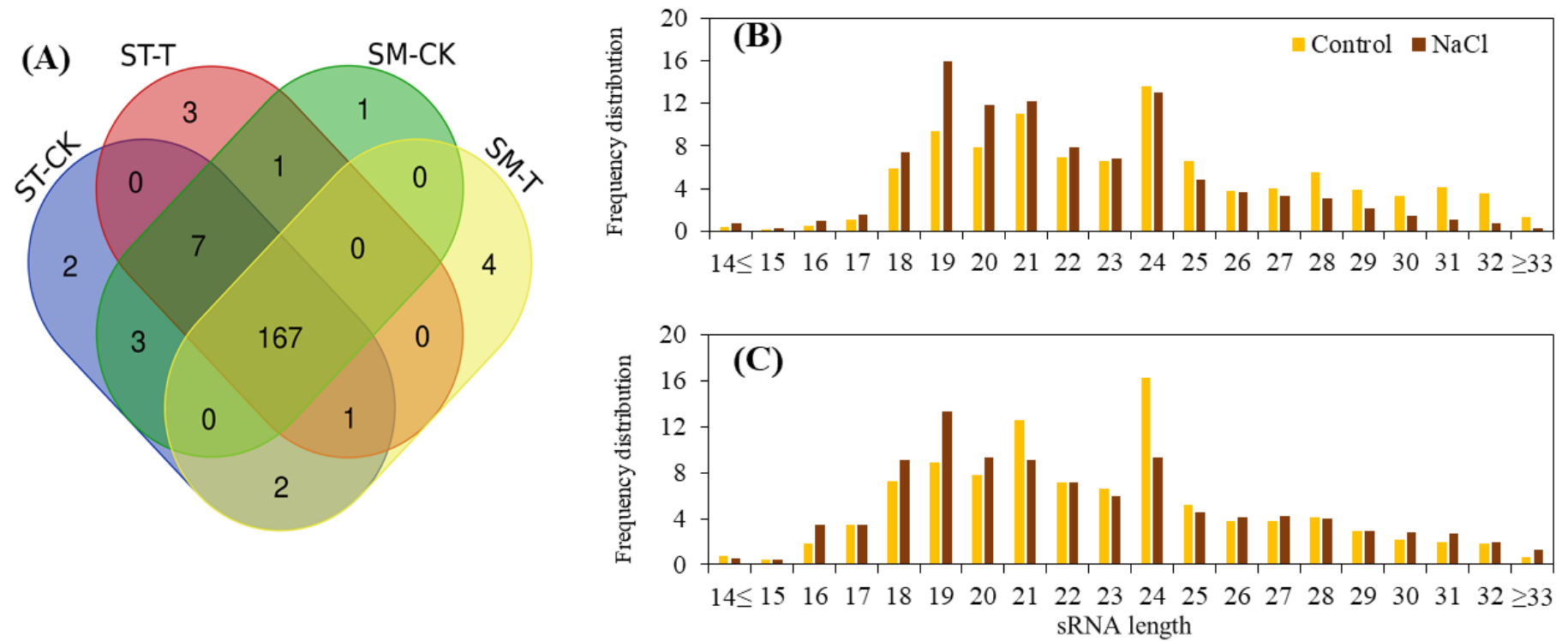


Figure S1. Number of miRNAs in two wheat cultivars under NaCl stress (T) and control conditions (CK) were counted in root (A). Length base distribution of total identified miRNAs in roots of two wheat cultivars (B) Suntop (ST), (C) Sunmate (SM).

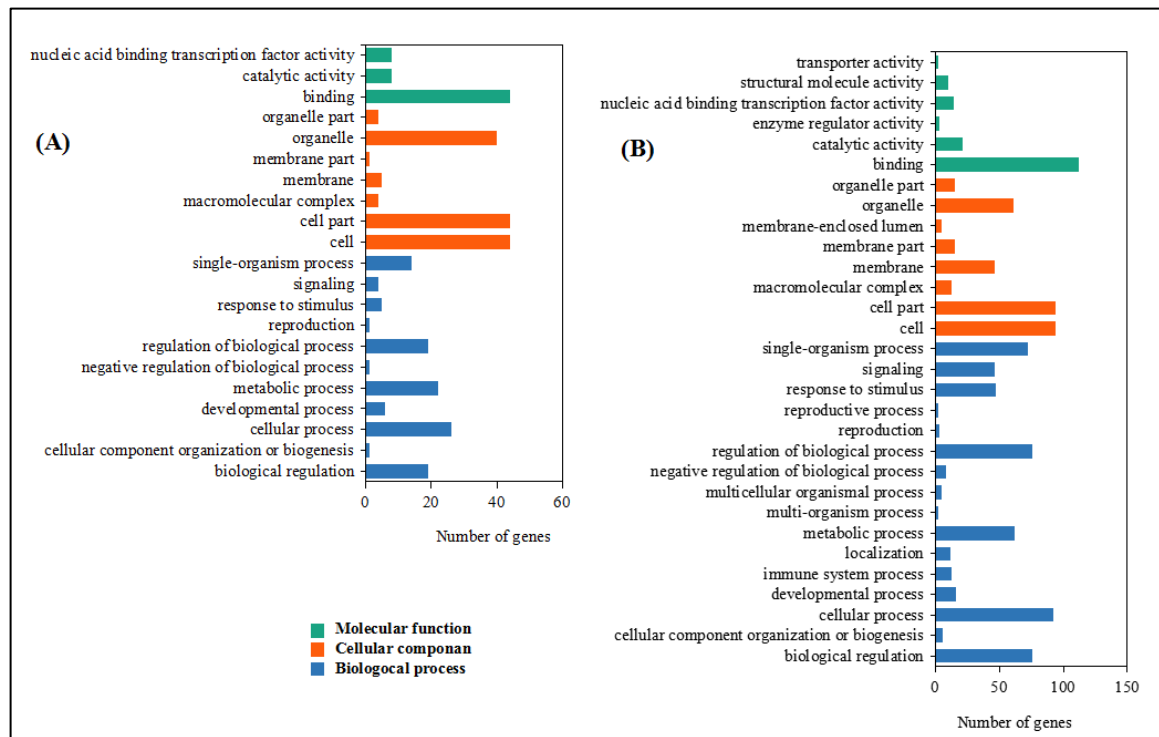


Figure S2. Go analysis of identified target genes in cultivar Suntop (A) and Sunmate (B). Target genes were classified into three categories: biological processes, cellular component, and molecular function. Values in the Y axis are the number of target genes in different functional categories