

Dr. Markus Kuhlmann



Transgenerational memory in plant stress biology

Get inside into epigenetic aspects of applied research

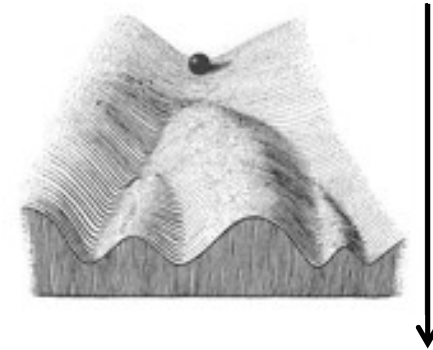
EPI-CATCH Cost action

Plant epigenetics

Workshop 2021

What is epigenetics?

- **Epigenetics:**
 - *epi* (Gk) upon
 - *genesis* (Gk) generation, birth, origin
 - *genes* (Gk) born
- ‘the interaction of genes with their environment, which bring the phenotype into being’ (Waddington, Endeavor 1:18,1942)
- ‘heritable changes in gene expression not attributable to nucleotide sequence variation’ (Murrell et al. Hum Mol Genet 14:R3, 2005)



Waddington, C. H. *The strategy of the genes. A discussion of some aspects of theoretical biology. With an appendix by H. Kacser.* London: George Allen & Unwin, Ltd., (1957).

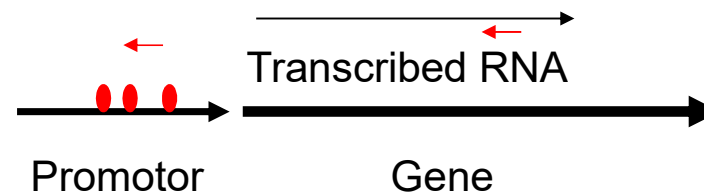
Definition:

“The study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in the DNA sequence.” (Riggs et al. 1996)

Mechanisms mediating an epigenetic response

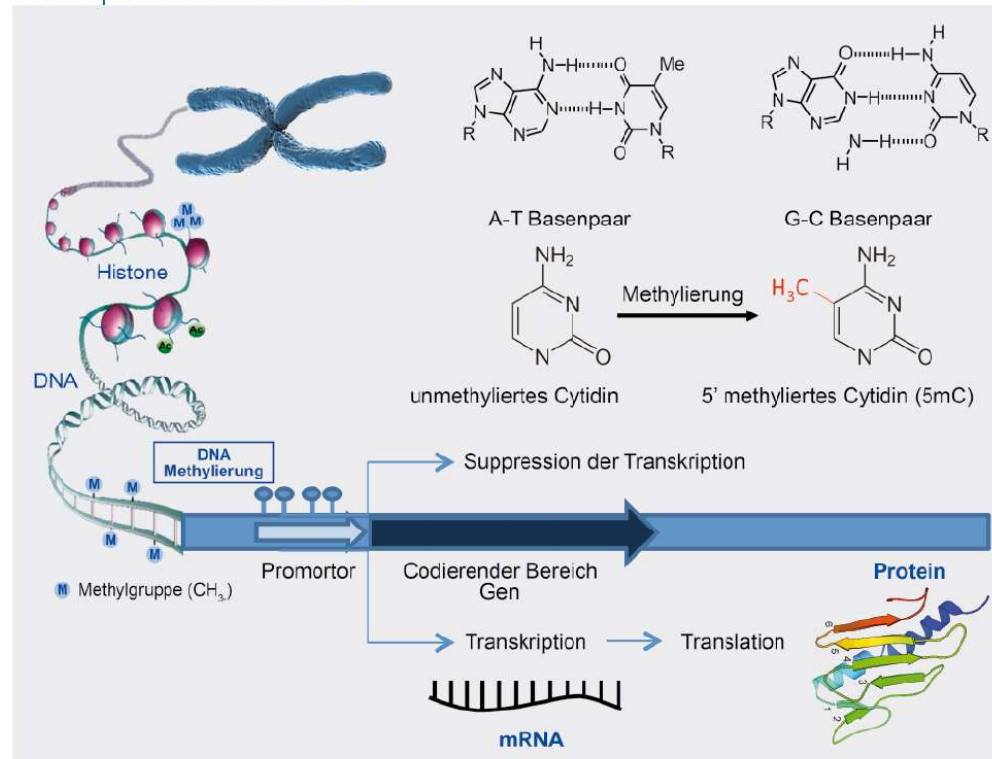
- PTGS – Post Transcriptional Gene Silencing
- **TGS – Transcriptional Gene Silencing**

Involving DNA-methylation, histone modification
and small regulatory RNA



DNA Methylation

ABB. 2 | DNA-METHYLIERUNG



Die genomische Information liegt in Eukaryonten in Form der DNA-Sequenz gespeichert im Zellkern vor. Das DNA-Molekül ist um Histone (Kernproteine) gewunden, mit weiteren Proteinen „dekoriert“ und als Chromosom organisiert. Die kodierenden Bereiche können in mRNA abgeschrieben (transkribiert) und in Proteine umgesetzt (translatiert) werden. Als DNA-Methylierung wird das enzymatische Ankoppeln einer Methylgruppe an das fünfte Kohlenstoffatom im Ring des Nucleosids Cytidin bzw. der Nucleinbase Cytosin bezeichnet. Es beeinflusst nicht die Basenpaarung der DNA (A-T und G-C), kann aber die Organisationsstruktur der DNA beeinflussen. Darstellung des Chromosoms: Fotolia.

Detection is possible by
Immunology

5 mC Antibody:

Histology

ChIP

ChIP-Seq

Chemistry

Bisulfite conversion

WGBS

Enzymes

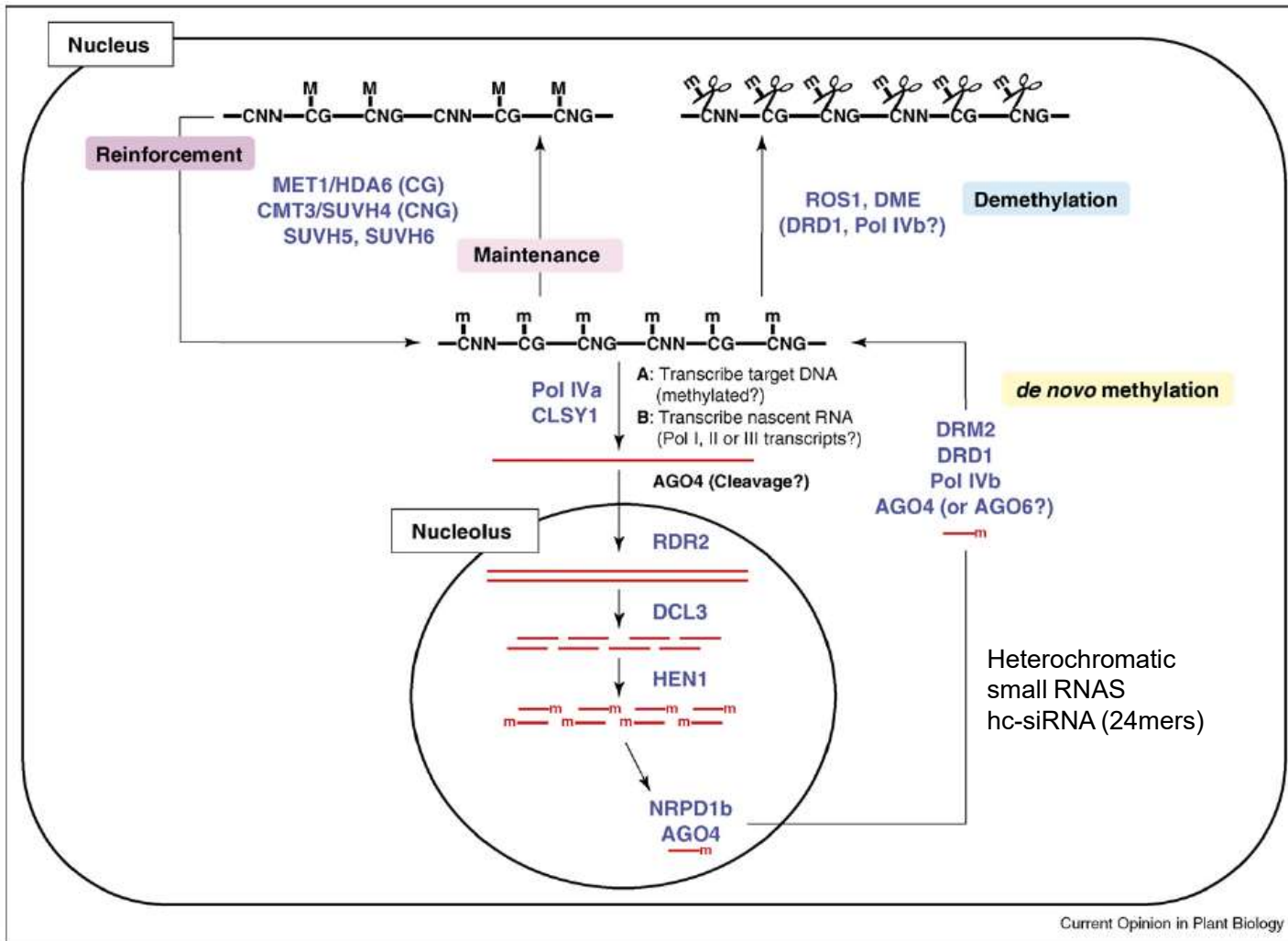
MSRE qPCR

enzymatic conversion

Enzymatic Methyl-seq

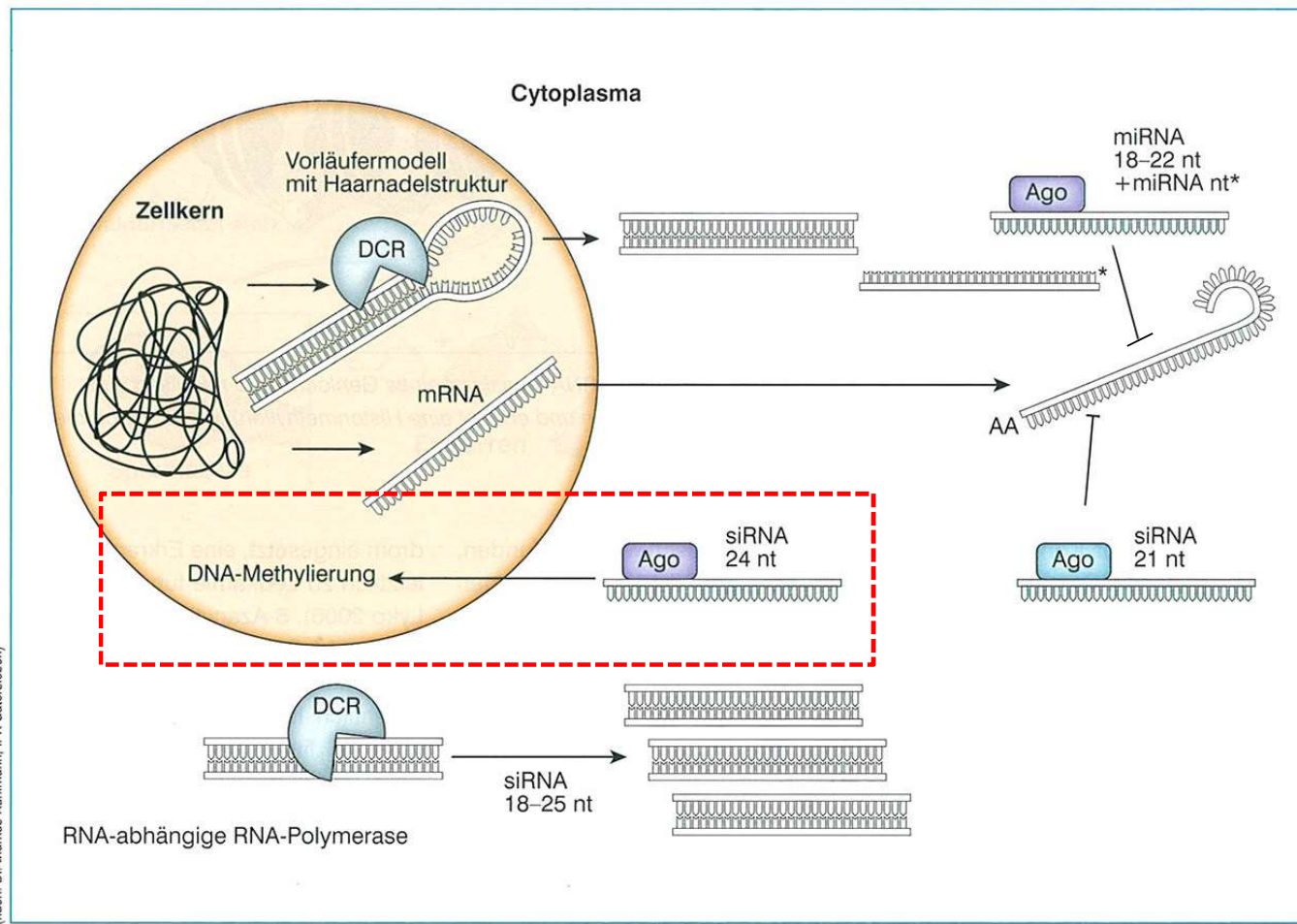
Regulation of DNA Methylation

De novo
Maintenance
Demethylation



mod. after „Targets of RNA directed DNA methylation“ Matzke et al., 2007; Curr. Opp in Plant Biol



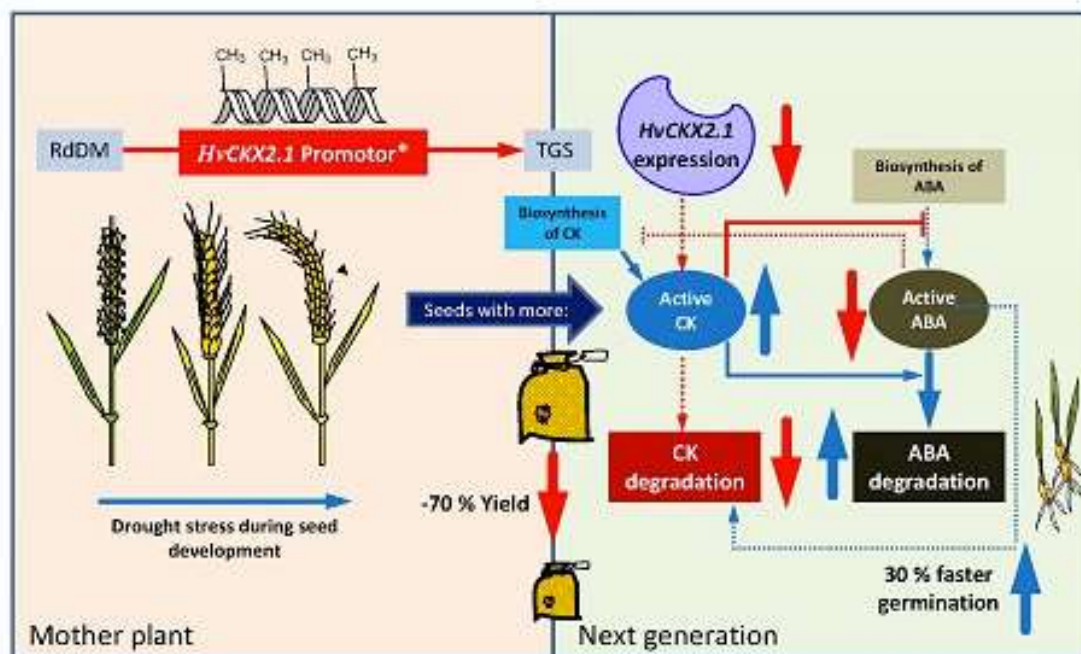


(nach: Dr. Markus Kuhlmann, IPK Gatersleben)

Unterricht Biologie, 2014



Example for transgenerational epigenetic effect in barley

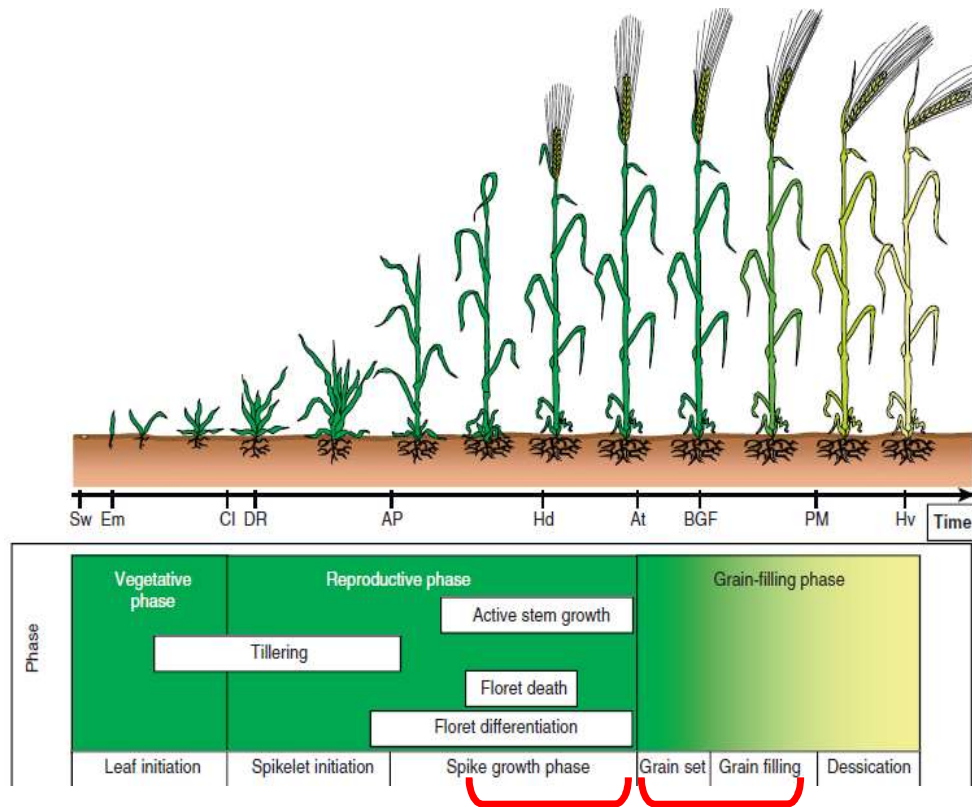


RNA-directed DNA methylation (RdDM) of HvCKX2.1 promoter leads to stable transcriptional gene silencing (TGS)



Terminal drought results in a loss of yield

Terminal Drought



Drought during the phase affect number of seeds size of seeds

Control Drought

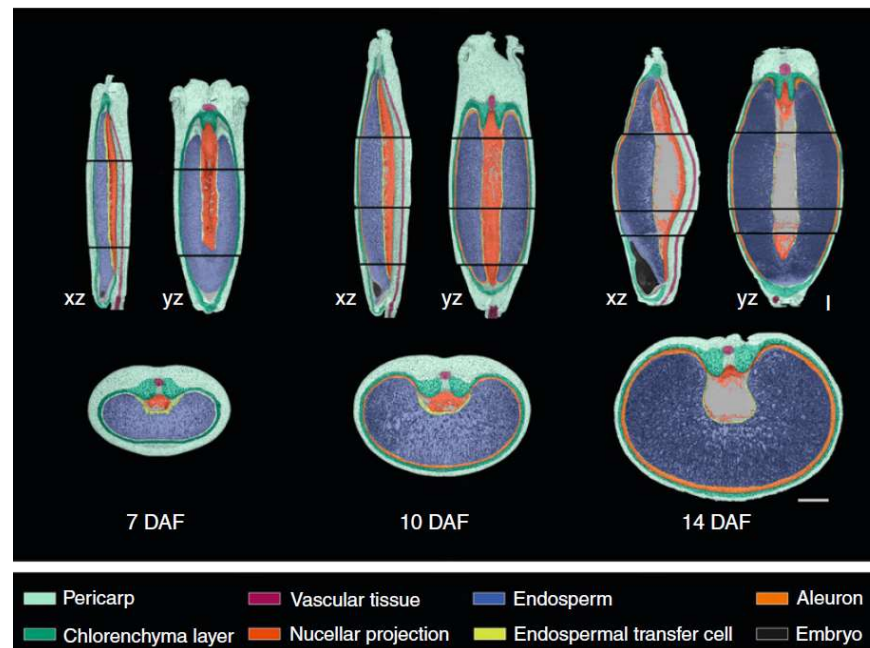
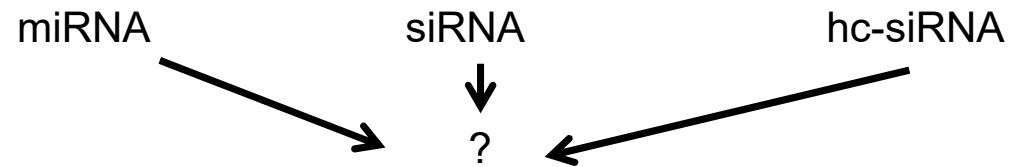


Mod. after Sreenivasulu & Schnurbusch 2012



Initial question:

Will the small RNAs contribute to drought stress induced change of the barley grain development?



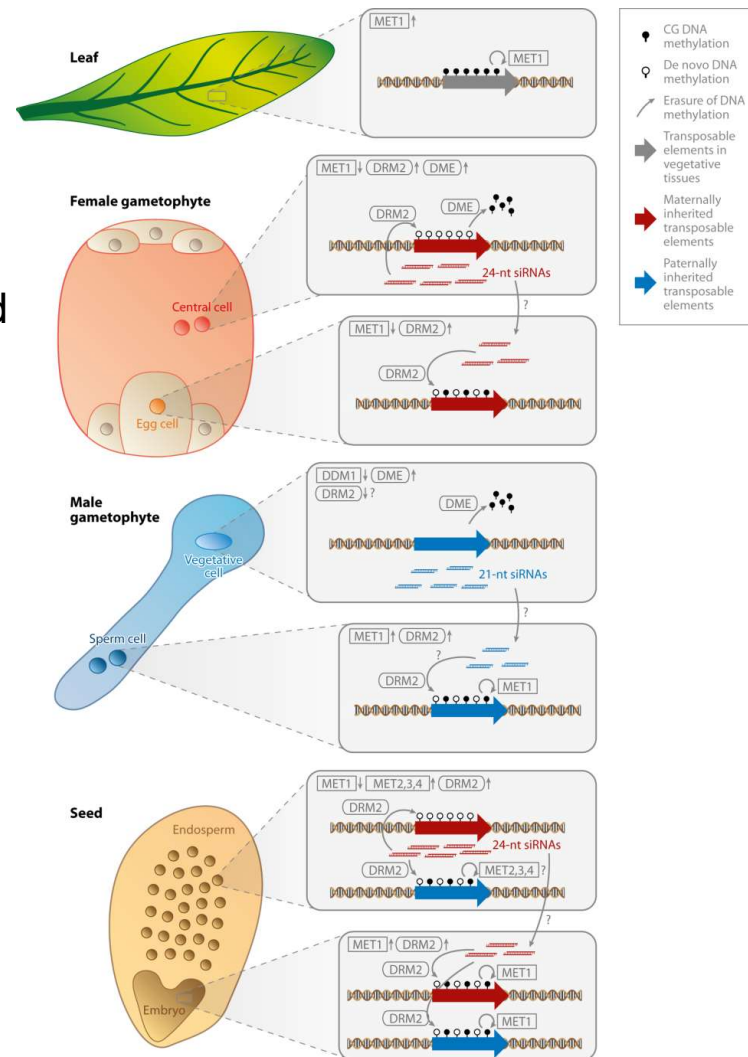
Sreenivasulu, et al., 2010



Importance of siRNA during seed development:

24mers are mainly contributed from the maternal site.

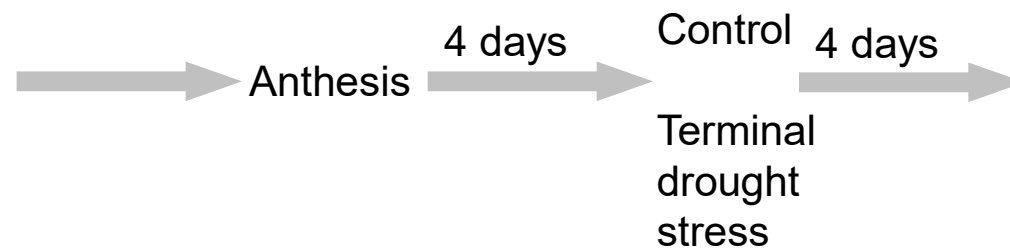
During seed development 24mers (heterochromatic small RNAs) are important triggers for DNA methylation in the embryo.



Kohler C, et al. 2012. Annu. Rev. Plant Biol. 63:331–52



Sequencing of small RNA in barley under terminal drought - experimental approach:



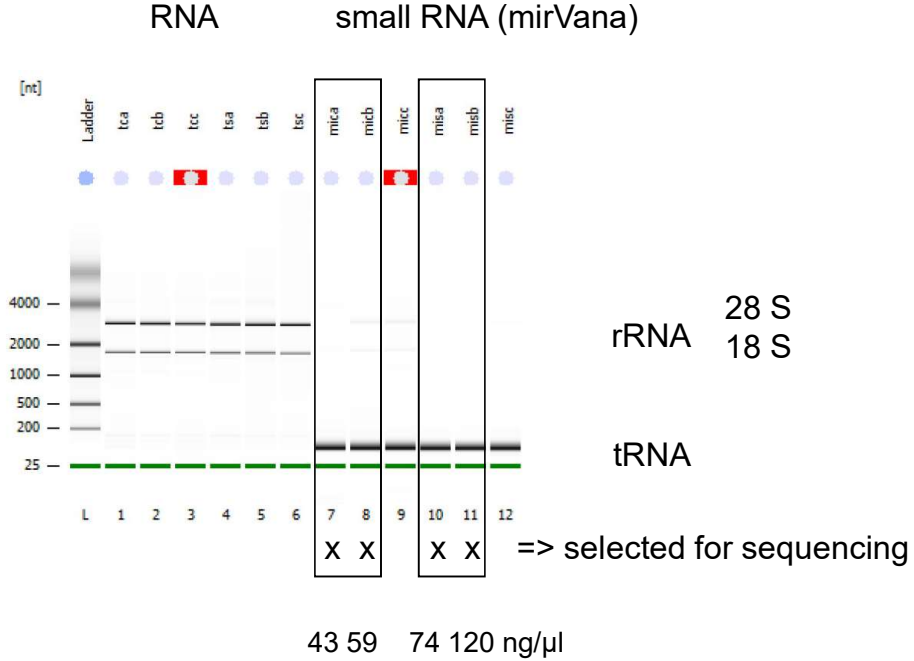
...to analyse the effect of the population of small RNAs
high throughput sequencing was performed on
barley caryopsis in duplicate.
Results obtained for sRNA, siRNA, heterochromatic-siRNA and miRNA



Control Stress



Sequencing of small RNA in barley under terminal drought sample preparation from caryopsis



Bioanalyser (Agilent)



Sequencing of small RNA in barley under terminal drought

Sequencing was performed in collaboration with GeneXPro

and resulted in 18/ 15.4 million reads for the control caryopsis
 11.3/ 8.6 million reads for the stressed caryopsis

Data analysis: GenXPro and CLC workbench



Sequencing of small RNA in barley at terminal drought

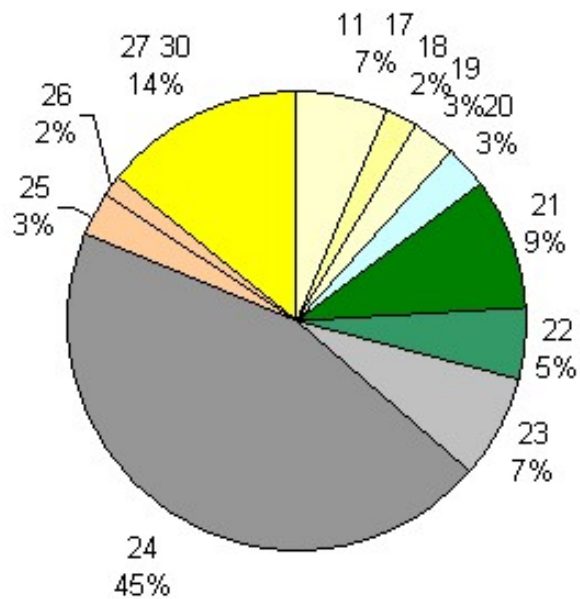
siRNA classes:

siRNA 21nt

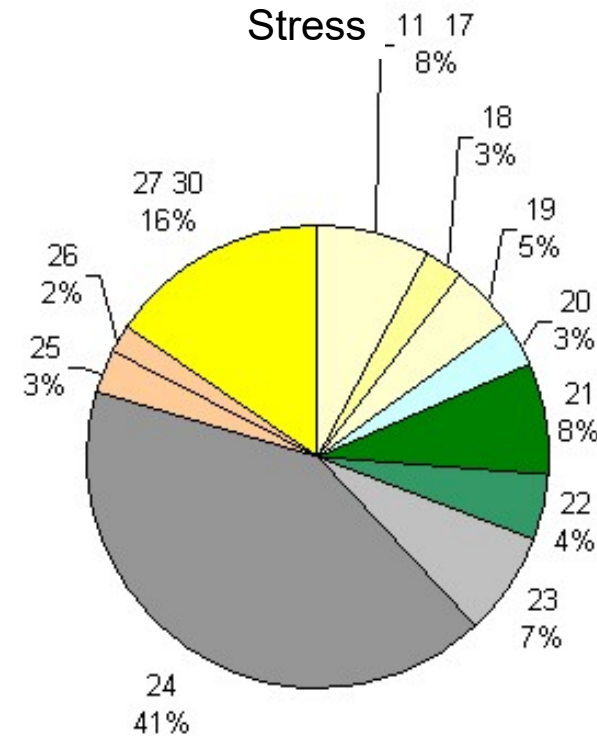
miRNA
18-24 nt

Heterochromatic siRNA 24 nt

Control



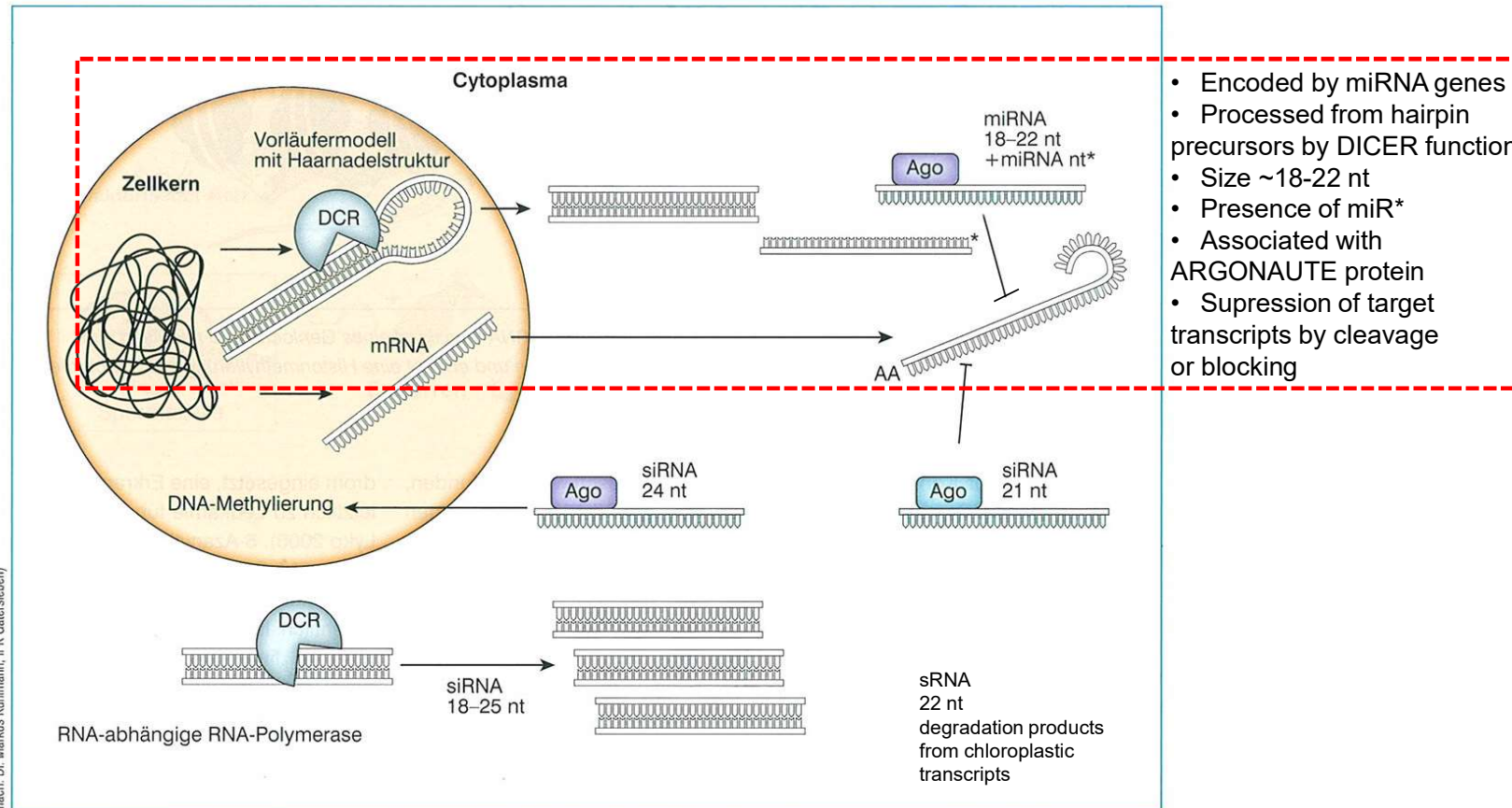
Stress



- 22 nt – leftover of degraded transcript of chloroplastic origin
- Class of 21mers/ siRNAs are not analysed yet.



microRNAS (miRNA)



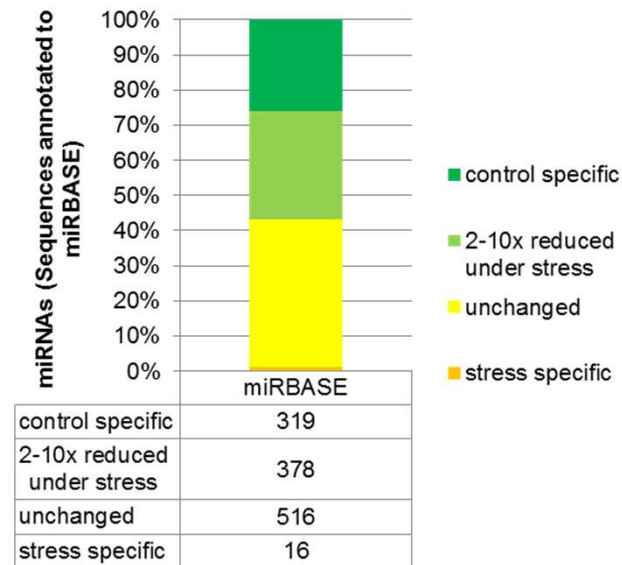
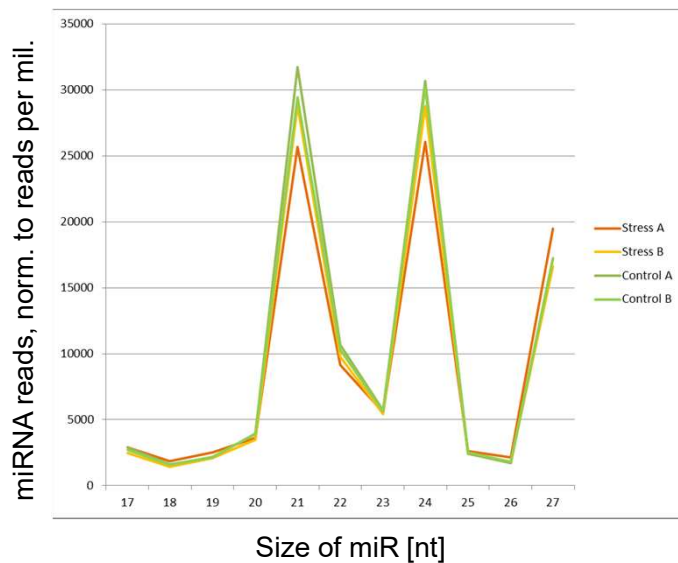
- Encoded by miRNA genes
- Processed from hairpin precursors by DICER function
- Size ~18-22 nt
- Presence of miR*
- Associated with ARGONAUTE protein
- Suppression of target transcripts by cleavage or blocking

(nach: Dr. Markus Kühmann, IPK Gatersleben)



Sequencing of small RNA in barley at terminal drought

analysis of sequences matching to hairpin database (miRBASE):
Selection for miRNA Sequences



⇒ minor changes in singletons, but
expression levels of several miRNAs are reduced during drought stress

16 miRNAs unique present under drought stress conditions.



miR-analysis

Unique miRs present only during drought stress
in comparison to published data

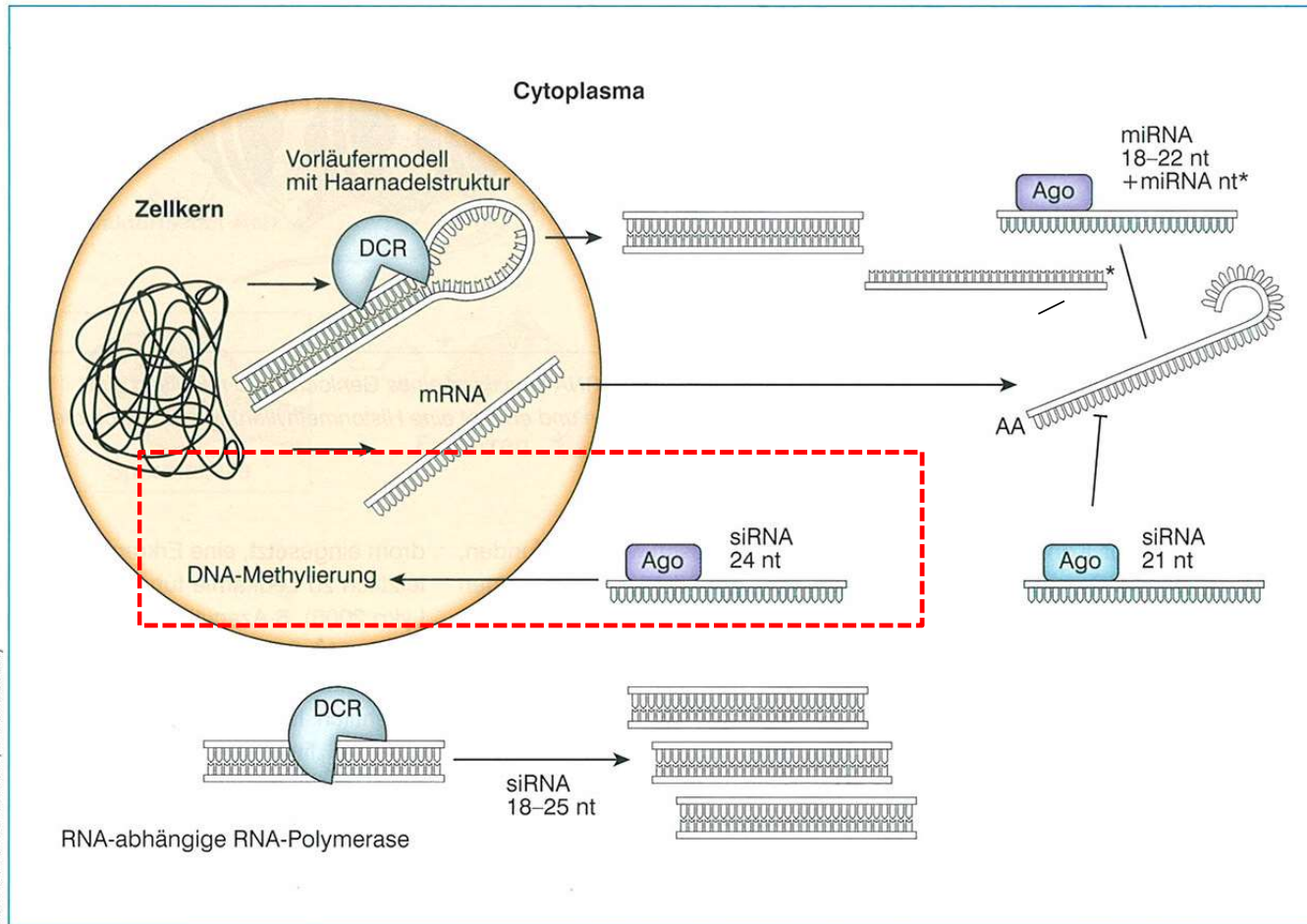


From Zhang 2015 acc. to Hackenberg et al., 2015

Feature ID	Small RNA -	Small RNA - Name	Small RNA - Resour	Small RNA -	Small RNA -	Experiment -	Experiment -	Experiment -	Experiment -	Control - mi	Control - mi	Control - Me	Stress - miR	Stress - miR	Stress - Mear	
AAGAGGAGAAAGGTT	15	mmu-miR-7086-5p	miRBASE2014	Exact		0	16	16	16	#DIV/0!	0	0	0	16	16	16
ACAAAACCTTCAGCTATCCATCG	23	tae-miR7757-5p	miRBASE2014	Variant		2	15	15	15	#DIV/0!	0	0	0	15	15	15
ACCTGTTTGTCAATAAATTTCTT	23	tae-miR9652-5p	miRBASE2014	Variant		2	20	20	20	#DIV/0!	0	0	0	20	20	20
AGGAATCTTGATGATGCTGCAT	22	ath-miR172e-3p//os	miRBASE2014	Variant		1	21	21	21	#DIV/0!	0	0	0	21	21	21
CAGGGATGGAGCAGAGCAAGG	21	ata-miR408-5p	miRBASE2014	Exact		0	15	15	15	#DIV/0!	0	0	0	15	15	15
CCTGGGCAGCAACACCA	17	bdi-miR5059	miRBASE2014	Variant		1	16	16	16	#DIV/0!	0	0	0	16	16	16
GCTCACTCCTTTCTGTGAG	21	ata-miR156d-3p	miRBASE2014	Exact		0	17	17	17	#DIV/0!	0	0	0	17	17	17
TCCACAGGCTTTCTTGAAGTGT	23	osa-miR396e-5p//zn	miRBASE2014	Variant		2	15	15	15	#DIV/0!	0	0	0	15	15	15
TCCACAGGCTTTCTTGAATT	20	osa-miR396e-5p//os	miRBASE2014	Variant		1	19	19	19	#DIV/0!	0	0	0	19	19	19
TCGCTTGGTGACAGATCGGGG	21	osa-miR168a-5p//sb	miRBASE2014	Variant		1	15	15	15	#DIV/0!	0	0	0	15	15	15
TCGGACCAGGCTTAAATCCCT	21	osa-miR166k-3p//os	miRBASE2014	Variant		1	15	15	15	#DIV/0!	0	0	0	15	15	15
TGGGGCTCGAAGACGA	17	peu-miR2916	miRBASE2014	Variant		1	17	17	17	#DIV/0!	0	0	0	17	17	17
TTCA CAGGCTTTCTTGAAGTGT	21	osa-miR396e-5p//zn	miRBASE2014	Variant		1	15	15	15	#DIV/0!	0	0	0	15	15	15
TTCACTCGGGTTCA	15	ppt-miR894	miRBASE2014	Exact		0	18	18	18	#DIV/0!	0	0	0	18	18	18
TTTGGCACCTTGAAACTGG	19	hvu-miR5051	miRBASE2014	Exact		0	18	18	18	#DIV/0!	0	0	0	18	18	18
TTTGGTTCTCCTAATATCTTA	22	mes-miR2275	miRBASE2014	Exact		0	21	21	21	#DIV/0!	0	0	0	21	21	21

miR5051(abiotic/boron stress), miR172e (flowering time), miR396e (GRF), miR156 (heat, drought)

hc-siRNAs (heterochromatic siRNA 24mers)

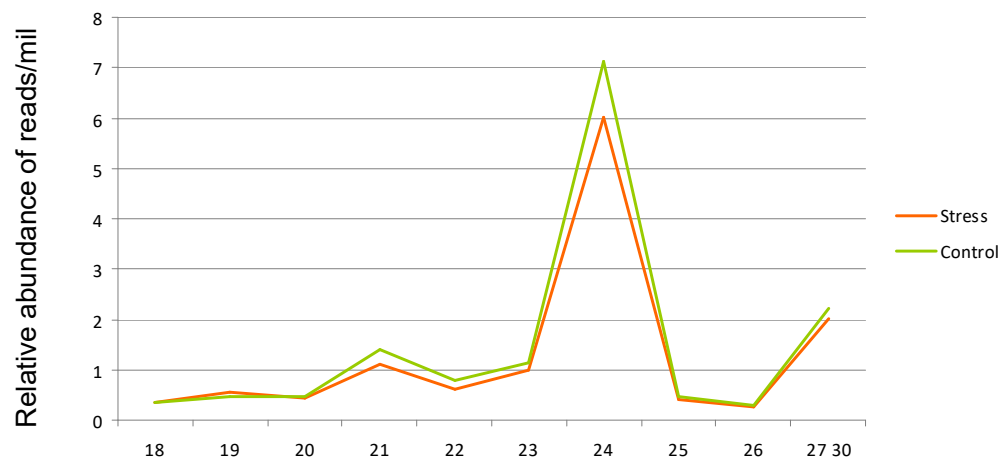


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mod. from Unterricht Biologie, 2014

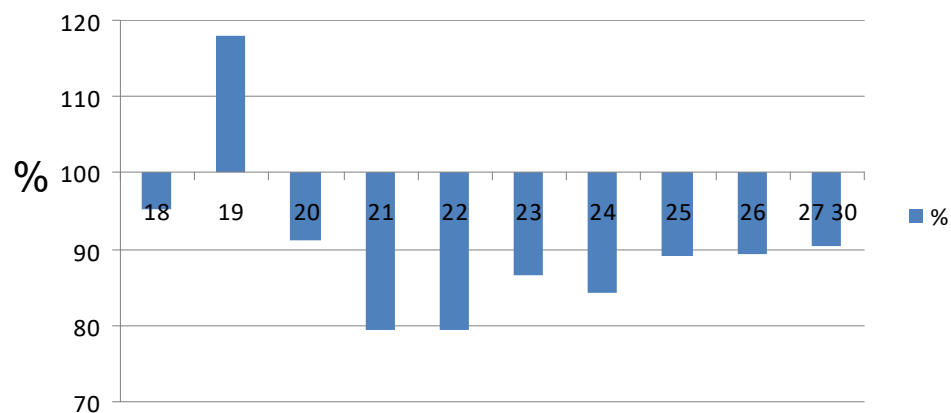


Sequencing of small RNAs in barley caryopsis under terminal drought



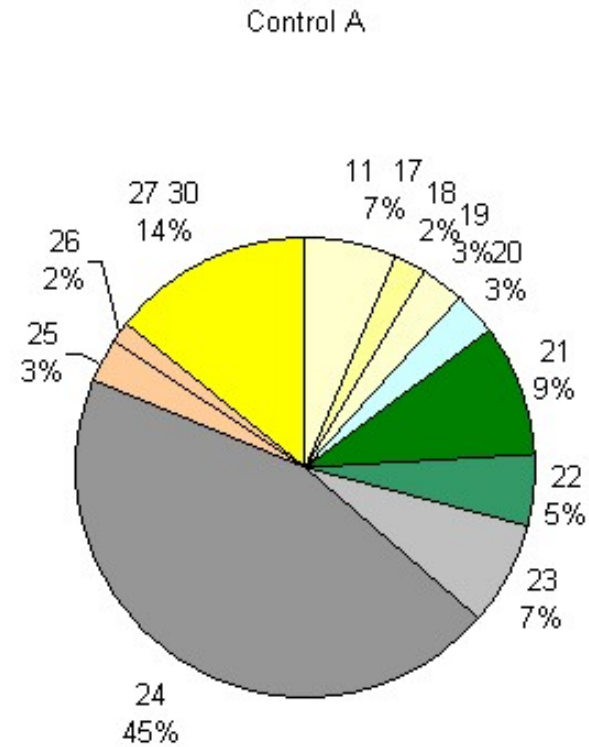
General profile of small RNAs: high abundance of 24mers in reproductive tissue, but relative reduction of 21 and 24mers under drought conditions

% of abundance compared to unstressed control



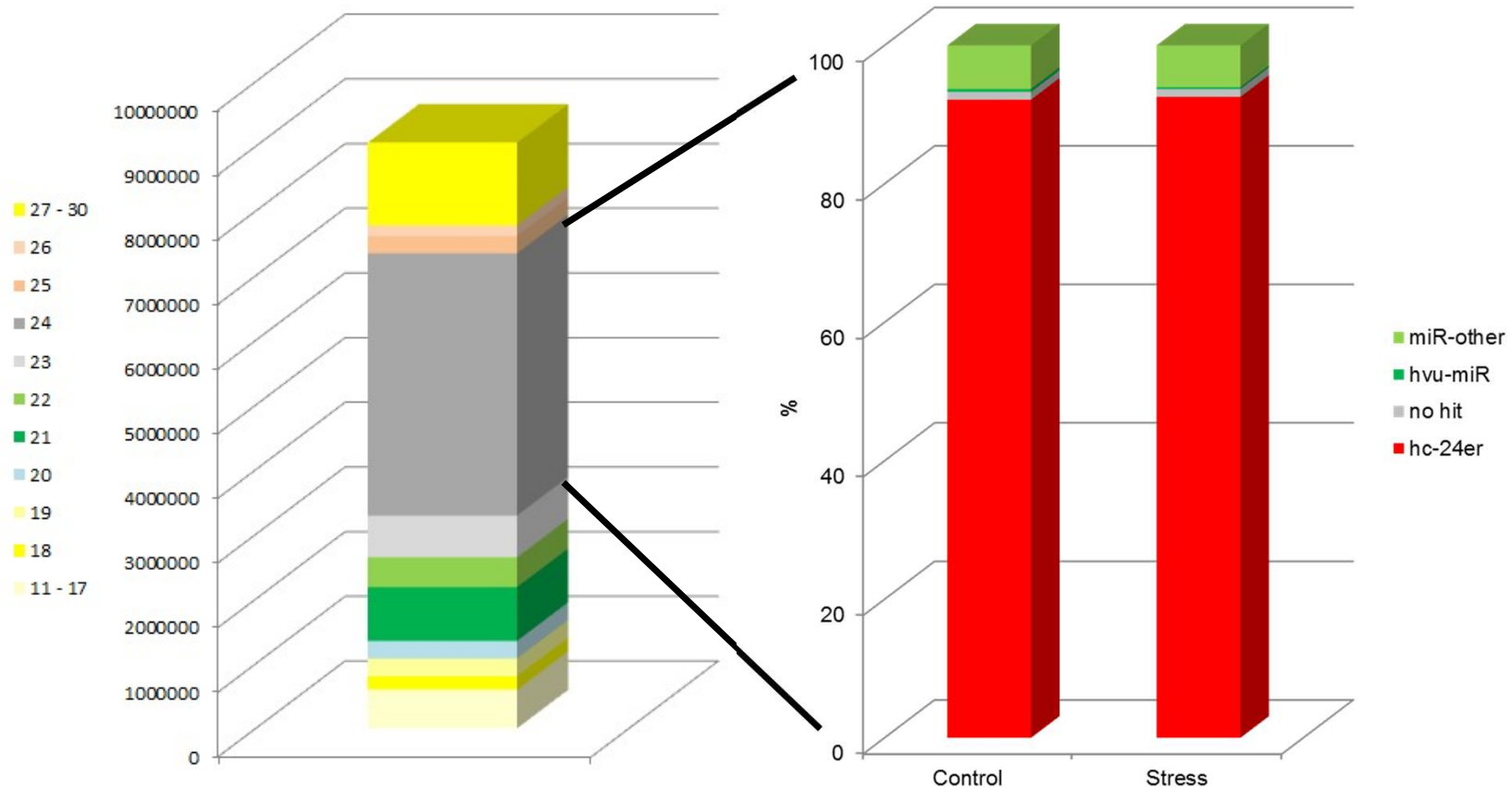
Identification of hc-small RNA

- Removal of miRNA sequences (based on hairpin database)
- Selection of 24mers (based on size)
- Mapping to genome
- Normalisation (reads/million)



Sequencing of small RNA in barley under terminal drought -

selection of hc-small RNA



45% of all reads are 24mers and ~ 92% are putative heterochromatic small RNAs.



Majority of high abundant 24mers reads is unchanged under control and stress conditions

High abundant under Control												
Identity	Clus miRNA	length of seccount	Short ID	description	percental ho	Blast score	Blast identit	database	L1_misa.miR	L2_misb.miR	L3_mica.miR	L4_micb.miR
482098	TTGAACTGTTTCCTCTGAAATTC	24	28574	minus503995 50399595	91,6667	38,1576	22	nt.viridiplan	4160	3761	11602	9051
183203	ATTGTATCCTTAACCAATTCCTTT	24	31016	409102295 #or# 409102295	100	48,0694	24	nt.viridiplan	6239	6843	9899	8035
208818	CACGACTCTCGGCAACGGATATCT	24	19665	387865909 #or# 387865909	100	48,0694	24	nt.viridiplan	4418	2800	6491	5956
185582	AITTTATTTCAAGCTATTTGCGATC	24	18177	118201020 #or# 118201020	100	48,0694	24	nt.viridiplan	3505	2922	6200	5550
439381	TCTTGGATTATGAAAGACGAACA	24	23019	507118450 #or# 507118450	100	48,0694	24	nt.viridiplan	4802	4950	7865	5402
214836	CAGCCCCATGTCGACGCGATTCTG	24	14704	169834 #or# 169834 gi	100	48,0694	24	nt.viridiplan	2583	2015	5011	5095
205637	CACACGACTCTCGGCAACGGATAT	24	15192	387865909 #or# 387865909	100	48,0694	24	nt.viridiplan	3400	2180	4737	4875
279399	CTAGCACTTTGAATTTGGGTTGAA	24	18865	minus63147801 63147801	83,3333	40,14	20	nt.viridiplan	2443	2504	9107	4811
400033	TACCTGGTTGATCCTGCCAGTAGT	24	20436	507413709 #or# 507413709	100	48,0694	24	nt.viridiplan	5956	4077	5695	4708
279374	CTAGCACTTTGAATTTAGGTTGAA	24	15116	20513849 #or# 20513849	83,3333	40,14	20	nt.viridiplan	2076	1943	6688	4409
493055	TTTAGTCGGGCGTTCTCCATCGCG	24	17708	minus24209507 24209507	75	36,1753	18	nt.viridiplan	4534	2723	6118	4333
183287	ATTGTATTCCTTAATTATTCTCT	24	15267	319412298 #or# 319412298	100	48,0694	24	nt.viridiplan	3970	3149	4274	3874
111059	AGCTTTTGACGTTGTTTGGCCAAG	24	13125	minus16799838 167998838	79,1667	38,1576	19	nt.viridiplan	2341	2001	5190	3593
485938	TTGCTCGTCAAAGTAGCTCCTCGAT	24	14615	478165359 #or# 478165359	75	36,1753	18	nt.viridiplan	3306	2522	5286	3501
238769	CCCGAGAGACCCGGGTTCAAGTCC	24	17300	335999261 #or# 335999261	95,8333	40,14	23	nt.viridiplan	6380	2831	4598	3491
466798	TTAAGACTAAAGGCTGCTGGAAGA	24	9789	302839568 #or# 302839568	75	36,1753	18	nt.viridiplan	1672	1344	4164	2609
173822	ATTAATTTGTGCTAATGCGTTTGT	24	8668	217332074 #or# 217332074	75	36,1753	18	nt.viridiplan	1419	1351	3468	2430
89148	ACTTAGGAATTCACACATGAGAAG	24	8122	323367034 #or# 323367034	75	36,1753	18	nt.viridiplan	1884	1460	2362	2416
94121	AGAAGTACTGCTAGTTCAAGTGGC	24	7886	minus509447377 509447377	91,6667	38,1576	22	nt.viridiplan	1291	1547	2682	2366

High abundant under Stress												
Identity	Clus miRNA	length of seccount	Short ID	description	percental ho	Blast score	Blast identit	database	L1_misa.miR	L2_misb.miR	L3_mica.miR	L4_micb.miR
183203	ATTGTATCCTTAACCAATTCCTTT	24	31016	409102295 #or# 410177745 #or# 410177832 #or# 4101779	100	48,0694	24	nt.viridiplan	6239	6843	9899	8035
439381	TCTTGGATTATGAAAGACGAACA	24	23019	507118450 #or# 507118451 #or# 507118452 #or# 5071184	100	48,0694	24	nt.viridiplan	4802	4950	7865	5402
400033	TACCTGGTTGATCCTGCCAGTAGT	24	20436	507413709 #or# 507413710 #or# 507413711 #or# 5074137	100	48,0694	24	nt.viridiplan	5956	4077	5695	4708
482098	TTGAACTGTTTCCTCTGAAATTC	24	28574	minus50399595 #or# 50872480	91,6667	38,1576	22	nt.viridiplan	4160	3761	11602	9051
183287	ATTGTATTCCTTAATTATTCTCT	24	15267	319412298 #or# 319412384 #or# 340802207 #or# 3408022	100	48,0694	24	nt.viridiplan	3970	3149	4274	3874
185582	AITTTATTTCAAGCTATTTGCGATC	24	18177	118201020 #or# 118201189 #or# 13928184 #or# 40910229	100	48,0694	24	nt.viridiplan	3505	2922	6200	5550
238769	CCCGAGAGACCCGGGTTCAAGTCC	24	17300	335999261 #or# 335999266 #or# 335999273 #or# 3377301	95,8333	40,14	23	nt.viridiplan	6380	2831	4598	3491
208818	CACGACTCTCGGCAACGGATATCT	24	19665	387865909 #or# 491649914 #or# 491649916 #or# 4916499	100	48,0694	24	nt.viridiplan	4418	2800	6491	5956
493055	TTTAGTCGGGCGTTCTCCATCGCG	24	17708	minus242095071	75	36,1753	18	nt.viridiplan	4534	2723	6118	4333
485938	TTGCTCGTCAAAGTAGCTCCTCGAT	24	14615	478165359 478165359	75	36,1753	18	nt.viridiplan	3306	2522	5286	3501
279399	CTAGCACTTTGAATTTGGGTTGAA	24	18865	minus63147801 63147801	83,3333	40,14	20	nt.viridiplan	2443	2504	9107	4811
205637	CACACGACTCTCGGCAACGGATAT	24	15192	387865909 #or# 491649914 #or# 491649916 #or# 4916499	100	48,0694	24	nt.viridiplan	3400	2180	4737	4875
214836	CAGCCCCATGTCGACGCGATTCTG	24	14704	169834 #or# 170767 #or# 21856 #or# 21973 #or# 24198866	100	48,0694	24	nt.viridiplan	2583	2015	5011	5095
111059	AGCTTTTGACGTTGTTTGGCCAAG	24	13125	minus167998838 #or# 168020616 #or# 168020616 #or# 1680206	79,1667	38,1576	19	nt.viridiplan	2341	2001	5190	3593
279374	CTAGCACTTTGAATTTAGGTTGAA	24	15116	20513849 #or# 20513849	83,3333	40,14	20	nt.viridiplan	2076	1943	6688	4409
94121	AGAAGTACTGCTAGTTCAAGTGGC	24	7886	minus509447377 #or# 509448471	91,6667	38,1576	22	nt.viridiplan	1291	1547	2682	2366
89148	ACTTAGGAATTCACACATGAGAAG	24	8122	323367034 #or# 332640072 #or# 509214914 #or# 7321030	75	36,1753	18	nt.viridiplan	1884	1460	2362	2416
339749	GCCGCGCGGGGACGGACCGGGAA	24	7490	195616217 #or# 253760054 #or# 308081571 #or# 3131036	100	48,0694	24	nt.viridiplan	1837	1460	2060	2133



51 heterochromatic siRNAs are exclusively detected at terminal drought stress condition

Stress Control

	313237	GAATACACAAAAAATGTCTAG	24	8	123694921	g	123694921	75	36,1753	18	nt.viridipla	2	6	0	0
	447423	TGATCTTCTACTCGGCCGCTC	24	8	no hit	no hit	no hit	no hit	no hit	no hit	no hit	3	5	0	0
	46833	AATCAGATAACACAGTTGTTAAC	24	8	minus4701;	g	4701247;	75	36,1753	18	nt.viridipla	3	5	0	0
	200420	CAAGGCCGCCGCTTCGCTCCGCT	24	8	509425433	g	5094254;	75	36,1753	18	nt.viridipla	3	5	0	0
	256972	CGATGGCCATCTTCGTCGACACGA	24	8	minus4494;	g	4494332;	75	36,1753	18	nt.viridipla	3	5	0	0
	457630	TGGTAGTGTTCGCTCTCTTAC	24	8	123683439	g	1236834;	79,1667	32,2105	19	nt.viridipla	3	5	0	0
	463090	TGTGTAGCGGCAATCACCCTACG	24	8	326526596	g	3265265;	66,6667	32,2105	16	nt.viridipla	3	5	0	0
	47144	AATCATGGGACAAATGAAGCAGGG	24	8	151419059	g	1514190;	79,1667	32,2105	19	nt.viridipla	4	4	0	0
	69725	ACATTTTTTGCACATGTTATGAA	24	8	388502393	g	3885023;	75	36,1753	18	nt.viridipla	4	4	0	0
	239965	CCCTTATGTCGCTGCTCACGAA	24	8	148469858	g	1484698;	66,6667	32,2105	16	nt.viridipla	4	4	0	0
	267323	CGGTGGGCTCCCTGACGGGGGCT	24	8	minus1514;	g	1514274;	70,8333	34,1929	17	nt.viridipla	4	4	0	0
	353360	GGATTTTGAAGGCTTTGACTC	24	8	minus5091;	g	5091933;	91,6667	44,1047	22	nt.viridipla	4	4	0	0
	422677	TCATGATTAGCGCATAATGGATG	24	8	157311787	g	1573117;	70,8333	34,1929	17	nt.viridipla	4	4	0	0
	441675	TGAACCTAATCGTACAAAAATGT	24	8	minus2249;	g	2249232;	79,1667	38,1576	19	nt.viridipla	4	4	0	0
	459922	TGTAGTGCAACTCTATAACACAC	24	8	61699076	g	6169907;	75	36,1753	18	nt.viridipla	4	4	0	0
	462046	TGTGACGTGCAAAAACAGCGACC	24	8	116308931	g	1163089;	70,8333	34,1929	17	nt.viridipla	4	4	0	0
	78518	ACGCTTTCTCAAATCCACCTTGA	24	8	29122710	g	2912271;	100	48,0694	24	nt.viridipla	5	3	0	0
	91970	AGAAAAAATTCAGACAGCCAG	24	8	333470784	g	3334707;	70,8333	34,1929	17	nt.viridipla	5	3	0	0
	114297	AGGATTTTTCTAGCTATTGTTTC	24	8	123671043	g	1236710;	79,1667	38,1576	19	nt.viridipla	5	3	0	0
	272950	CGTGTGTTGCTAGTGGTGAAGAT	24	8	minus4405;	g	4405774;	87,5	36,1753	21	nt.viridipla	5	3	0	0
	319881	GACCTTATGCTGAAGCAAGCAATGA	24	8	166234948	g	1662349;	75	36,1753	18	nt.viridipla	5	3	0	0
	433989	TCTAATGCCCTCATGTAATGATGT	24	8	194267329	g	1942673;	87,5	42,1223	21	nt.viridipla	5	3	0	0
	480950	TTCTCCGGACCGATCACTGACGT	24	8	110742206	g	1107422;	79,1667	32,2105	19	nt.viridipla	5	3	0	0
	137920	ATAGATCCACCACTAGACATACA	24	8	25900578	g	2590057;	70,8333	34,1929	17	nt.viridipla	6	2	0	0
	229543	CATTCTGACTAAAGCTTTTTGAA	24	8	minus1236;	g	1236492;	79,1667	38,1576	19	nt.viridipla	6	2	0	0
	244447	CCGTTGTGTTGCCATAGAACAA	24	8	minus3006;	g	3006815;	75	36,1753	18	nt.viridipla	6	2	0	0
	285448	CTCCGTGCCGAATCCCCCTCCG	24	8	218664762	g	2186647;	100	48,0694	24	nt.viridipla	6	2	0	0
	353989	GGCATTGTTGCTCCTTAGTCTACT	24	8	297611087	g	2976110;	75	36,1753	18	nt.viridipla	6	2	0	0
	421129	TCAGGTGGACGCTAGGTTCAATGGA	24	8	356544835	g	3565448;	70,8333	34,1929	17	nt.viridipla	6	2	0	0
	424638	TCCAGCGAGGTAAGTCTAATGAAA	24	8	minus4001;	g	4001730;	70,8333	34,1929	17	nt.viridipla	6	2	0	0
	492816	TTTAGGAGGAGGAATGACAGAGT	24	8	minus3995;	g	3995719;	79,1667	38,1576	19	nt.viridipla	6	2	0	0
	317447	GACCTGTATGGGCCAGCCTTTTT	24	8	168052750	g	1680527;	70,8333	34,1929	17	nt.viridipla	7	1	0	0
	506899	TTTTTCTCTGCTATTAATTTGT	24	8	482662084	g	4826620;	83,3333	40,14	20	nt.viridipla	7	1	0	0
	451943	TGCTAATGAGCTCACTAGACTAGC	24	8	224122325	g	2241223;	83,3333	40,14	20	nt.viridipla	8	0	0	0
CCA1	298581	CTCCCTTTTGGGATGGTGGCT	24	9	502136179	g	5021361;	75	36,1753	18	nt.viridipla	3	6	0	0
	140359	ATATAAGACGTCCTGCAACAATG	24	9	147790313	g	1477903;	91,6667	38,1576	22	nt.viridipla	4	5	0	0
	188639	ATTTGTTTCTGCTACTCTATGAC	24	9	326523548	g	3265235;	79,1667	38,1576	19	nt.viridipla	4	5	0	0
	16003	AAATAGGAATGGCTCTGGCACT	24	9	19172013	g	1917201;	95,8333	40,14	23	nt.viridipla	5	4	0	0
	133289	ATAATATCAAGAACTGCTCCATT	24	9	minus2170;	g	2170758;	83,3333	40,14	20	nt.viridipla	5	4	0	0
	461214	TGTGGCGTCTCAGCACTGGC	24	9	no hit	no hit	no hit	no hit	no hit	no hit	no hit	6	3	0	0
	66350	ACATACGATTACCTATCTATCGG	24	9	332656411	g	3326564;	66,6667	32,2105	16	nt.viridipla	6	3	0	0
	441175	TGAACAACATCACATCTGATTA	24	9	minus1478;	g	1478152;	79,1667	38,1576	19	nt.viridipla	6	3	0	0
	493052	TTTGTGCGGCGTTCCTCCATGCC	24	9	minus3571;	g	3571336;	70,8333	34,1929	17	nt.viridipla	7	2	0	0
	163543	ATGACTTCTAAATATCGTGGG	24	9	196259863	g	1962598;	70,8333	34,1929	17	nt.viridipla	9	0	0	0
Sandra2	379988	GTGTAGTTTTGCTCATCGTGAAT	24	10	minus1680;	g	1680246;	70,8333	34,1929	17	nt.viridipla	3	7	0	0
DEMETER	103665	AGATGGTAGTACTTGCCTGGGG	24	10	118485797	g	1184857;	79,1667	32,2105	19	nt.viridipla	5	5	0	0
HOX1 VRS1	139428	ATAGTCAAAAGTTGTAAGGAGA	24	10	123663132	g	1236631;	75	36,1753	18	nt.viridipla	6	4	0	0
HOX1 Sandra5 Hordein	397325	TAATTGTTTAAAGAACGTCACAT	24	10	minus2253;	g	2253192;	75	36,1753	18	nt.viridipla	7	3	0	0
Lks2 (short internodes)	423240	TCATCCGACAGAGGGAAGGACG	24	10	147865233	g	1478652;	79,1667	32,2105	19	nt.viridipla	7	3	0	0
CCA1/TOC1	153923	ATCGTCAATGCGGGAAGGAGCTC	24	11	240254173	g	2402541;	75	36,1753	18	nt.viridipla	7	4	0	0
	387039	GTTGTGTGAGATGCTTAGCATATA	24	12	147821593	g	1478215;	70,8333	34,1929	17	nt.viridipla	7	5	0	0
Lks2 (short internodes)	90352	ACTTGCCAAAGTGAATCTACTCT	24	14	359751507	g	3597515;	70,8333	34,1929	17	nt.viridipla	9	5	0	0
CKX2.1 MLOC_53923 und Hox1	54486	AATTCAGAGGAAATAGCGTGATT	24	15	194267329	g	1942673;	95,8333	40,14	23	nt.viridipla	8	7	0	0
											Summe	275	189	0	0

Genes associated with 24mer hits (putative candidate genes):

CKX2.1
 cytokinin oxidase/dehydrogenase
 CCA1
 Circadian Clock Associated 1
 LKS2
 Short internodes TKF



Putative target CKX2.1 Cytokinin oxidase

Integration of epigenetic and genetic controls of seed size by cytokinin in *Arabidopsis*

Jing Li^{a,b}, Xin Nie^{a,b}, Jeanie Li Hui Tan^a, and Frédéric Berger^{a,b,1}

^aTemasek Life Sciences Laboratory, National University of Singapore, Singapore 117604; and ^bDepartment of Biological Sciences, National University of Singapore, Singapore 117543

Edited by Robert L. Fischer, University of California, Berkeley, CA, and approved August 14, 2013 (received for review March 18, 2013)

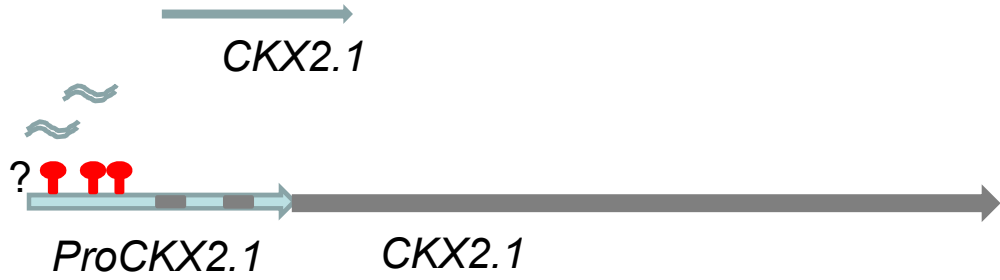
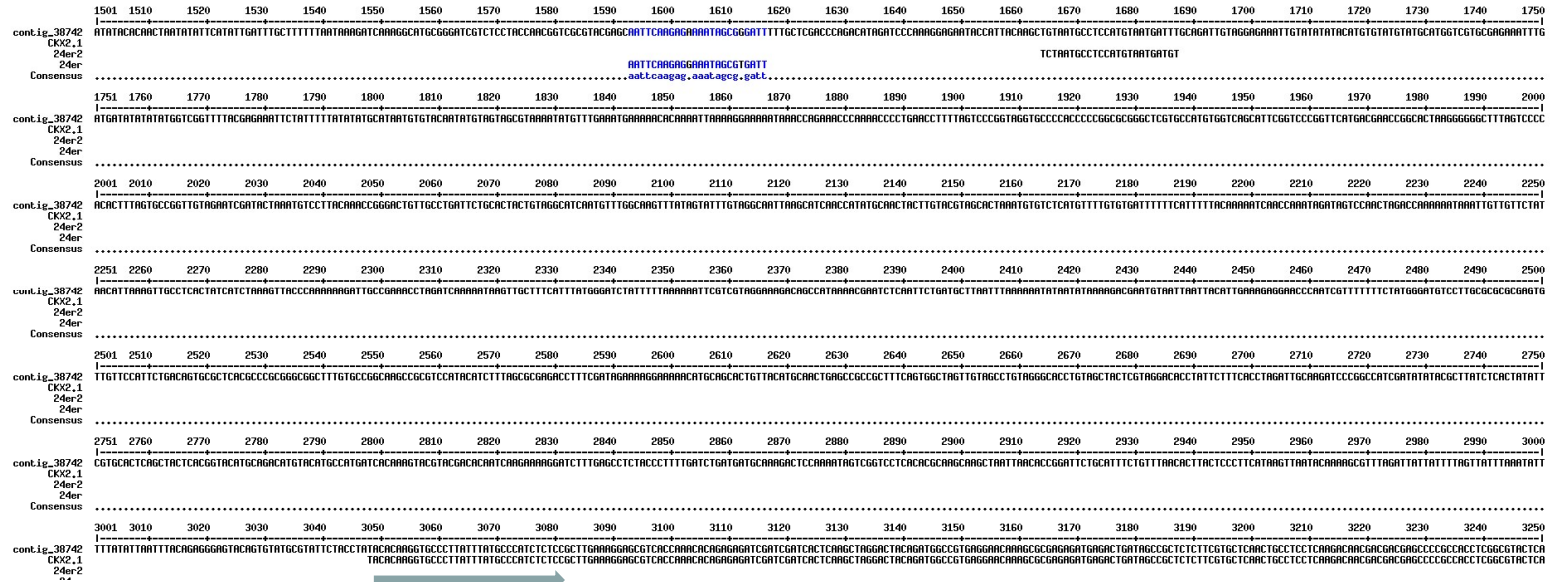
The development of seeds in flowering plants is placed under complex interactions between maternal tissues, the embryo, and the endosperm. The endosperm plays a major role in the regulation of seed size. In *Arabidopsis thaliana*, endosperm size depends on the coordination of the genetic pathway HAIKU (IKU) with epigenetic controls comprising genome dosage, DNA methylation, and trimethylated lysine 27 on histone H3 (H3K27me3) deposition. However, the effectors that integrate these pathways have remained unknown. Here, we identify a target of the IKU pathway, the cytokinin oxidase CKX2, that affects cytokinin signaling. CKX2 expression is activated by the IKU transcription factor WRKY10 directly and promotes endosperm growth. CKX2 expression also depends on H3K27me3 deposition, which fluctuates in response to maternal genome dosage imbalance and DNA demethylation of male gametes. Hence, the control of endosperm growth by CKX2 integrates genetic and epigenetic regulations. In angiosperms, cytokinins are highly active in endosperm, and we propose that IKU effectors coordinate environmental and physiological factors, resulting in modulation of seed size.

27 on histone H3 (H3K27me3) might be responsible for the restriction of endosperm and seed growth (18). However, additional results have questioned this idea and proposed rather that the dosage of AGL62 and associated AGLs are primarily involved in responses to maternal dosage excess (19).

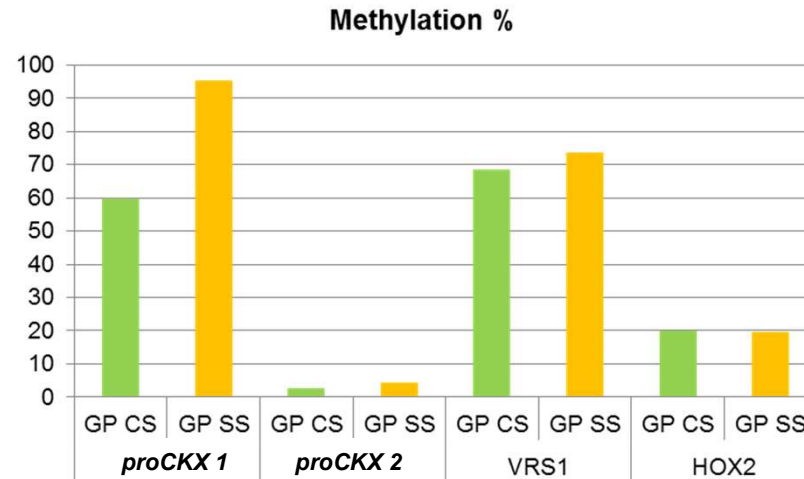
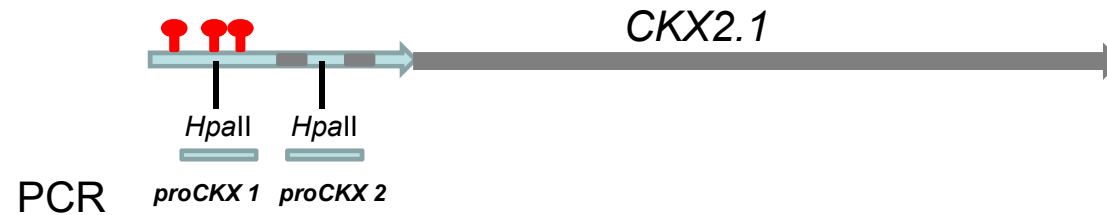
To investigate further the link between epigenetic regulations and endosperm growth, we identified the series of *haiku* (*iku*) mutants that phenocopy the excess of maternal genome dosage and the fertilization of WT ovules by *met1* pollen (20). Molecular analyses of the *iku* mutants have identified three genes (2). *IKU1* encodes a VQ domain protein containing a conserved FxxxVQxLTG (VQ motif) of unknown function (21). *IKU2* and *MIN3* encode a leucine-rich repeat transmembrane kinase and the WRKY10 transcription factor, respectively (22). WRKY proteins constitute a class of transcription factors characterized by the conserved WRKY domain, which contains an almost invariant WRKYGQK sequence followed by a zinc-finger motif (23, 24). All *IKU*-class genes are expressed in the syncytial endosperm after fertilization (21, 22). Double mutants *iku1;iku2* (20) and *iku2;min3* (21) show the same phenotype as single



Position of drought stress specific 24mers upstream of *CKX2.1*

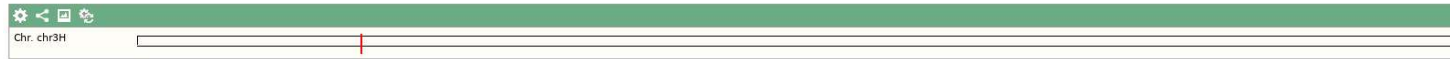


Methylation quantified by MSRE in the region upstream of *CKX2.1*

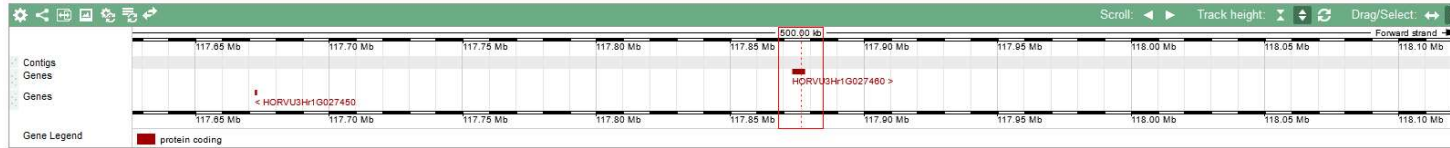


- DNA-methylation in *CKX2.1* promoter region is elevated after drought stress
- => *HvCKX2.1* is transcriptionally active during germination

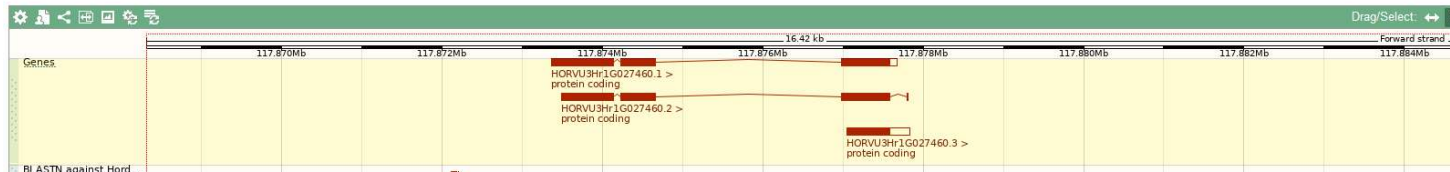
Chromosome chr3H: 117,868,317-117,884,738




Region in detail



Location: chr3H:117868317-117884738 Gene:





Expression Atlas

Gene expression across species and biological conditions

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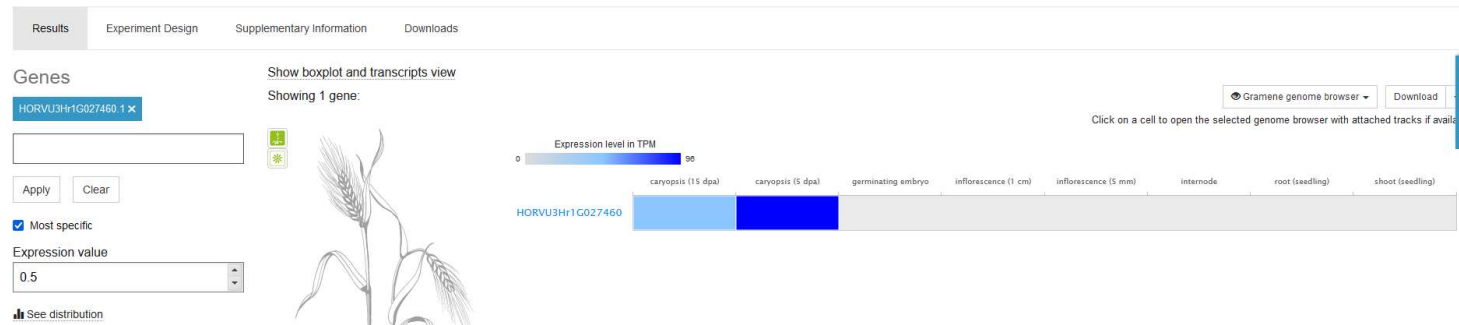
Query single cell expression

RNA-seq of coding RNA of eight barley tissues from different developmental stages

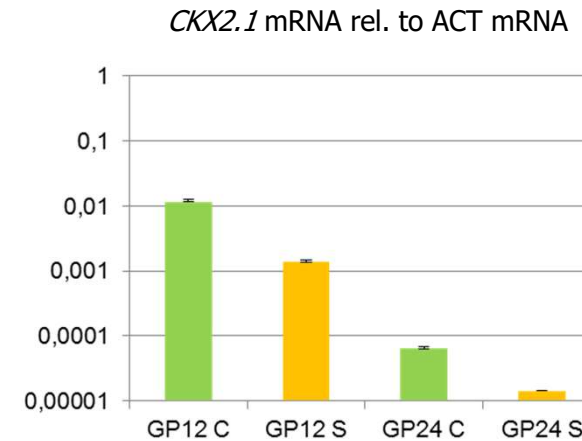
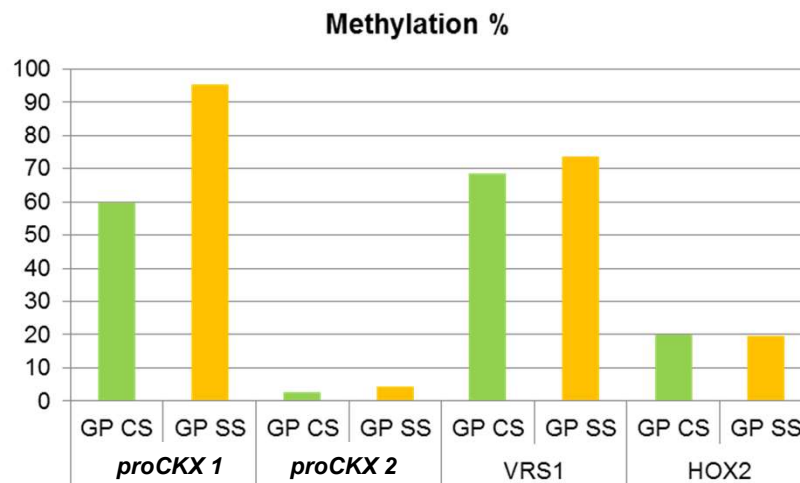
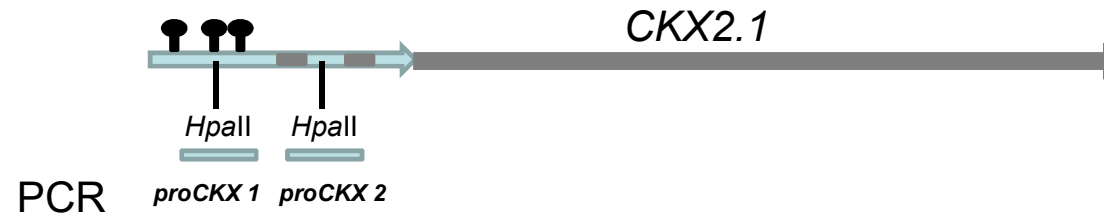
RNA-Seq mRNA baseline

Organism: *Hordeum vulgare subsp. vulgare*

Reference(s): 23075845 (Filter by genes in paper)



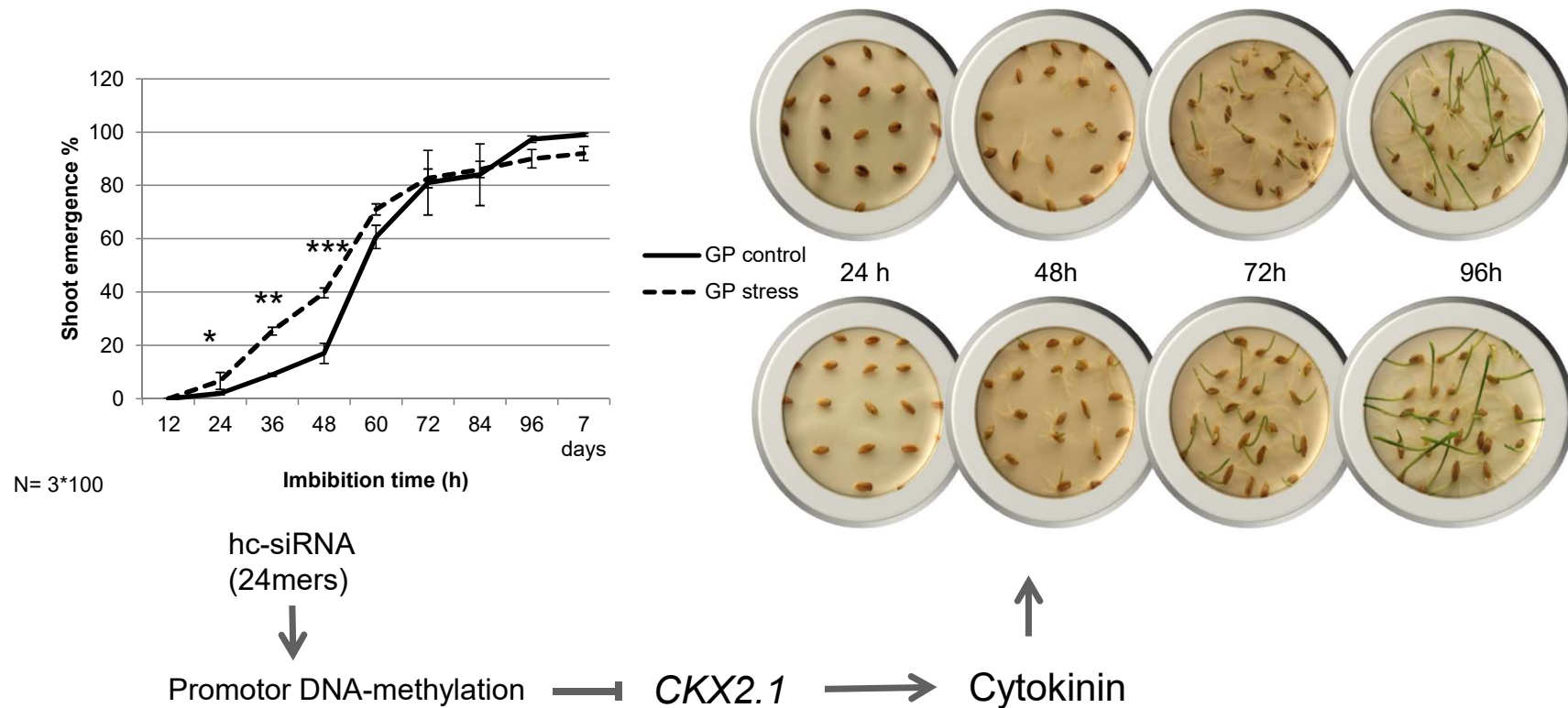
Methylation quantified by MSRE in the region upstream of *CKX2.1*



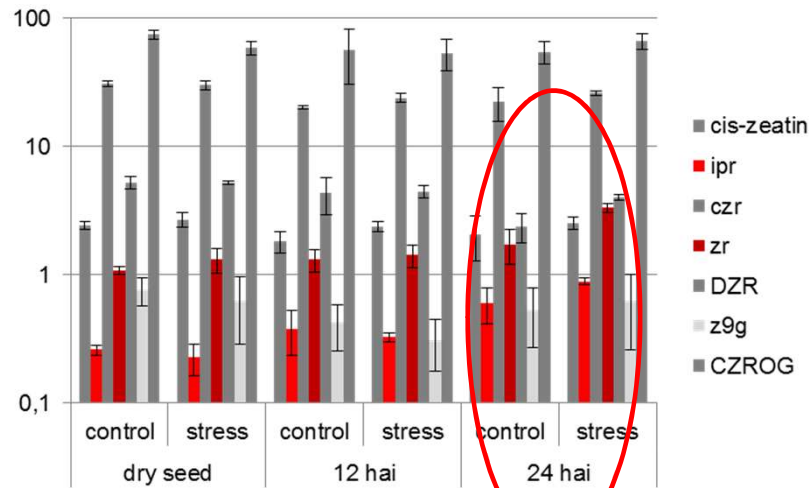
12/24 h after imbibition; N=3

- DNA-methylation in *CKX2.1* promoter region is elevated after drought stress
- Transcription of *CKX2.1* is reduced during imbibition.

Germination speed of barley seeds is increased after application of terminal drought stress on the mother plant

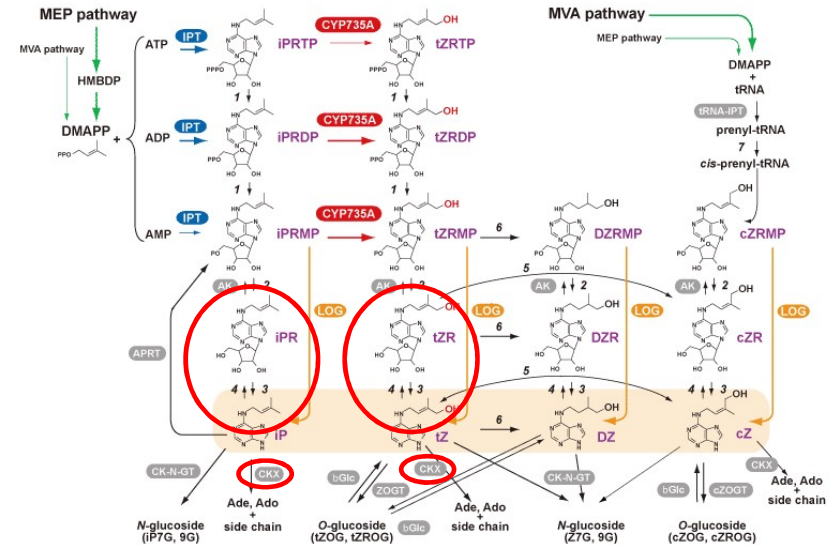


Cytokinin level is elevated in seeds after drought stress applied to the motherplant



N=4
Coll. Kai Eggert, IPK, RG MP

Reduced expression of *CKX2.1* might lead to elevated level of cytokinin



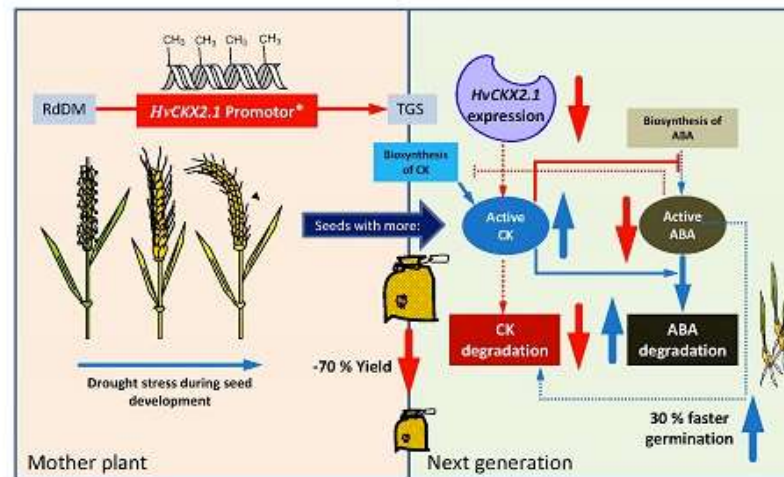
Isoprenoid side chains of *N*⁶-(Δ^2 -isopentenyl)adenine (iP) and *trans*-zeatin (tZ) predominantly originate from the methylerythritol phosphate (MEP) pathway, whereas a large fraction of the *cis*-zeatin (cZ) side chain is derived from the mevalonate (MVA) pathway (green arrows). Plant adenosine phosphate-isopentenyltransferases (IPTs) preferably utilize ATP or ADP as isoprenoid acceptors to form iPRTP and iPRDP, respectively (blue arrows). Dephosphorylation of iPRTP and iPRDP by phosphatase (1), phosphorylation of iPR by adenosine kinase (AK), and conjugation of phosphoribosyl moieties to iP by adenosine phosphoribosyltransferase (APRT) create the metabolic pool of iPRMP and iPRDP. APRT utilizes not only iP but also other CK nucleobases. The CK nucleotides are converted into the corresponding tZ-nucleotides by CYP735A (red arrows). CK nucleoside 5' -monophosphates, such as iPRMP, tZMP, DZMP, and cZMP, are activated to CK nucleobases by two-step reaction catalyzed by 5' -ribonucleotide phosphohydrolase (2) and adenosine nucleosidase (3), or by one-step reaction catalyzed by LONELY GUY (LOG) (orange arrows). iP, tZ, and the nucleosides can be catabolized by CKX to adenine (Ade) or adenosine (Ado). cZ and tZ can be enzymatically interconverted by zeatin *cis-trans* isomerase (5). tZ can be reversibly converted to the *O*-glucoside by zeatin *O*-glucosyltransferase (ZOGT) and β -glucosidase (β Glc). CK nucleobases also can be converted to the *N*-glucoside by CK *N*-glucosyltransferase (CK-N-GT).

The width of the arrowheads and lines in the green, blue, and red arrows indicates the strength of metabolic flow. Flows indicated by black arrows are not well characterized to date. tZRPD, tZR 5' -diphosphate; tZRT, tZR 5' -triphosphate; 2, 5' -ribonucleotide phosphohydrolase; 3, adenosine nucleosidase; 4, purine nucleoside phosphorylase; 6, zeatin reductase; 7, CK *cis*-hydroxylase. Modified from a previous review (Sakakibara 2006).

Article

Increase of DNA Methylation at the *HvCKX2.1* Promoter by Terminal Drought Stress in Barley

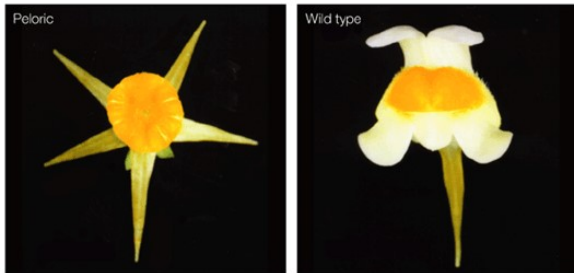
Korana Surdonja ¹, Kai Eggert ², Mohammad-Reza Hajirezaei ²,
Vokkaliga Thammegowda Harshavardhan ^{1,4}, Christiane Seiler ¹, Nicolaus von Wirén ²,
Nese Sreenivasulu ^{1,3} and Markus Kuhlmann ^{1,*} 2017



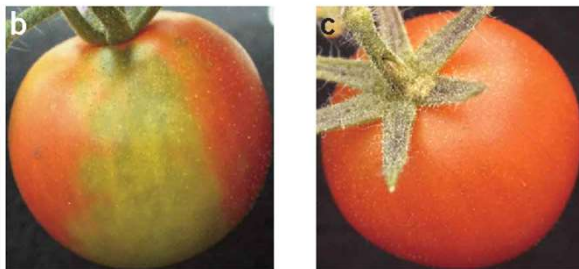
RNA-directed DNA methylation (RdDM) of *HvCKX2.1* promoter leads to stable transcriptional gene silencing (TGS)

„famous“ phenotypes associated with epigenetics (changing pattern of DNA methylation)

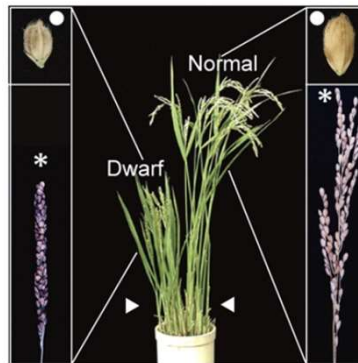
Peloric in *Linaria vulgaris*



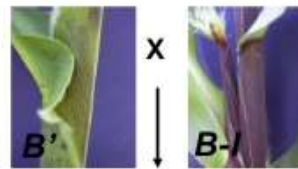
CNR in *Solanum lycopersicum*



Dwarf1 in *Oriza sativa*



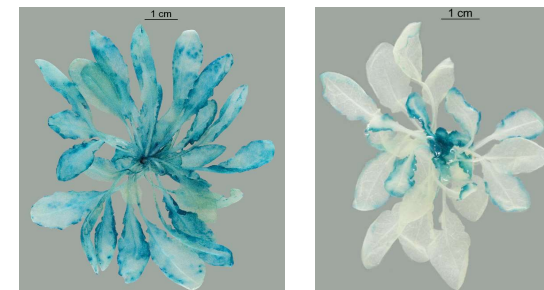
Paramutation (B) in *Zea mays*



DFR in *Petunia*



GUS (transgene Reporter) in *Arabidopsis thaliana*



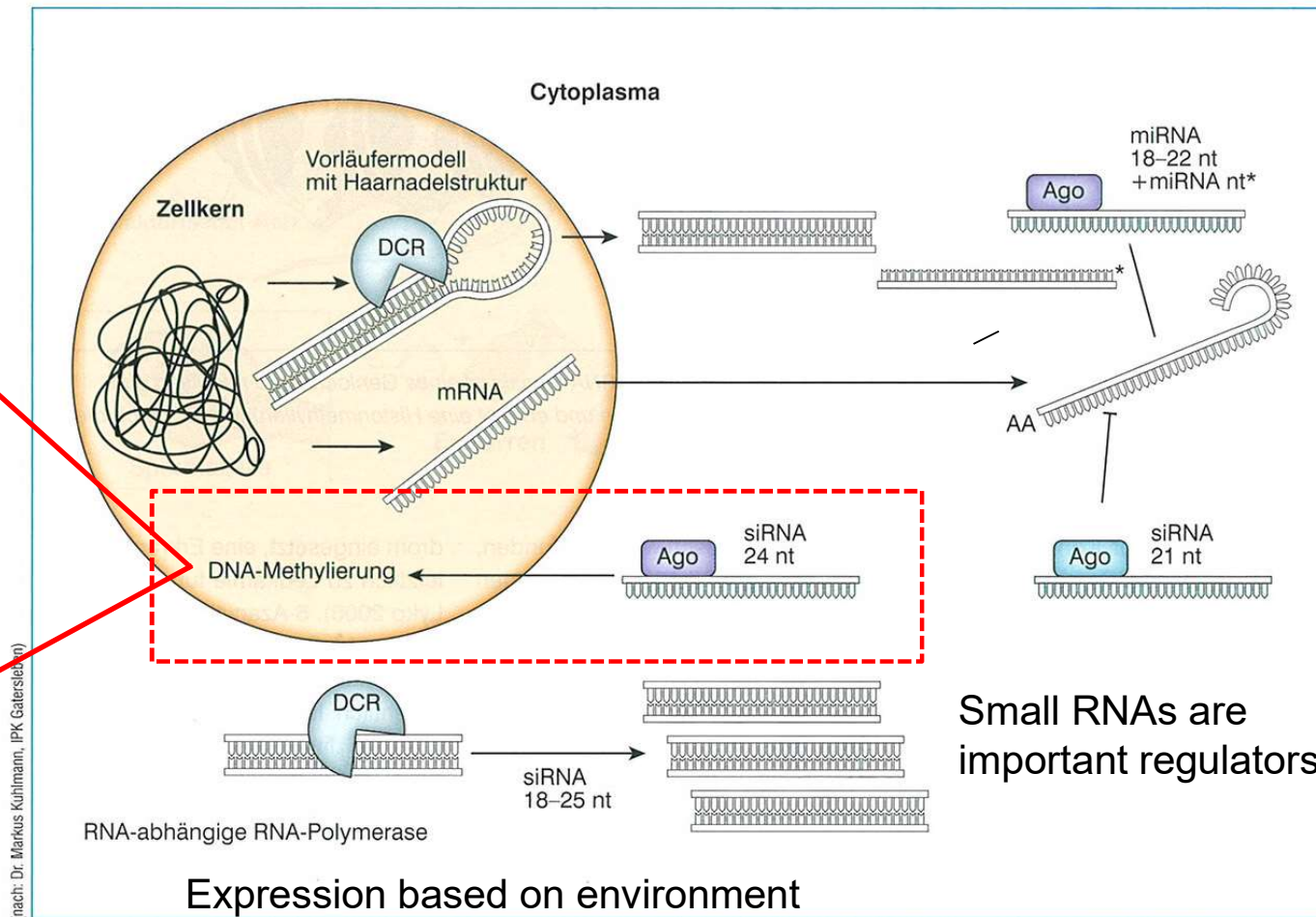
Take home message

DNA Methylation is regulated via

De novo

Maintenance

Demethylation



Thanks to

RG Heterosis

Thomas Altmann
Rhonda Meyer
Lothar Altschmid



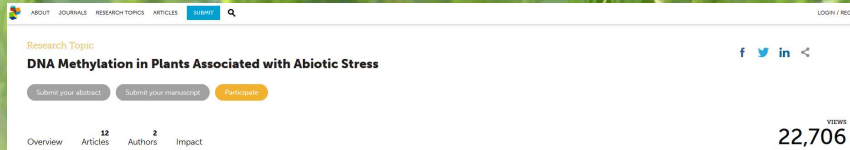
...and former
RG Abiotic Stress Genomics
Korana Surdonja



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