Improvement and Identification of Drought- & Salinity-Tolerance on Cotton

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Outline

1. Background
2. Germplasm identification
3. Mechanism of salinity tolerance
4. Salinity-tolerant genes isolated

Germplasm identification

Cotton germplasm (US *cv* China)

Species	US	China
G.hirsutum L.	3400	7522
G.barbadense L.	1600	565
G.herbaceum L.	190	17
G.arboreum L.	1700	378
G.hirsutum L.*landrances*	2200	350
others	250	41
total	9340	8873

Establishment of screen system on salt-/draught-stress

Stress identification and screening system

Cotton germplasm and salinity resistance in China

1. Germplasm: 8873.
2. Few of them are resistant to salinity, as well as to drought, chilling.
3. Distribution of salinity-resisted:

G.herbceum, 33.33%

G.barbadense, 3.86%

G.hirsutum, 0.09%

G.arboreum, none

Mechanism of salinity-tolerance

1. DNA methylation analysis under different types of salt

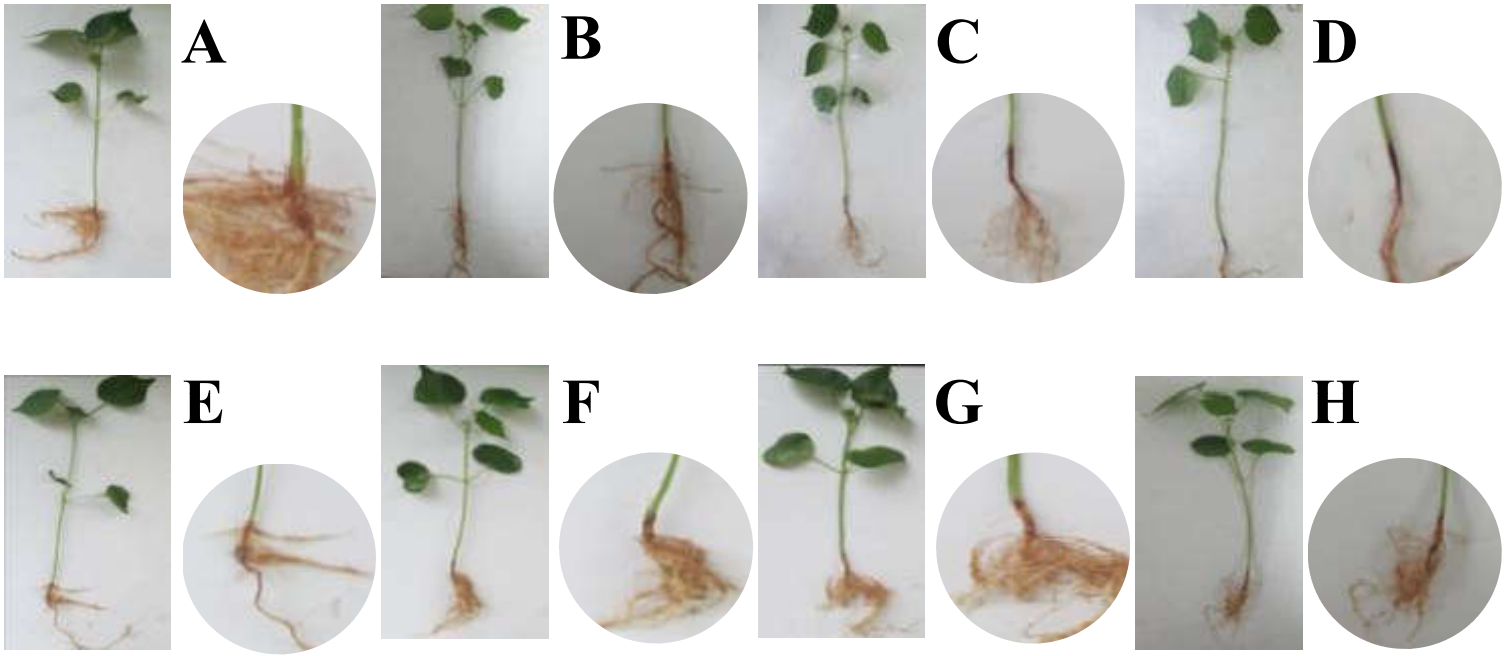


Fig. 2-1 Cotton seedlings under different salt stresses

A) Zhong07–dH_2O; **B)** Zhong07–NaCl; **C)** Zhong07–$NaHCO_3$; **D)** Zhong07–Na_2CO_3; **E)** ZhongS9612–dH_2O; **F)** ZhongS9612–NaCl; **G)** ZhongS9612–$NaHCO_3$; **H)** ZhongS9612–Na_2CO_3

2. DNA methylation analysis in different cotton accessions under salt stress

Table 1 Salt-tolerance level of different cotton accessions

Cotton accession	Germination ratio under salt treatment (%)	Germination ratio of control (%)	Relative germination ratio (RGR, %)	Salt tolerance level
Zhong 07	66.53	83.67	79.51[a]	Tolerant
CCRI 35	64.32	84.38	76.23[a]	Tolerant
CCRI 12	25.21	80.44	31.34[b]	Sensitive

Table 2 DNA methylation patterns of different cotton accessions detected by MSAP

Type	Enzyme digestion		Methylation pattern	Number or ratio of loci					
	H	M		CCRI 35		Zhong 07		CCRI 12	
				Salt stress	Control	Salt stress	Control	Salt stress	Control
I	0	1	C<u>C</u>GG GG<u>C</u>C	339	838	410	977	793	588
II	1	0	*C*CGG or *C*CGG GGCC···GGCC	658	792	679	496	375	222
III	0	0	*C*CGG or *C*CGG or *C*CGG or *C*CGG or C*C*GG GG*CC*···GG*C*C···GG*C*C GG*CC*···GG*CC*	1668	356	1191	447	311	838
IV	1	1	CCGG or C*C*GG GGCC···GGCC	425	1104	410	770	694	525
Type I loci no./primer combination				10.6**	26.2	12.8**	30.5	24.8*	18.4
Type II loci no./primer combination				20.6	24.8	21.2	15.5	11.7*	6.9
Type III loci no./primer combination				52.1**	11.1	37.2**	14	9.7**	26.2
Methylation loci no./primer combination				83.3**	62.1	71.3**	60	46.2	51.5
Total methylation loci (I + II + III)/ratio to total loci				2665**/ 86.2 %	1986/ 64.3 %	2280**/ 84.8 %	1920/ 71.4 %	1479/ 68.1 %	1648/ 75.8 %
Total amplified loci (I + II + III + IV)				3090	3090	2690	2690	2173	2173

3. Epigenetic mechanisms of salt Tolerance and heterosis in upland cotton

Table 4 Salt-tolerance level of CCRI29 and its two parents

Cotton material	Salt-tolerance index (%)*	Salt-tolerance level
CCRI29	60.84a	Tolerant
P1	54.50b	Tolerant
RP4	42.39c	Sensitive
Mid-parent heterosis of salt-tolerance level	25.59	

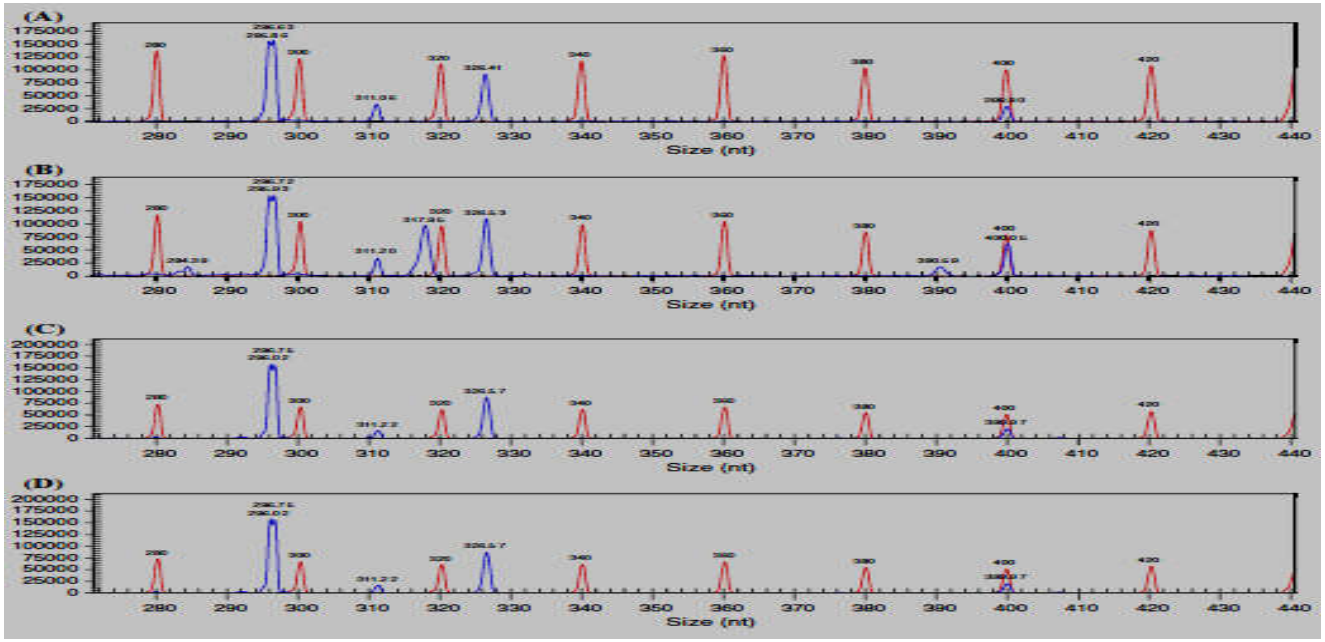
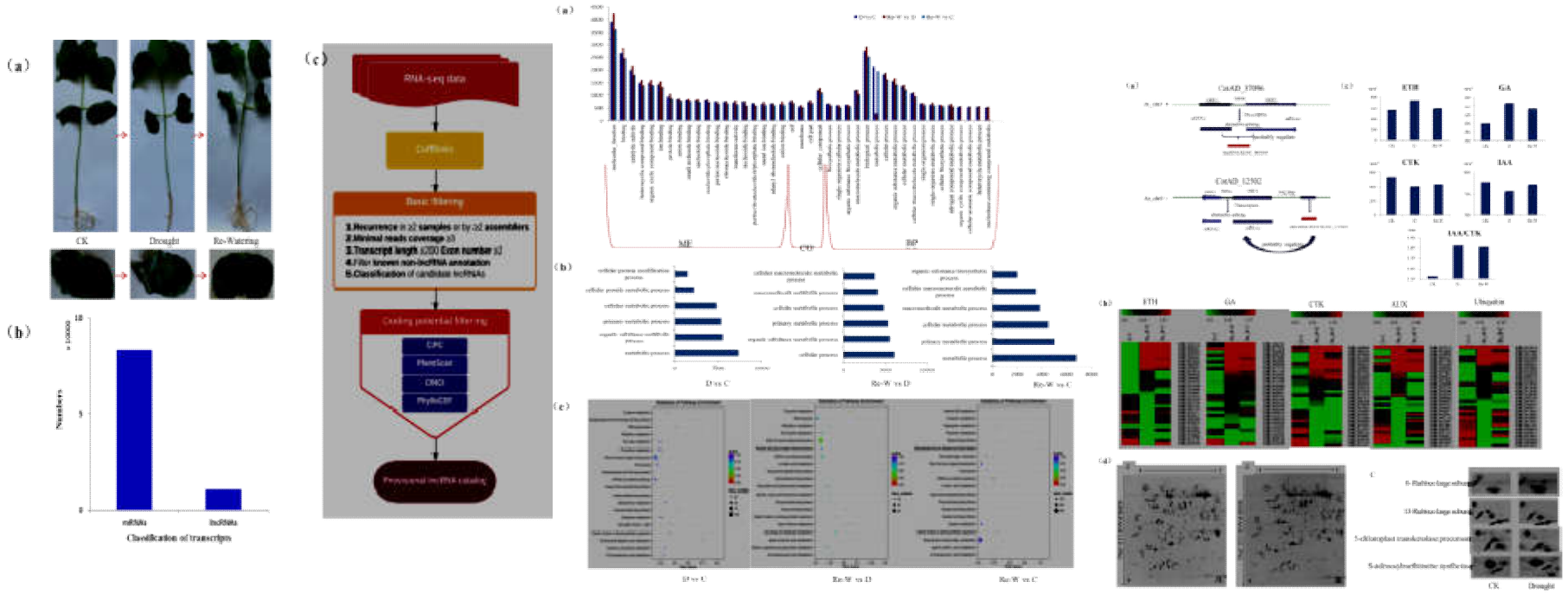


Fig.2-6 DNA methylation patterns of CCRI 29 detected in CE with the primer combination

4. lncRNAs responding to drought

•10,820 lncRNAs of high-confidence were observed, Small RNAs revealed that 196 lncRNAs may be the precursors to small RNAs, most of which (35.7%, 70) were miRNAs.

•LncRNAs may be likely to involve in regulating plant hormones pathway in response to drought stress.



5. DNA methylation helps to understand the responding to salt stress

- **Salt-tolerant cotton might have a mechanism of increasing the methylation level when responding to salt stress;**
- **Increasing level of DNA methylation and different methylation patterns might play important roles in active responding to salt stress in cotton.**

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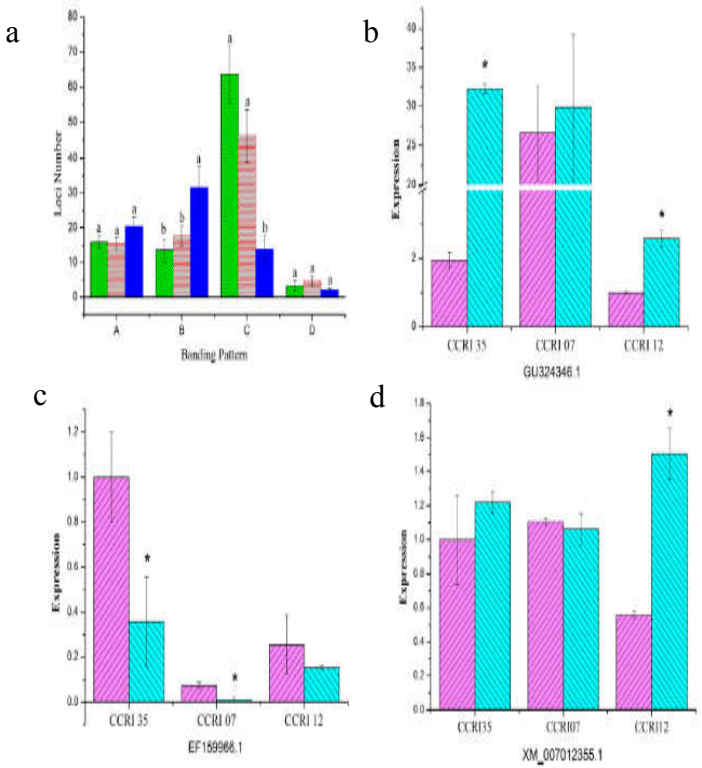


Genes of salinity-tolerance

Transgenetic seeds (2016)

No.	receptor	genes	No.of seeds
1	ccri45	*GhVP*	180
2	ccri63	*GhVP*	60
3	ymz21	*GhVP*	96
4	sGK958	*GhVP*	87
5	ccri45	*GhSAMS*	99
6	ccri63	*GhSAMS*	120
7	ymz21	*GhSAMS*	78
8	sGK958	*GhSAMS*	56

Transgenetic seeds screened under the salinity stress(0.4%NaCl)

Some salinity toeralant races

Race No.	Salinity resistance index/%	Salinity resistance level	Race No.	Salinity resistance index/%	Salinity resistance level
ccri9806	76.5	resistant	804129	58.2	tolerant
806081	74.9	tolerant	805021	55.3	tolerant
810151	72.3	tolerant	810011	50.6	tolerant
807071	72.3	tolerant	804137	64.3	tolerant
807017	71.2	tolerant	805133	63.2	tolerant
810121	65.3	tolerant	806011	58.9	tolerant
807067	55.2	tolerant	ccri9612	21.5	sensitive

salinity tolerant

CRI9806

salinity sensitive

CCRI12

CRI9806 grown in the saline(0.4%NaCl) land.

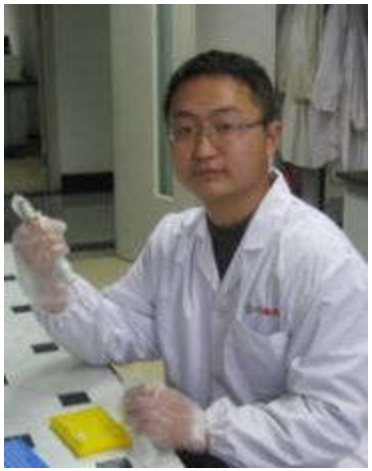
CRI9806 growing in the saline land.

Ccri9806----0.52% saline land, Changzhou, Hebai province

ccr i9806

control

1	The draft genome of a diploid cotton Gossypium raimondii	Nature Genetics	2012,44(10):1098-1103
2	Genome sequence of the cultivated cotton Gossypium arboreium	Nature Genetics	2014,46, 567-574
3	Genome sequence of cultivated Upland cotton (Gossypium hirsutum TM-1) provides insights into genome evolution	Nature biotechnology	2015,33(5):524-530
4	Genome-wide Identification and Structural Analysis of Pyrophosphatase Gene Family in Cotton	Crop Science	2016,56:1-10
5	Genome-Wide Analysis of Long Noncoding RNAs and Their Responses to Drought Stress in Cotton (G. hirsutum L.)	PLOS ONE	2016,11(6):e0156723
6	Mining and Analysis of SNP in Response to Salinity Stress in Upland Cotton (Gossypiumhirsutum L.)	PLOS ONE	2016,11(6):e0158142
7	Genome-wide Identification and analysis of the stress-resistance function of the TPS (Trehalose-6-Phosphate Synthase) gene family in cotton	BMC Genetics	2016,17:54
8	In vitro regeneration protocol for synthetic seed production in upland cotton (Gossypium hirsutum L.)	Plant Cell, Tissue & Organ Culture	2016,123:673-679
9	Genome-wide identification and expression analysis of CIPK genes in diploid cottons	Genetics & Mol. Research	2016,15(4)gmr15048852
10	Epigenetic mechanisms of salt tolerance and heterosis in Upland cotton (Gossypium hirsutum L.) revealed by methylation-sensitive amplified polymorphism analysis	Euphytica	2016, 208:477–491
11	Cloning of SjCA gene and its expression analysis on upland cottons	Journal of Biomedical Engineering and Informatics	2016, 2(2):150-162




Thanks!!