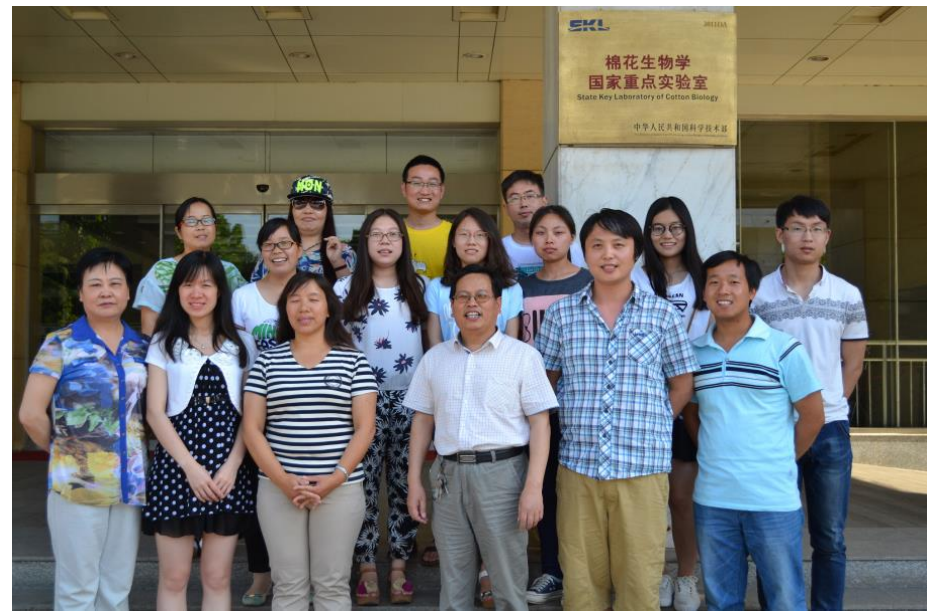


Cotton DNA methylation and its analysis under the salt- and draught-stress

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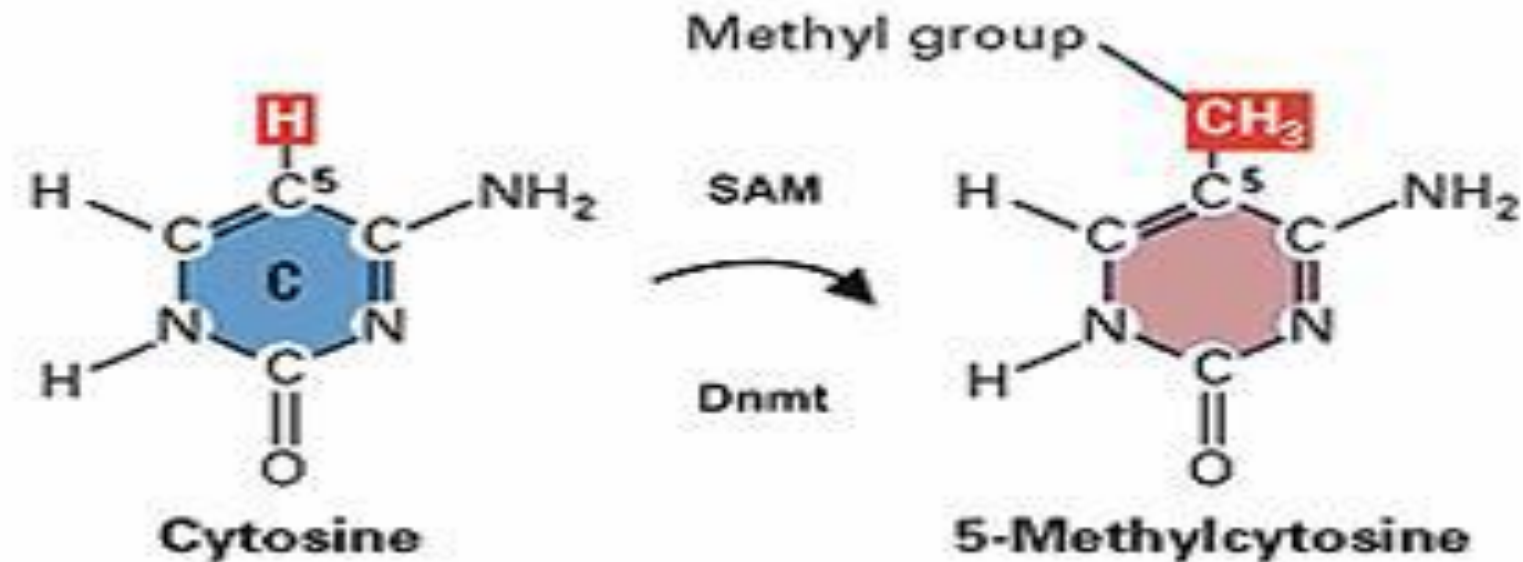
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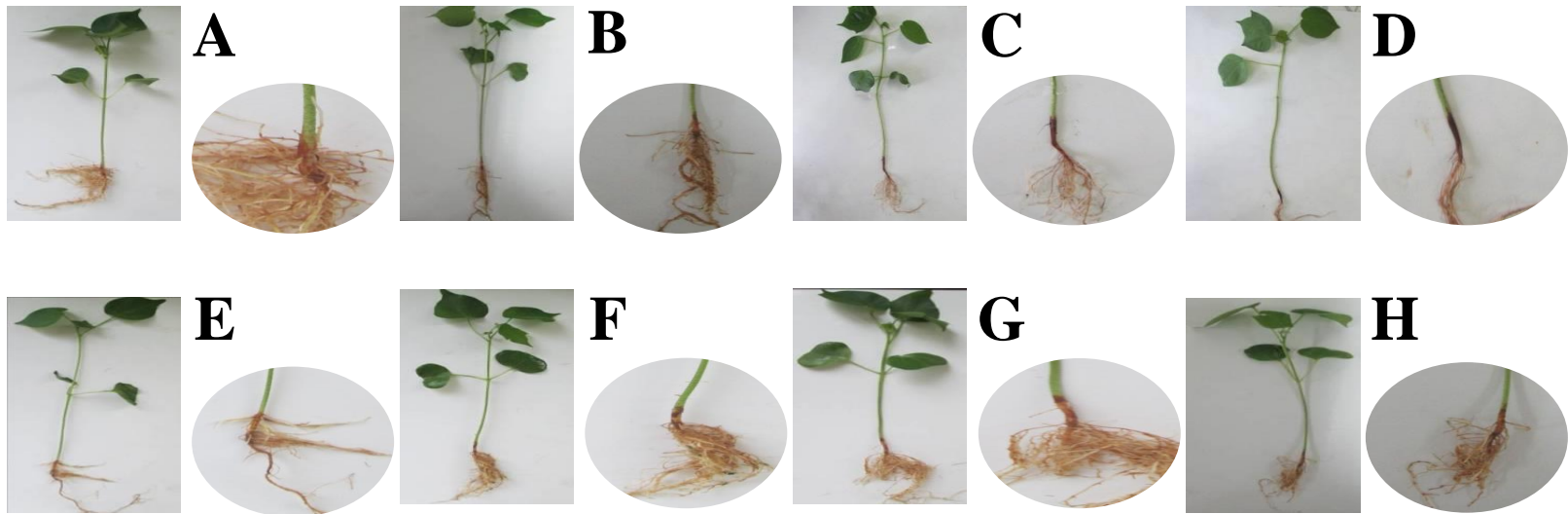
- Epigenetics
- DNA methylation
 - Histone modifications/variants
 - Non-coding RNAs
 - Other mechanisms

DNA methylation



1. DNA methylation analysis under different types of salt

- Different salt stresses exerted distinct effects on cotton seedling growth.
- Neutral salt NaCl < alkalescent salt NaHCO_3 < alkaline salt Na_2CO_3



A) Zhong07-dH₂O; B) Zhong07-NaCl; C) Zhong07-NaHCO₃; D) Zhong07-Na₂CO₃; E) ZhongS9612-dH₂O; F) ZhongS9612-NaCl; G) ZhongS9612-NaHCO₃; H) ZhongS9612-Na₂CO₃

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

- The higher DNA methylation diversity (CHG methylation being more diverse than CG methylation) in cotton genotypes suggest epigenetic regulation may be important for cotton.
- The change in DNA methylation between genotypes indicates epigenetically developed in various *spp*.

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>CGG</u> GG <u>CC</u>	339	838	410	977	793	588
II	1	0	C <u>CGG</u> or C <u>CGG</u> GGCC--GGCC	658	792	679	496	375	222
III	0	0	C <u>CGG</u> or C <u>CGG</u> or C <u>CGG</u> or C <u>CGG</u> or C <u>CGG</u> GG <u>CC</u> --GGCC--GGCC GG <u>CC</u> --GGCC	1668	356	1191	447	311	838
IV	1	1	C <u>CGG</u> or C <u>CGG</u> 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

- Most cytosine methylation sites in hybrid CCRI 29 shared the same status as that of at least one of the parents
- The number of parents hypomethylation is significantly higher than that of hypermethylation in CCRI 29 under both control and salt stress.
- Demethylation could be the mechanism to explain heterosis in cotton hybrid.

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	

4. 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.

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

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 ₅ CGG GGCC	339	838	410	977	793	588
II	1	0	C ₅ CGG or C ₅ CGG GGCC--GGCC	658	792	679	496	375	222
III	0	0	C ₅ CGG or C ₅ CGG or C ₅ CGG or C ₅ CGG GGCC--GGCC--GGCC GGCC--GGCC	1668	356	1191	447	311	838
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