

通过调控糊粉层发育提高 水稻种子营养品质

刘金鑫

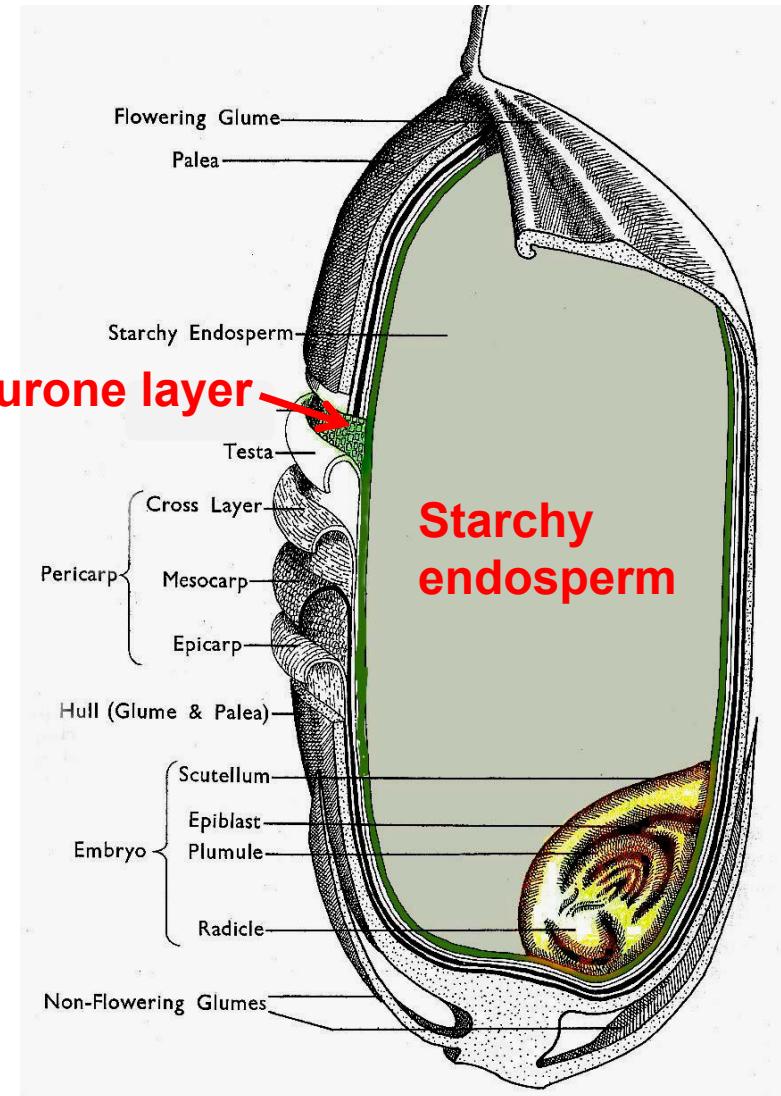
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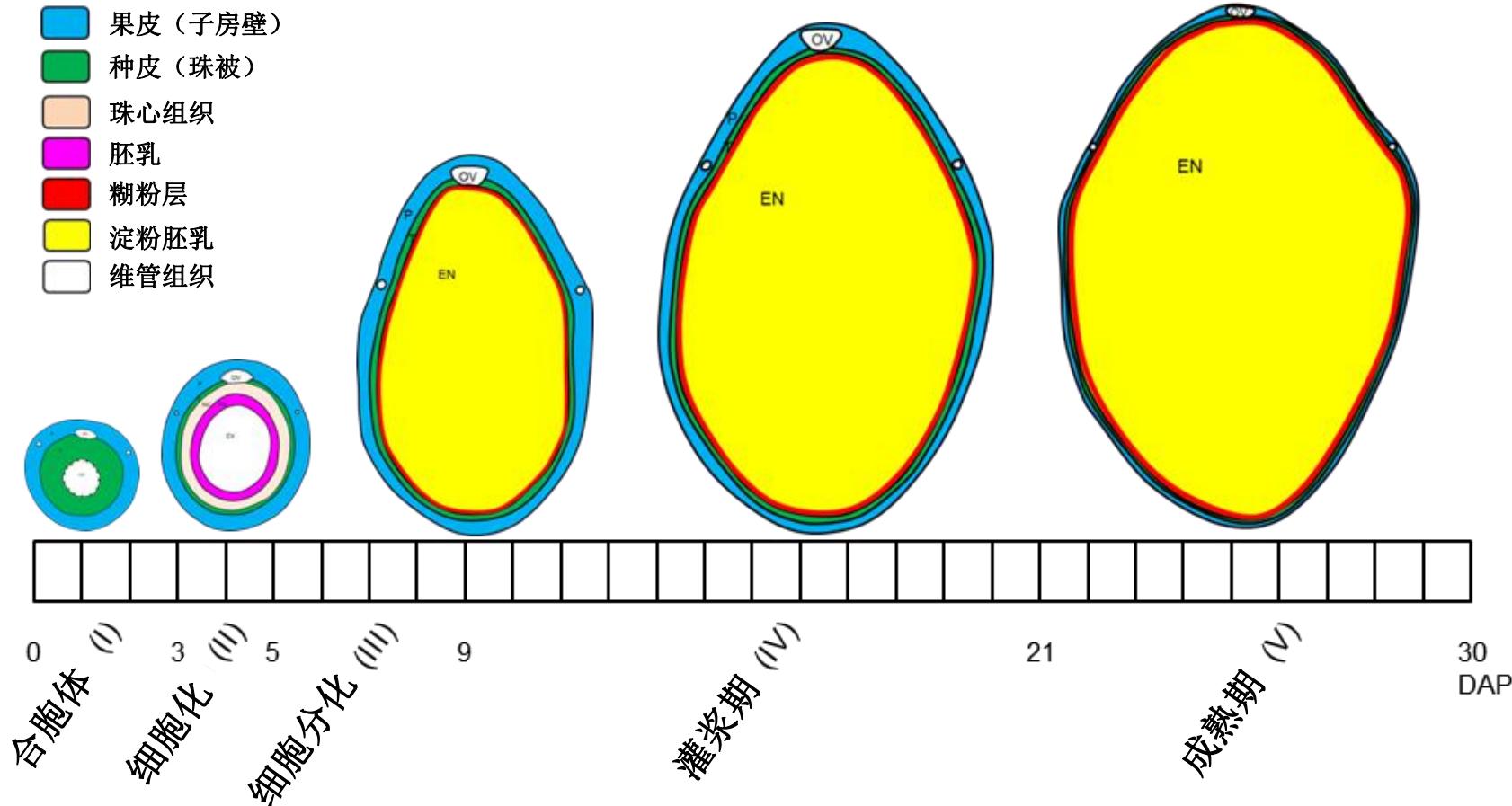
2019.8.8

水稻种子的结构

- Palea (内稃) and lemma (外稃)
- Pericarp (果皮)
- Testa (种皮)
- Embryo (胚)
- Endosperm (胚乳)
 - Starchy endosperm (淀粉胚乳)
 - Aleurone layer (糊粉层)

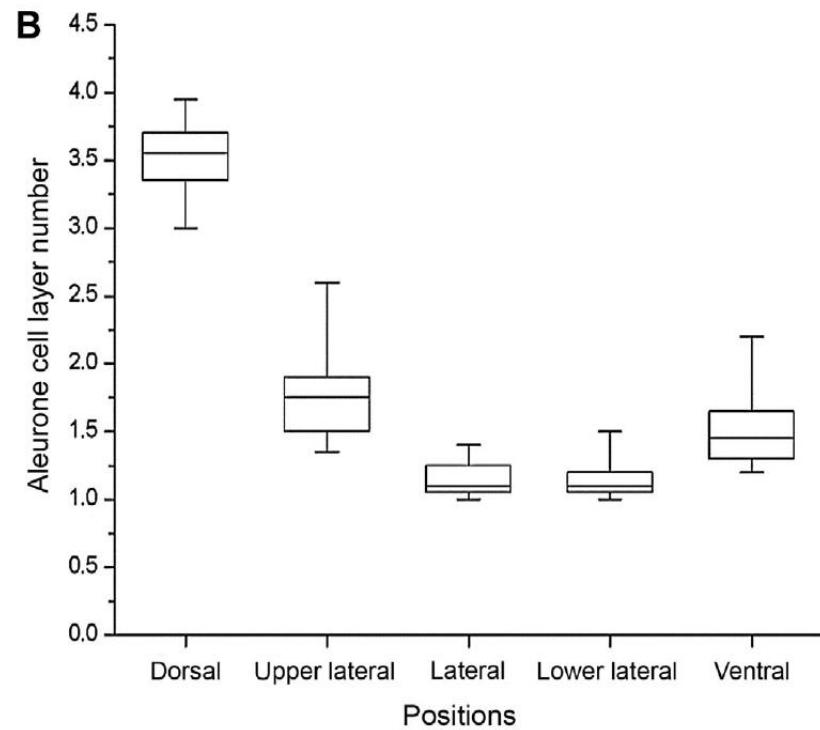
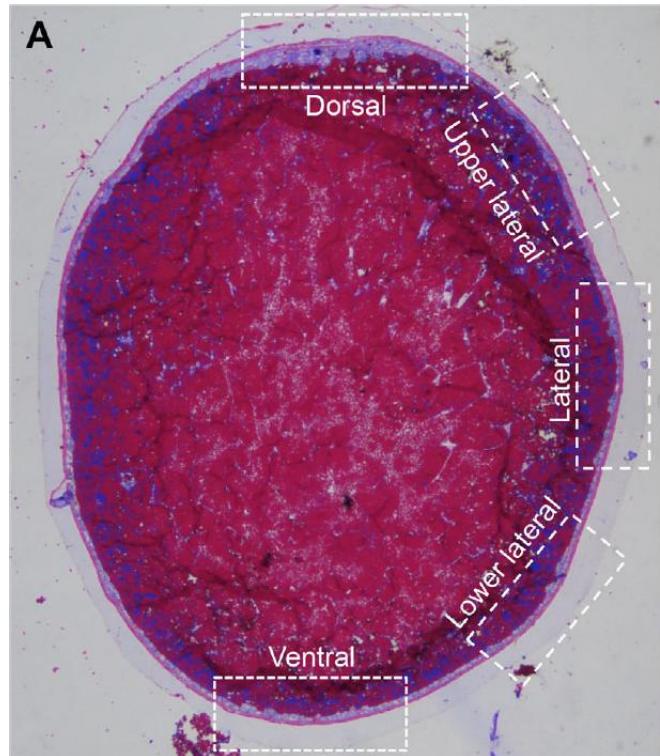


水稻胚乳发育时期



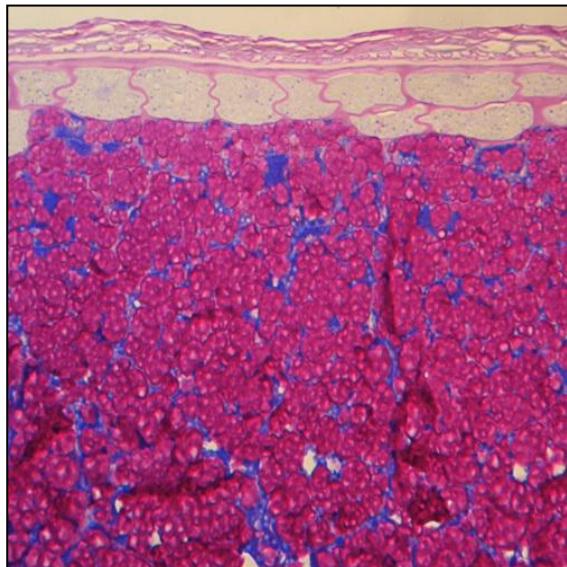
Modified from Wu et al., JIPB, 2016a,
2016b

水稻胚乳不同位置的糊粉层细胞



Wu et al, JIPB, 2016b

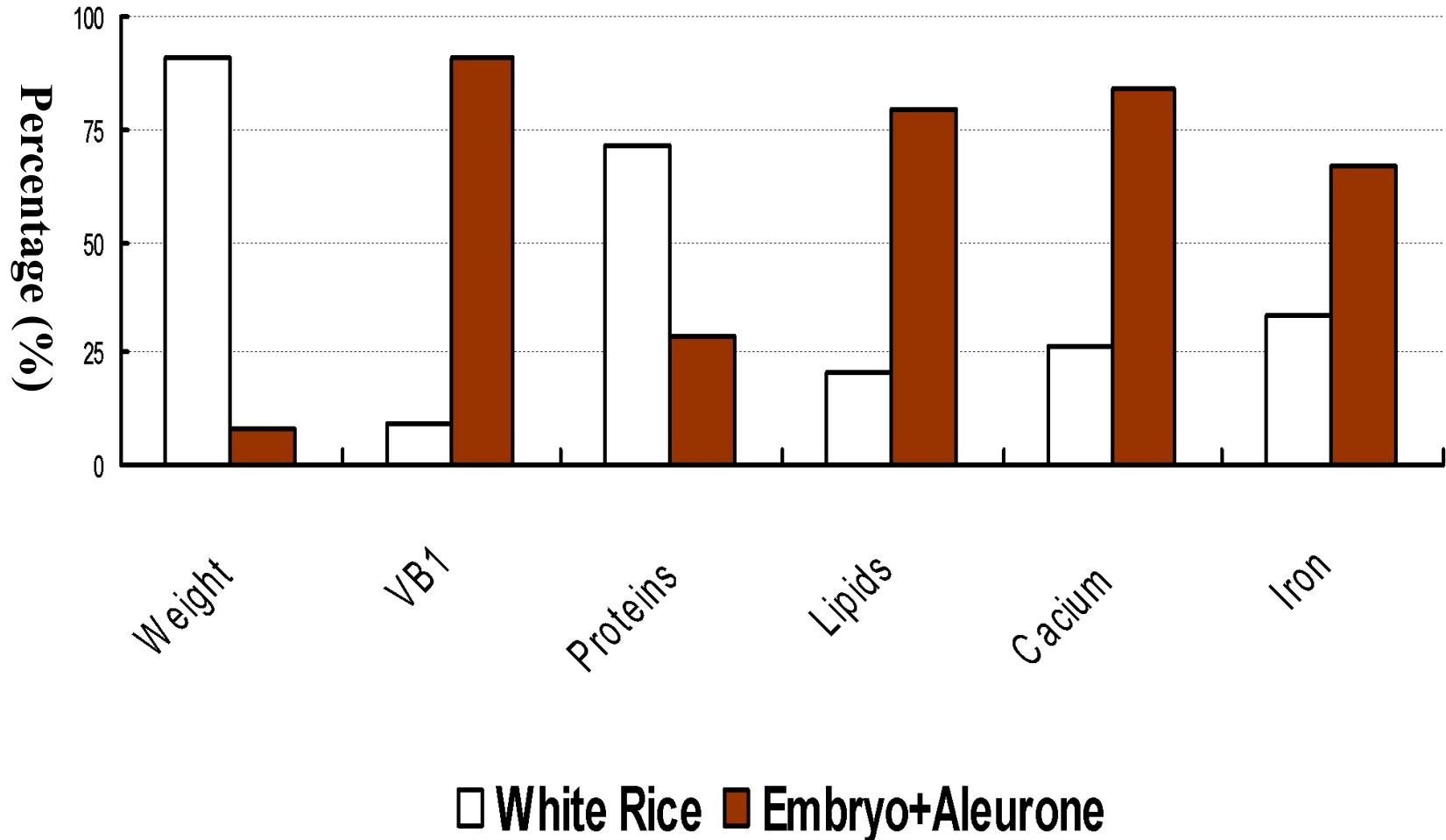
截然不同的细胞命运和营养累积



Aleurone (live cell)
Nutrition: proteins,
lipids, vitamins,
micronutrients

Starchy endosperm
(PCD)
Nutrition: starch,
proteins

水稻种子营养储存分布



食用保留糊粉层和胚的糙米均衡营养



水稻种子



糙米



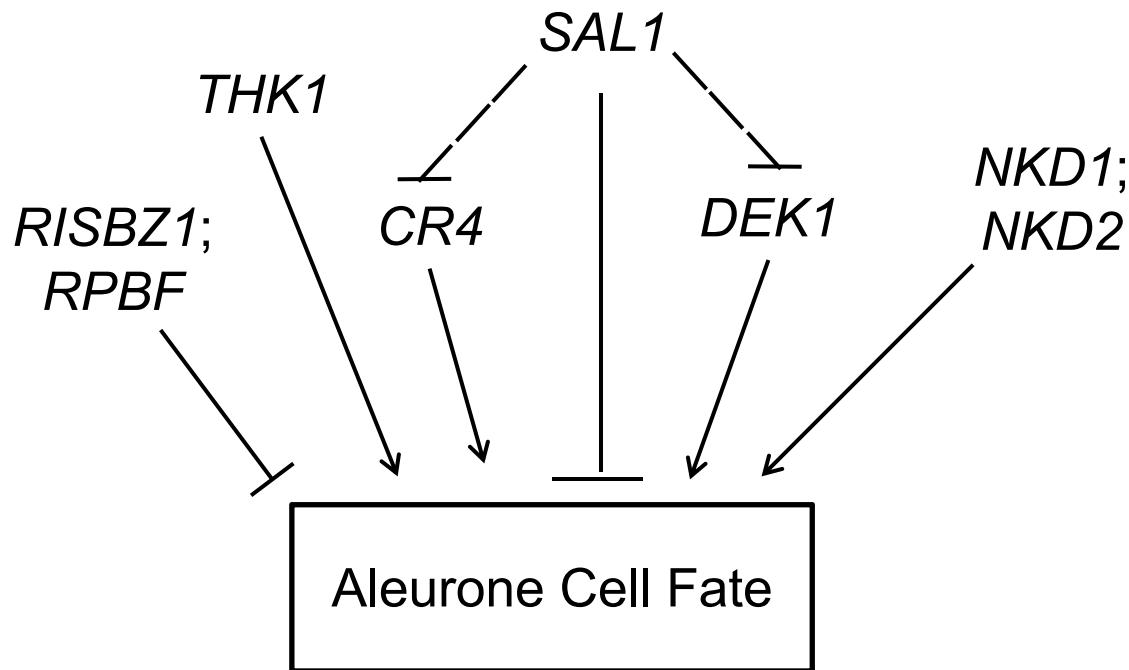
白米

脱壳

抛光

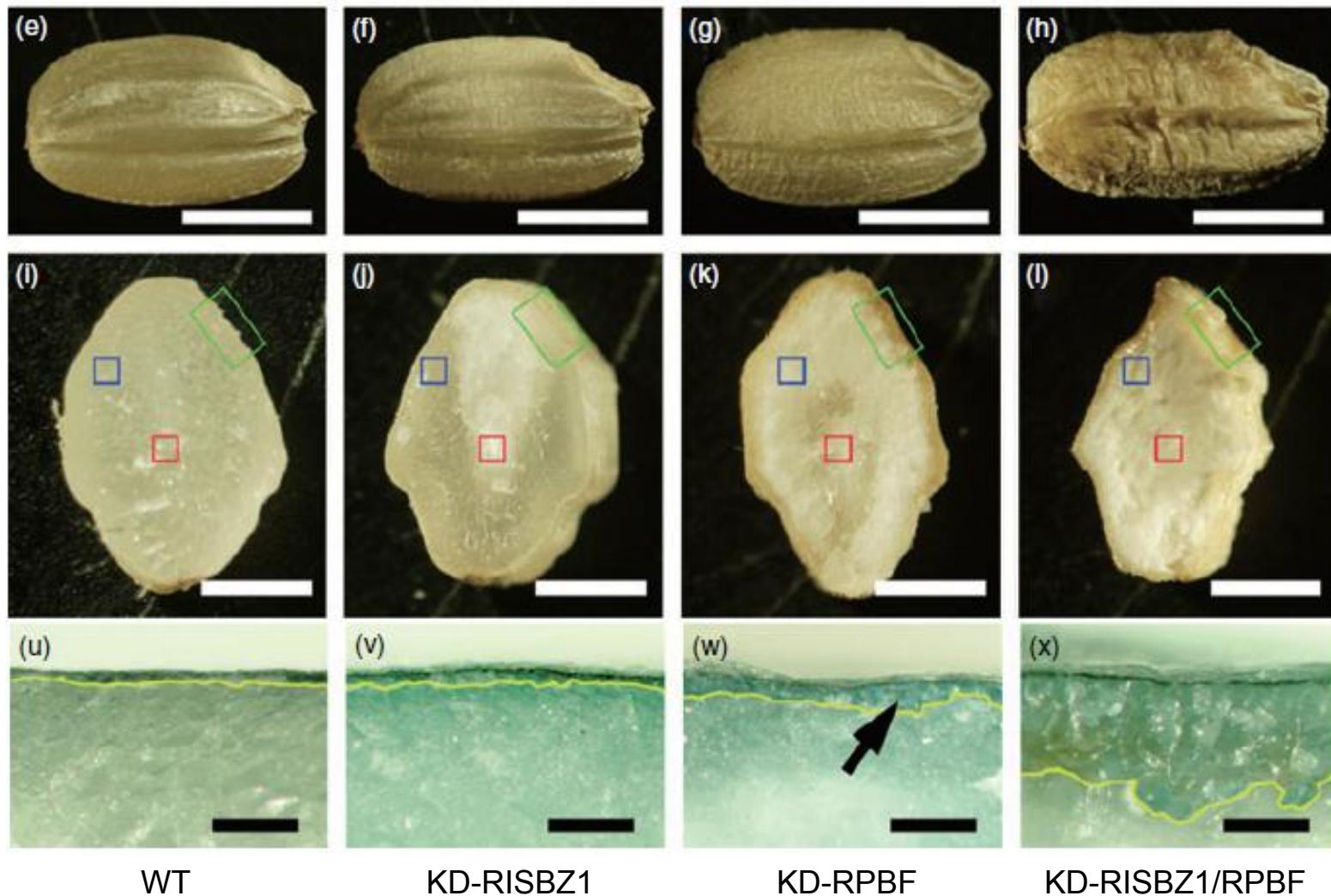
- 白米是水稻的淀粉胚乳，是其能量储存单元
- 糊粉层是水稻的主要营养元素如蛋白质、不饱和脂肪酸、维生素和微量元素等主要储存场所

胚乳糊粉层发育的基因调控网络

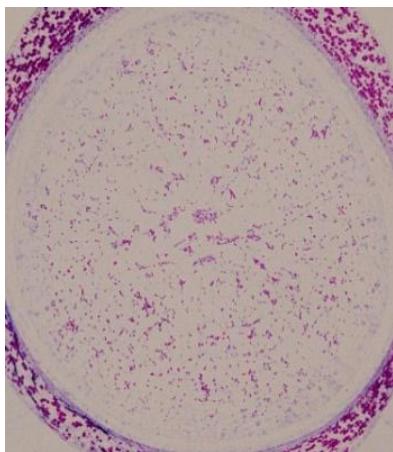


- Becraft et al., science 2002
Lid et al., PNAS 2002
Shen et al., PNAS 2003
Yi et al., Plant Physiol 2003
Kawakatsu et al., Plant J 2009
Hibara et al., Dev Biol 2009

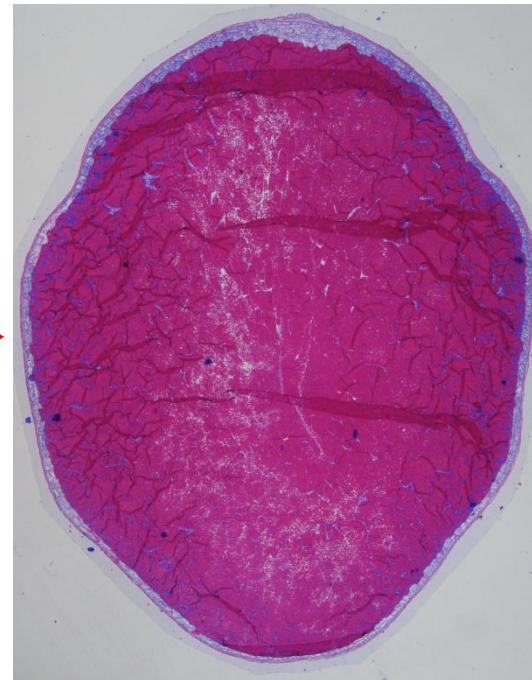
抑制*RISBZ1*和*RPBF*表达导致水稻糊粉层加厚表型



解析水稻胚乳糊粉层细胞分化的分子机制



?

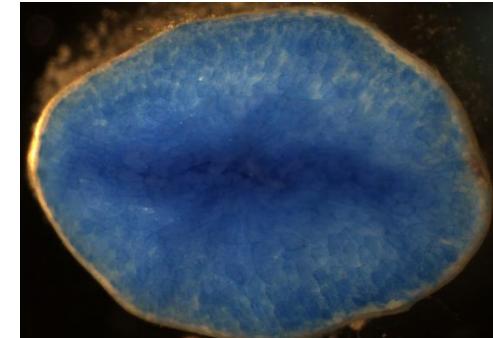
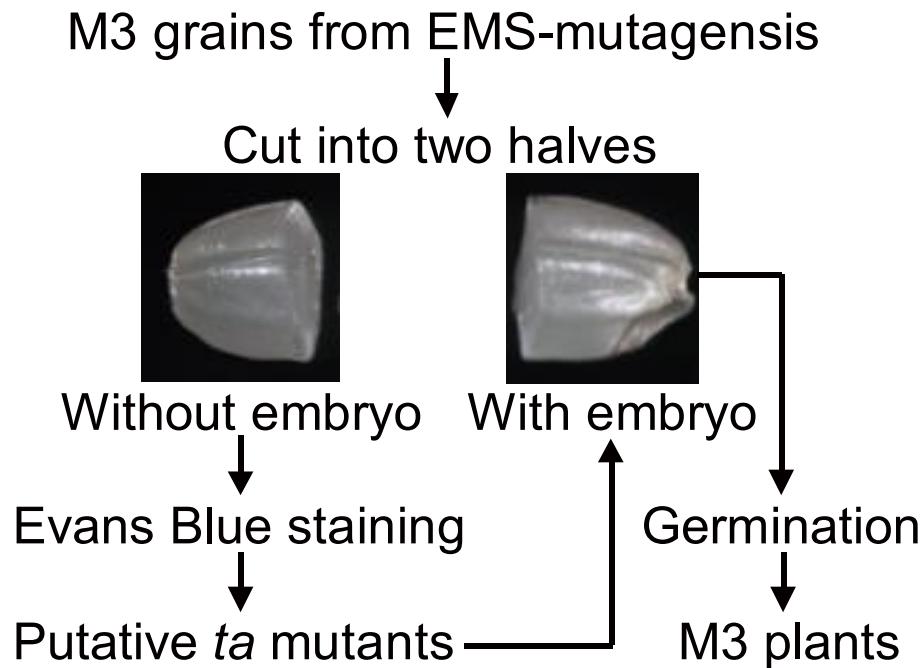


哪些关键基因参与调控其分化过程？

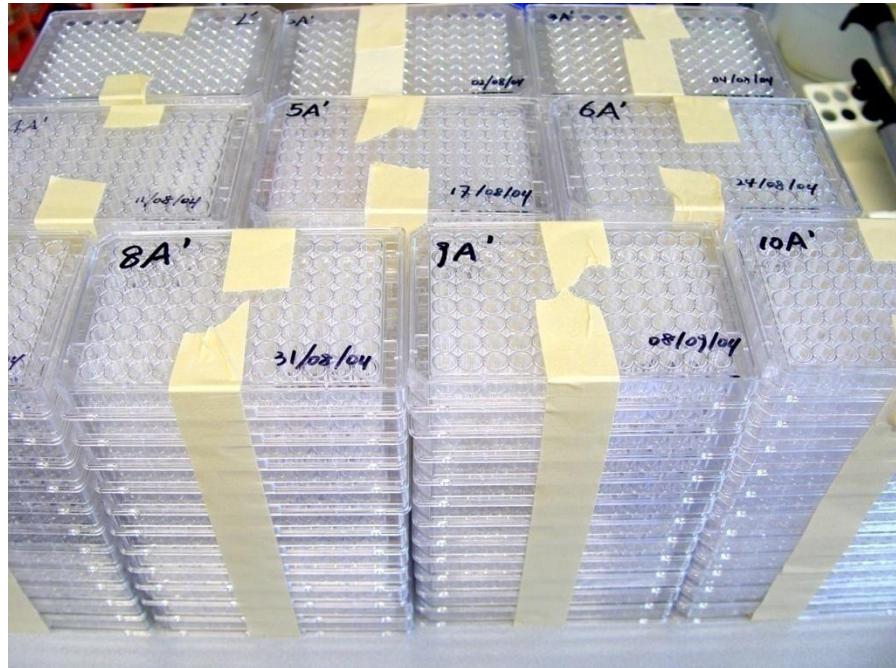
水稻糊粉层和淀粉胚乳细胞分化的分子机制？

如何通过改造胚乳结构改善水稻健康品质？

水稻半粒种子筛选方法



水稻半粒种子筛选方法



germline collections



PCD assay plates



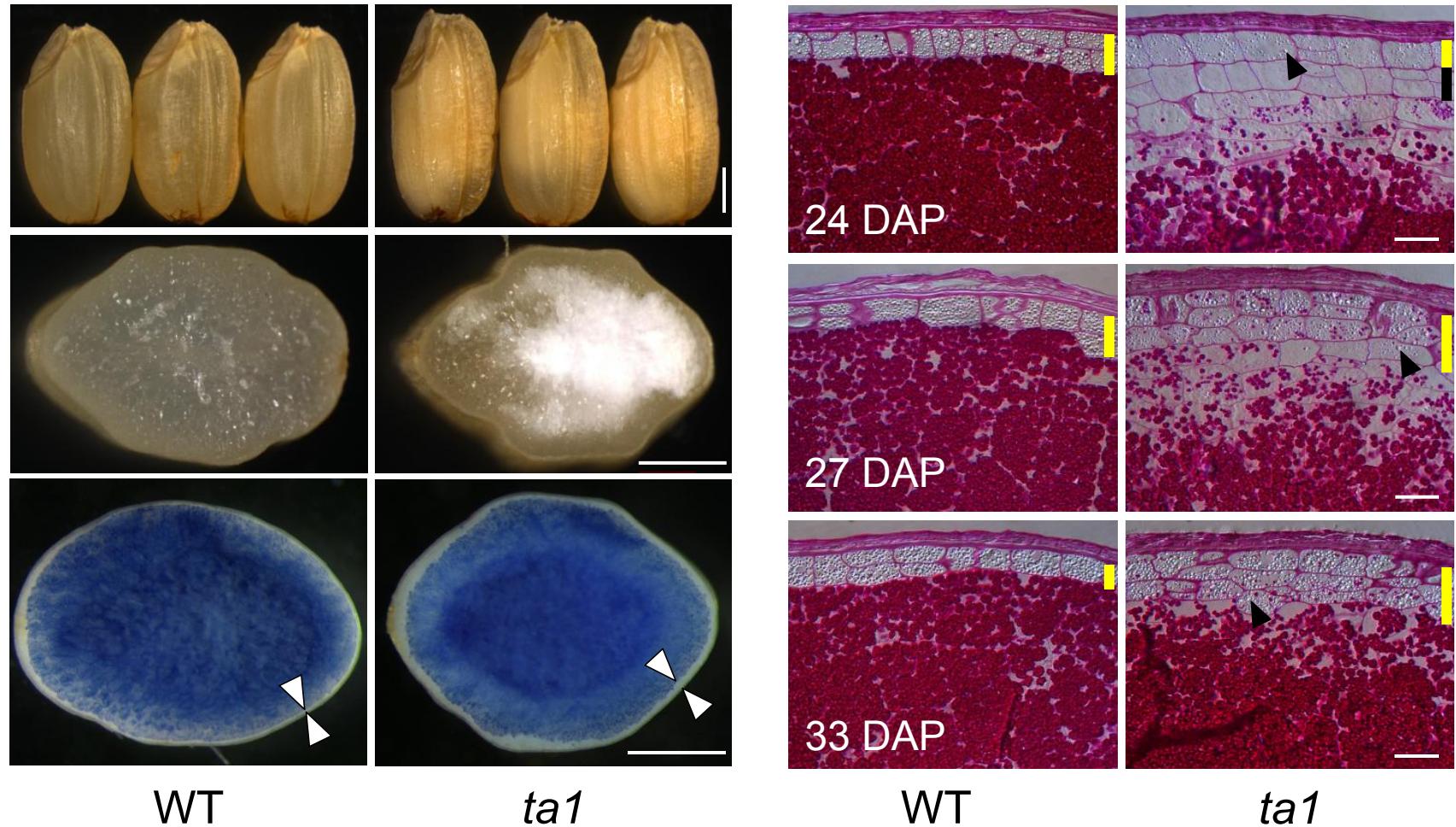
获得预选 *ta* 突变体 》 体外萌发

获得糊粉层加厚突变株系(*thick aleurone, ta*)

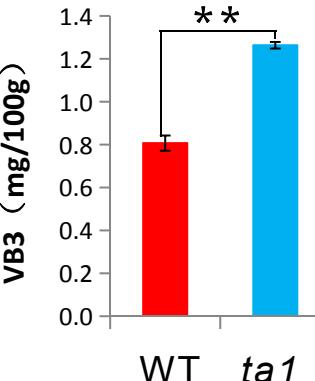
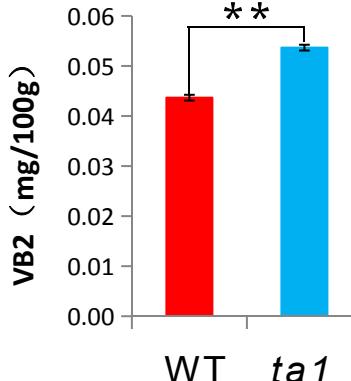
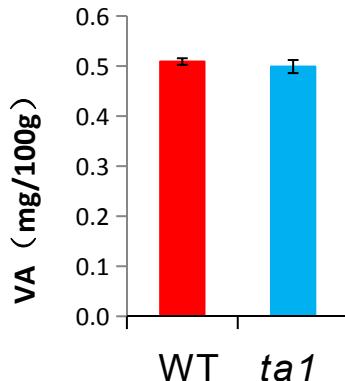
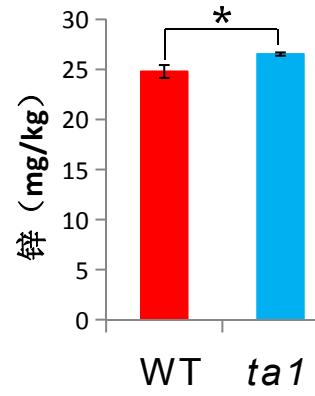
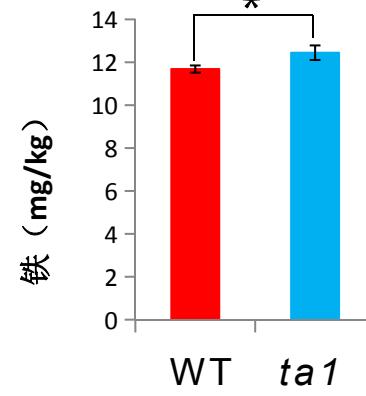
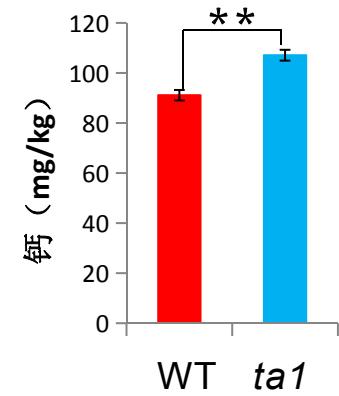
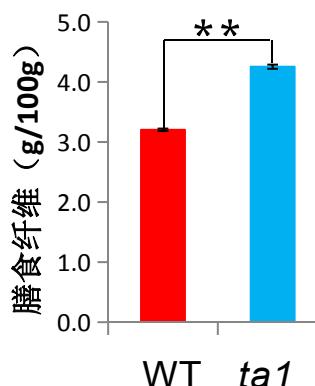
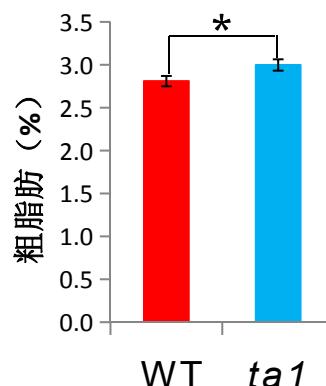
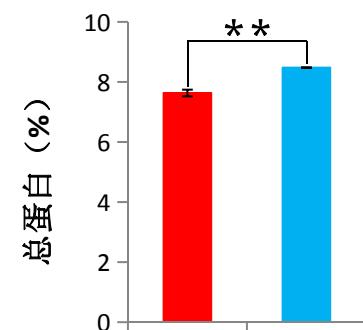
M2 lines screened	8,925
M3 seeds screened	36,000
Candidate <i>ta</i> obtained	23
Confirmed <i>ta</i> mutants	2

(Background material: Zhonghua 11, japonica type)

*ta1*胚乳呈现糊粉层加厚表型



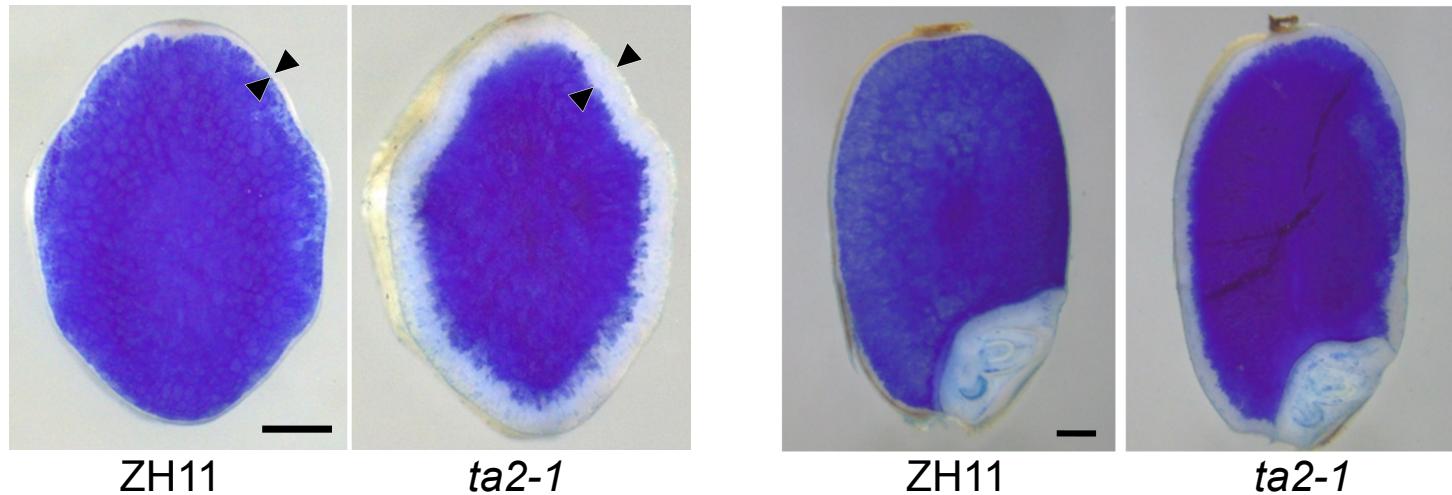
*ta1*成熟颖果营养物质含量增加



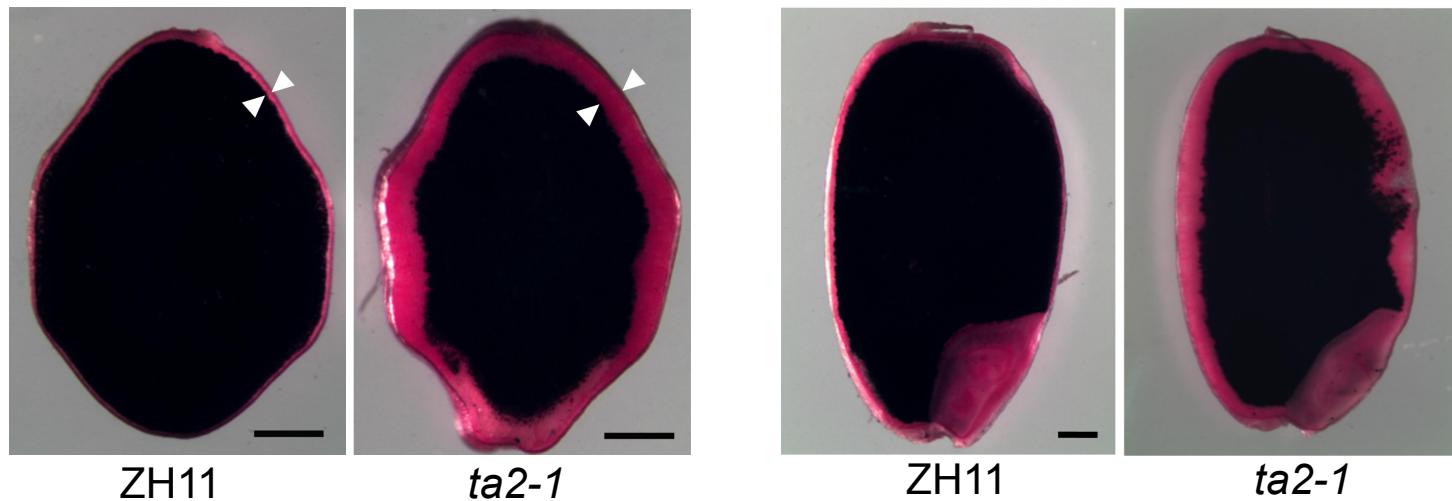
Unpublished data

*ta2-1*突变体糊粉层明显加厚

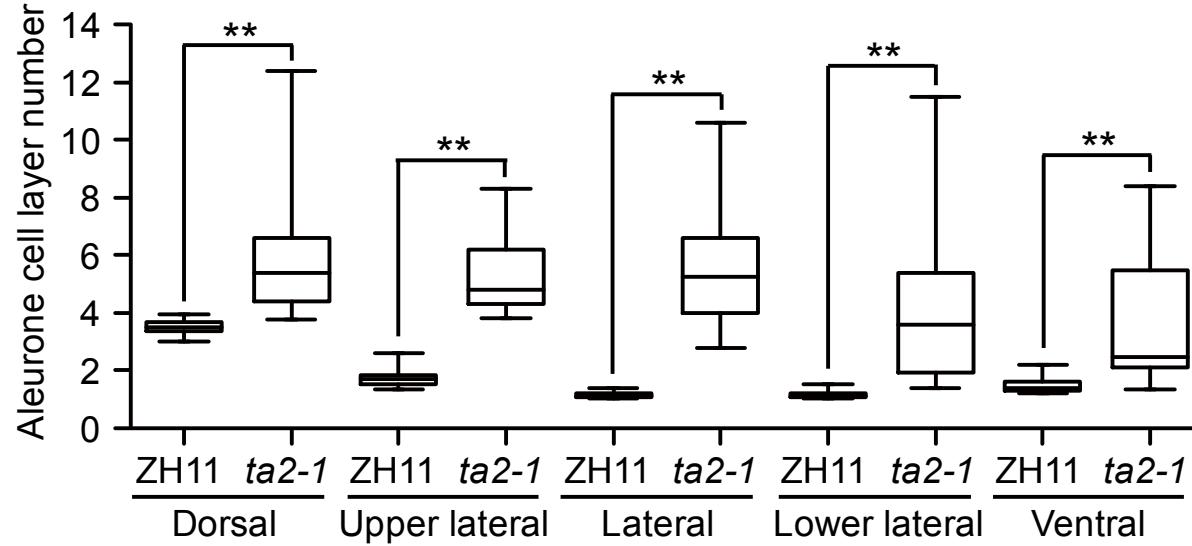
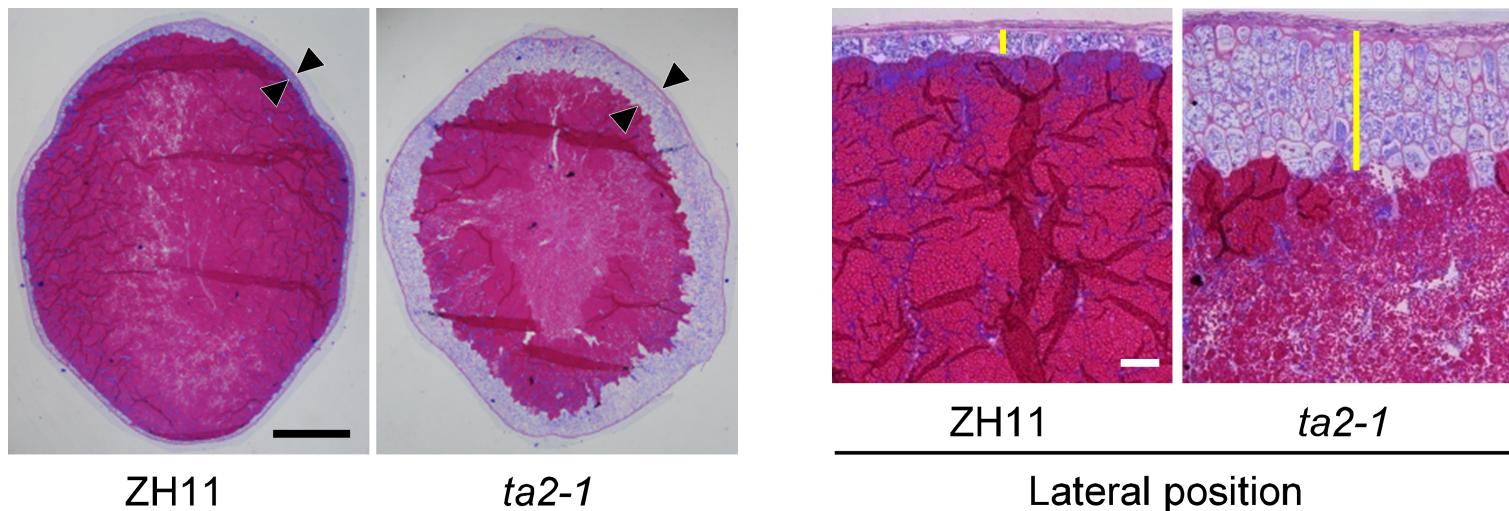
Evans Blue



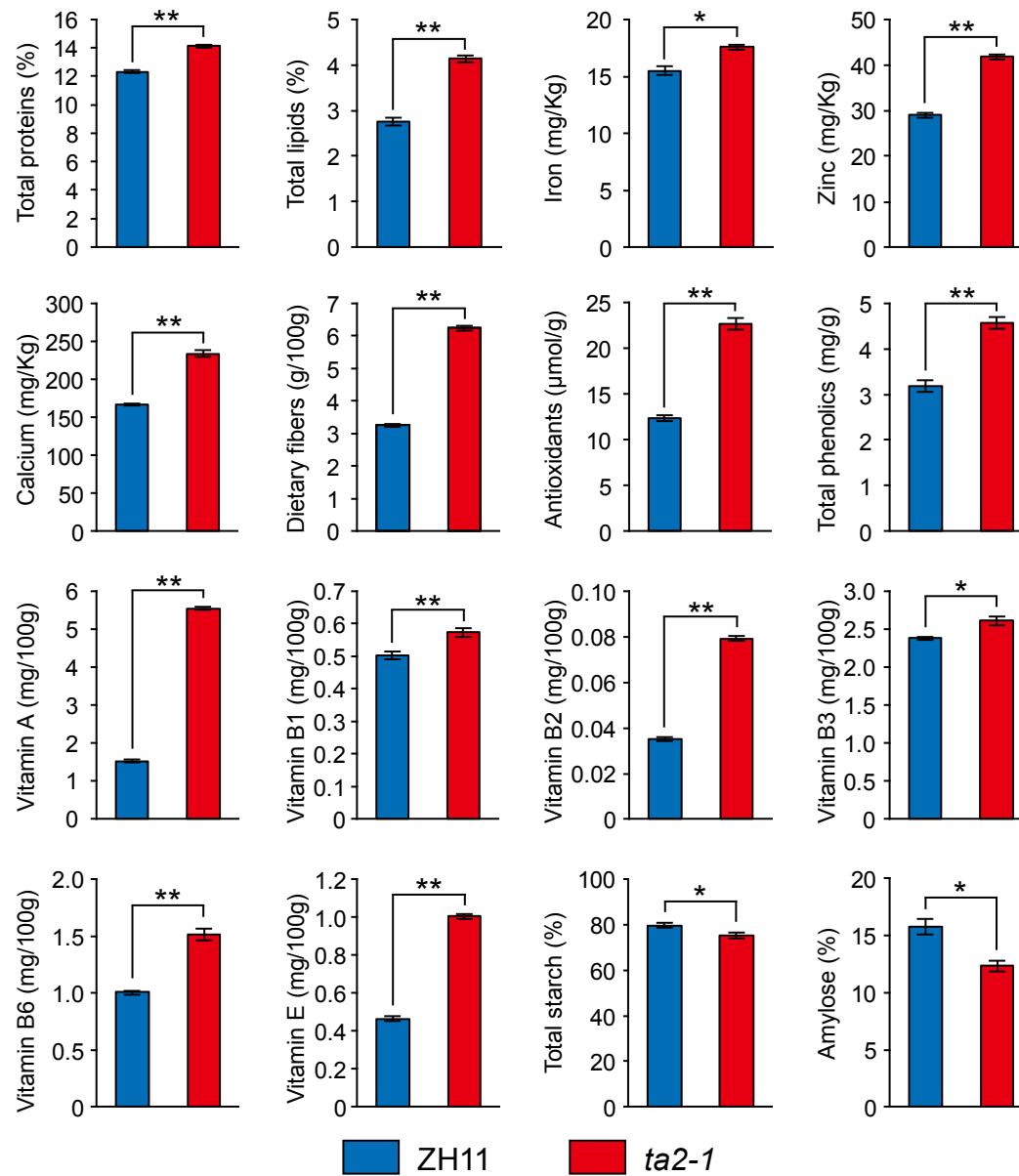
Lugo + Sudan Red



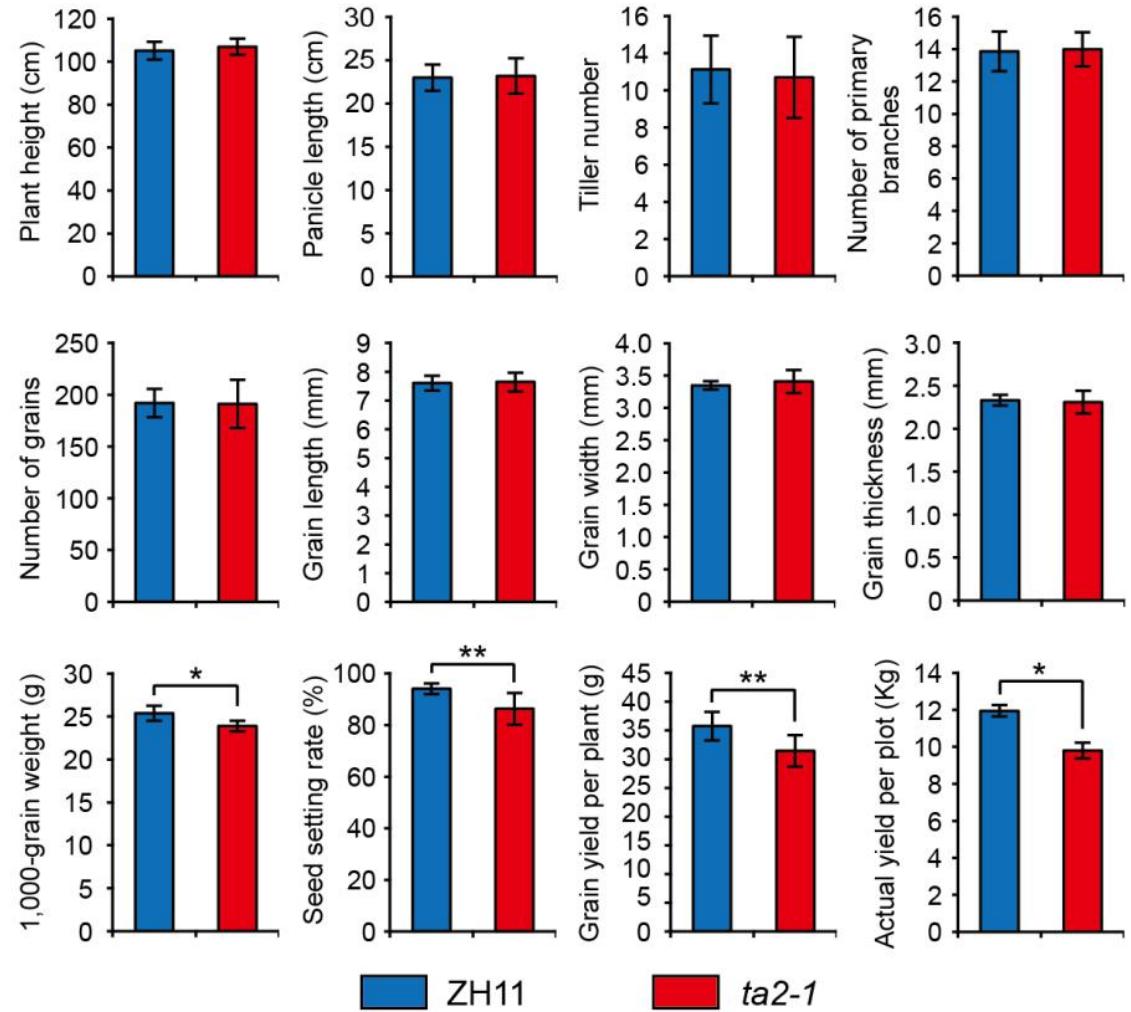
*ta2-1*糊粉层细胞层数显著增加



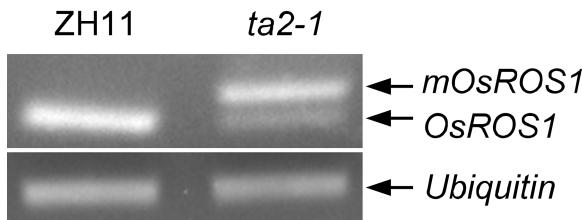
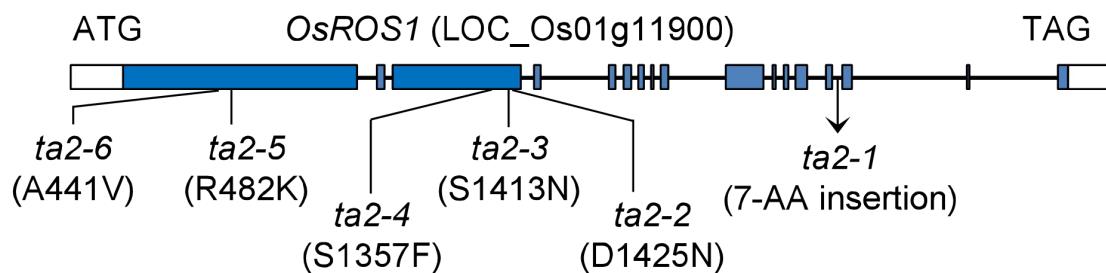
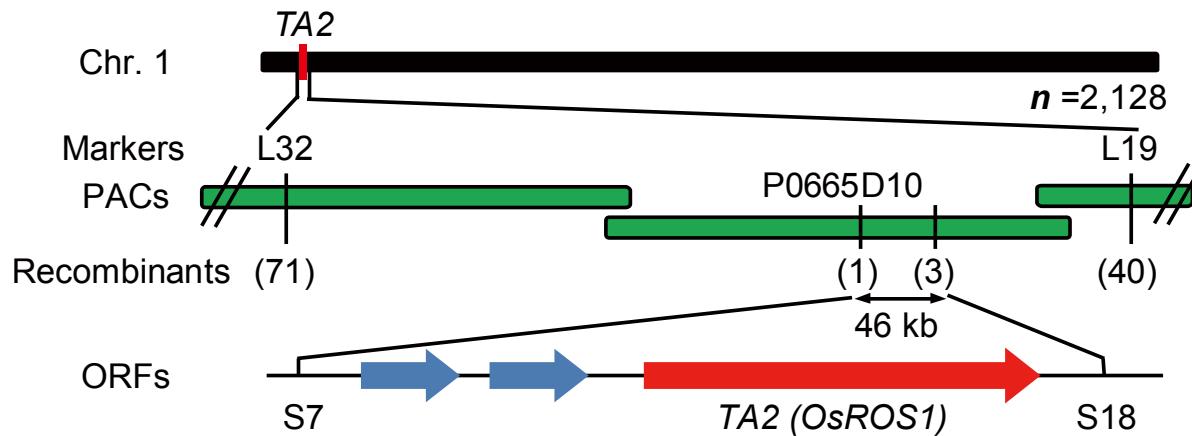
*ta2-1*糙米中营养成分含量明显提升



*ta2-1*的主要农艺形状



TA2 编码DNA去甲基化酶OsROS1



	N	E	-	-	-	-	-	-	-	V	F
OsROS1	AAT	GAG	-	-	-	-	-	-	-	GTA	TTT
<i>mOsROS1</i>	AAT	GAG	TGT	TCA	AAT	GTT	ATG	CGG	CAG	GTA	TTT
	N	E	C	S	N	V	M	R	Q	V	F

mOsROS1在C-terminal domain插入7个氨基酸

OsROS1	VGTQNAVEAEDGSLEGVSSENSTISQSNSDYLFLMDSMSLNLTAEDIGSRNMPKHAIRTYTELLRMQELQNSNSETIYSEHGVPHCSCNNIKHLNGQHLSLHSISVHQHLDQVWHLPLDVHSAESLTYCQTGNNLRLVDLSNTQTSVPSHMPGIACNNETQKADSLSNMLYGIIDRSRDKTTSLSLEPTI	1261
OsROS1b	VGTQNAVEAEDGSLEGVSSENSTISQSNSDYLFLMDSMSLNLTAEDIGSRNMPKHAIRTYTELLRMQELQNSNSETIYSEHGVPHCSCNNIKHLNGQHLSLHSISVHQHLDQVWHLPLDVHSAESLTYCQTGNNLRLVDLSNTQTSVPSHMPGIACNNETQKADSLSNMLYGIIDRSRDKTTSLSLEPTI	1261
OsROS1bGSLEGVLSSQNSVSPRNFSKYLNGITYIMGSSSLVKTQEVGSSGCQHCVQSVLPITSDLNKAAFPDLDTTYQICLGDHGVNISDEVAQSEVS.....LYQQHPIFASINRNKK.....AKVTDYSSGFLYDNK...DGSLSQMHYSSFFQ...PSQEAECS.....AT	1088
OsROS1cHEITZIDHHAKSISIDSFAVELTACHMNLNQHAFQKEISLSVSVTISESILQFGFLPSLSDMHNRNVFGSISUQTSQGSNFSDLGSNDLGNQDNTANETEYHGKRAAINTNTVYDFEGPISGSSLYTFPSADICLHQDRNDHIVSSTSFCNSC1CSASS.	1194
OsROS1dHEITRDNHAKSISIDSFAVELTACHMNLNQHAFQKEISLSVSVTISESILQFGFLPSLSDMHNRNVFGSISUQTSQGSNFSDLGSNDLGNQDNTANETEYHGKRAAINTNTVYDFEGPISGSSLYTFPSADICLHQDRNDHIVSSTSFCNSC1CSASS.	1161
OsMLJ3aPKRHKNAEACSMSLNLQDMLRNLNLLNEQSGWIDBGS.....HMSDNVFLAQNQNSGNVPLLADQHGDEQFCQHCOLQSLAQCS.....	592
OsMLJ3aHMSDNVFLAQNQNSGNVPLLADQHGDEQFCQHCOLQSLAQCS.....	378
DME	CSCSK5DAEFTTCTCE1VKTGTSQSVCQGSPNLSEICLQGRNPHYERGGSUQKQETINAVQKPFDRDLCMNNWSVCFEGPKFRNDTCQNCITPFSSEYYEQCATRGPHVHLLIDFGMGEGLGYSWMSISLSPRVDRVNRNFRPFYRQGGSVPREFTQGCLIPFSTH.	1323
ROS1PCHNNHSVTNLNTQFDEEKD.....YVSEMSNTSSELSAIESAHESVDTKTSKVEDSDRSRKS.....SVEUDTIDEKCVNLFFPSEDA.....	741
DML2GETILNLDFRIG.....VSTRIRNPFRIVIIIZIDDENDIADVCQSSESKTSDS.....SITSADQSKMLIDP.....FNTVLMMNEQVDSQM/VKGSHGHIPIFYIDDLNDLNSLQGIS.....	714
DML3LSYIEEPQDAKSECIIDSEISKVEHEH.....	483

ta2-4 (S1357F)

ta2-3 (S1413N) ta2-2 (D1425N)

Glycosylase domain

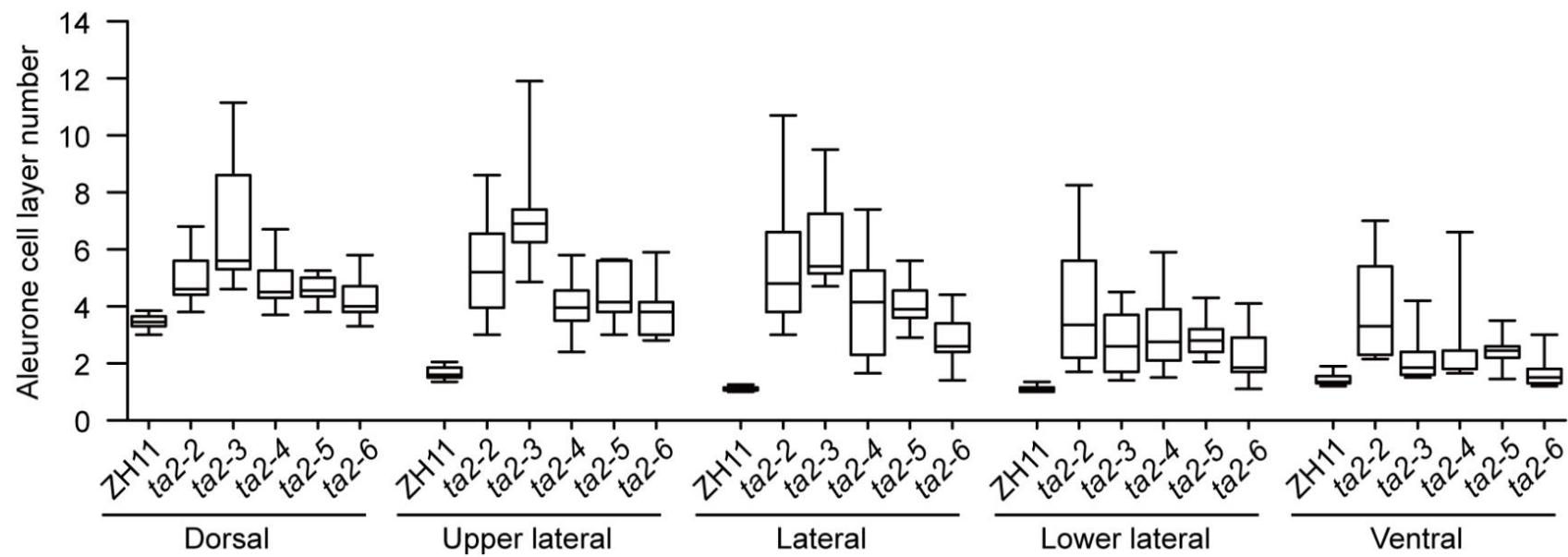
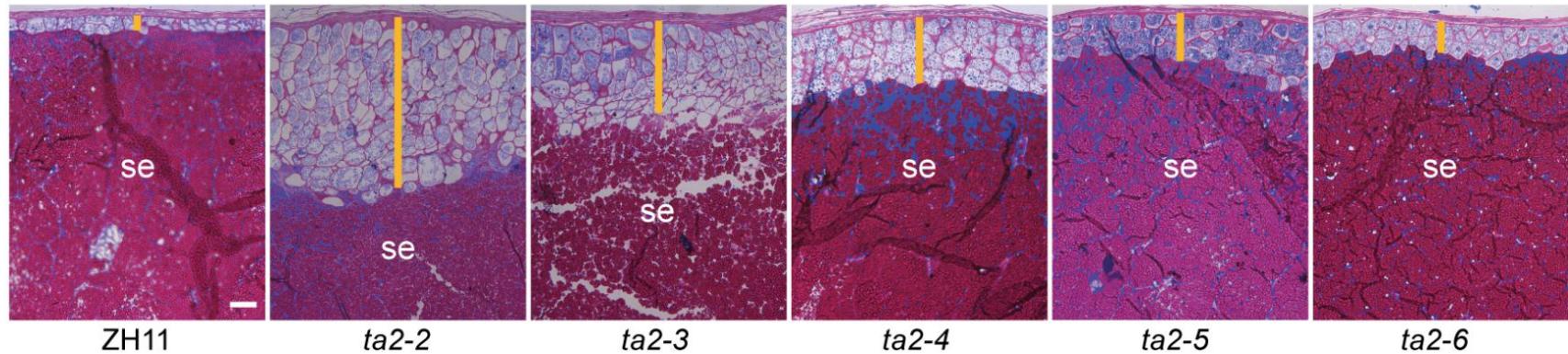
Osr051	CHH2MMNRQFIEEEASPFVPEHTEEMRCAIEDSFVDPEEPIIPIK...INFEETQNLNSYHQANN...	IEEDADMALKALWITPEVASIIFTP...	KLKNVSRSLRTERHEVIEPEPSPLEGFRNQ...	SEEDCIVLISINTP...	GETAQSTDAPKS...VCNS	1803	
mOsR051	CHH2MMNRQFIEEEASPFVPEHTEEMRCAIEDSFVDPEEPIIPIK...INFEETQNLNSYHQANN...	IEEDADMALKALWITPEVASIIFTP...	KLKNVSRSLRTERHEVIEPEPSPLEGFRNQ...	SEEDCIVLISINTP...	GETAQSTDAPKS...VCNS	1803	
Osr051b	PGH...	...YEAREAAEEDPFSDEPDIPIK...	KLKNVSRSLRTERHEVIEPEPSPLEGFRNQ...	SEEDCIVLISINTP...	GETAQSTDAPKS...VCNS	1803	
Osr051c	RDHVHNNTTNIIEEEASPFRECRELLIEDPDTEDIEPIIPIK...	LNNEFAASQNLCEKIRENSKD...	FDS...	DIDTAKVALAISEANSATIVE...	KLKNVHRRLTPEHYYIEPEPSPLEGFRNQ...	1597	
Osr051d	RDHVHNNTTNIIEEEASPFRECRELLIEDPDTEDIEPIIPIK...	LNNEFAASQNLCEKIRENSKD...	FDS...	DIDTAKVALAISEANSATIVE...	KLKNVHRRLTPEHYYIEPEPSPLEGFRNQ...	1597	
GML3	GRESVYCEK...PESVYSPEDLEEDCDLDEDIEPIIPIK...	YTFDLCLESLKHTVNG...	WTPNKGSKLNLINQHQSVAQNC...	KLKNVHRRLTPEHYYIEPEPSPLEGFRNQ...	SEEDCIVLISINTP...	1701	
oGML3	NTENNYCEPVIEPIFPFHNGNETSLEDENGGYDNEYDMDIGHRHYDMEIDYHDMEDYLRSAKPTTNSQAGATPGKEMIFINPRAKTNTVKKFLSRLIEYIAITYSWA...	WTPLLEEF...	WTPLLEEF...	SEEDCIVLISINTP...	DELKUDTRAPKF...CNP	1685	
DME	AFPSNCREEDLIEEPASQPCQTCETT...ESQEDAYNDKPPPIFTK...	LNEIEQGKMSKALVALPHIPTT...	KLKNVSRSLRTERHEVIEPEPSPLEGFRNQ...	SEEDCIVLISINTP...	GETANASQPFK...KCG...	841	
ROS1	PARKVTCOEEAEEPEPTAIEVS...	IADEEAEPE...DEPEELPIF...	LNNDAPTSNLKHKM...	KEIQLDGMSNALVALTAETASLIMP...	KLKNVSRSLRTERHEVIEPEPSPLEGFRNQ...	GETAQSTDAPKS...VCNS	1803
DML2	EAJQRSCPEQNT...	YIEHDIEVPPDRNQHGS...SEDWPWDNPVITIINREAGTSHD...	LVNREAGTSHDWLVSTYAAPEP...	KLKNVSRSLRTERHEVIEPEPSPLEGFRNQ...	GETADASIQPSVS...TICF	1252	
DML3	CSDQAYCYK...WEFSS...	PAFPIESTEDVFPNMNLQSYASVYFRIK...	SDLDALKRSVEEDALVISGR...	MSSDEEAKALVINGEPEPPI...	KLKNVSRSLRTERHEVIEPEPSPLEGFRNQ...	GETVADASIQPSVS...TICF	1191
					SEISSSSVFEFKR...KCSS	902	

C-terminal domain

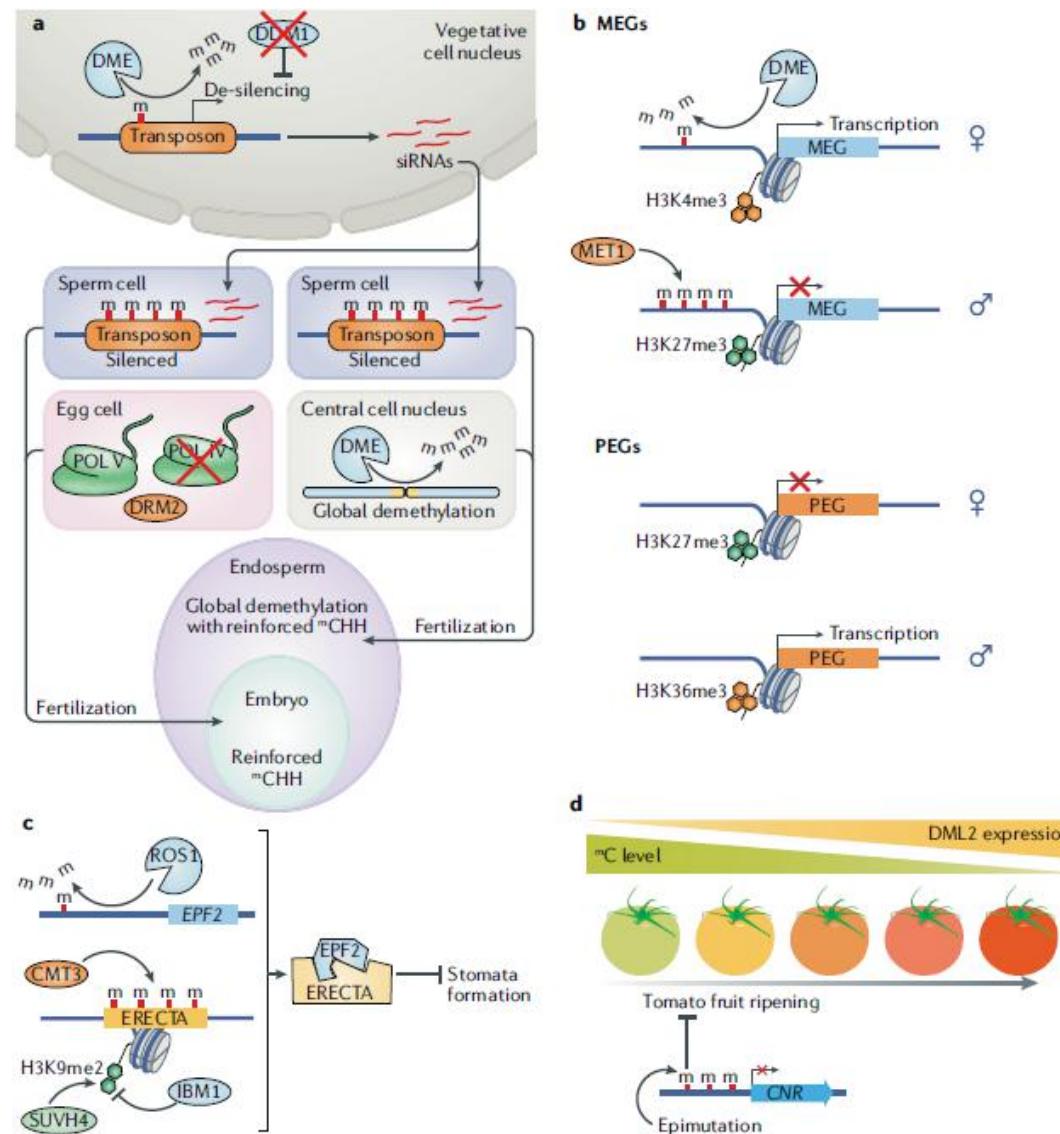
OsRos1	GENELGCASTNTFCNSNIREAQKVRCG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1952
OsRos1	GENELGCASTNTFCNSNIREAQKVRCG... PORTA-RGSFFLNGTYQVNE CSNVNMRG	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1953
OsRos1b	KETKGKCESSTFCSNSTRHEMQSQFVRG... DASS	LLRVP	1634
OsRos1d	QTGGGLCNSMEWHRNCBENQRQIYRG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1847
OsRos1d	QTGGGLCNSMEWHRNCBENQRQIYRG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1828
OsMl3.1 DEHTYKG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1206
OsMl3.1 DEHTYKG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	960
DME	KASGRMFETTFCNSNIREAQKVRCG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1987
R0S1	QANSHLDEETFCNSNIREAQKVRCG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1393
DML2	ESNNENKFCQDNKTRTEESQFVRG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1332
DML3	DGS... KLKQJNQSYCWIRESQNSNIFG... PORTA-RGSFFLNGTYQVNE	LEADHSSRPLDVTRSWINLFRP... T/FGISIPTFNGLITEEIQCHFWFGEVCRGEDETSRSHAIFIYARLHFPAKSLTRNKKSAGSAPGRDDE	1044

7-AA insertion in mOsROS1

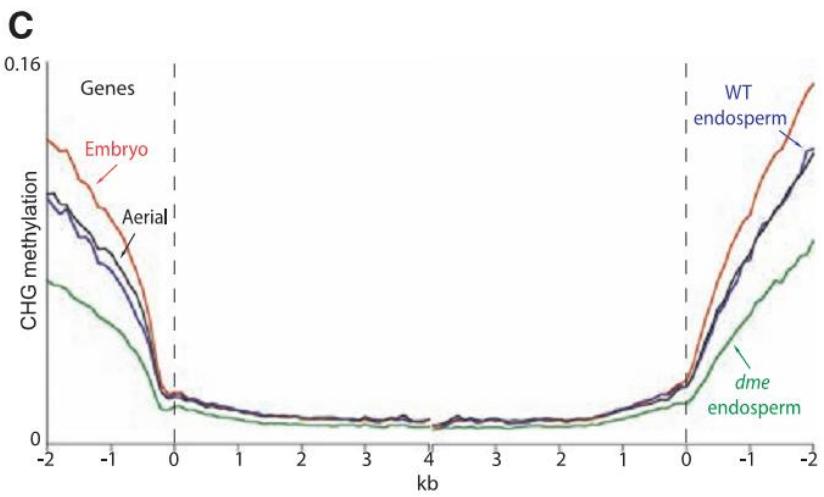
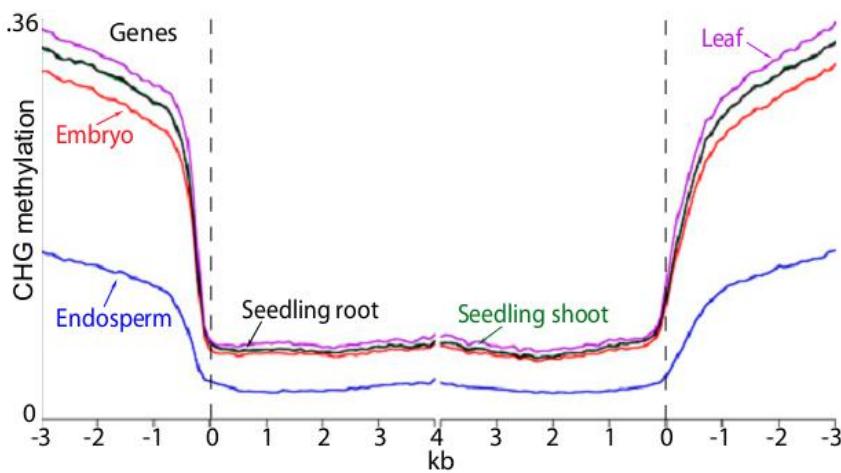
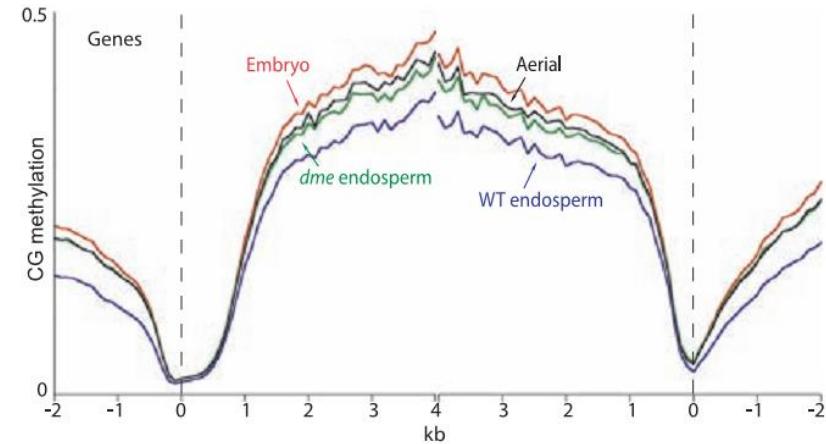
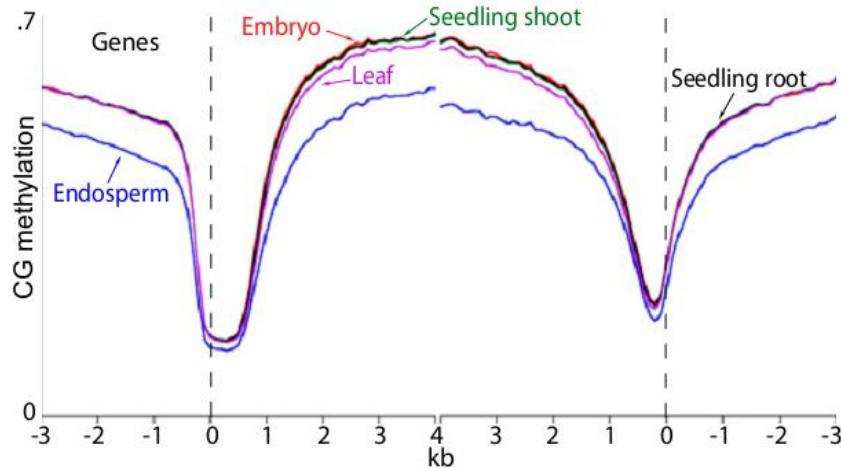
*ta2*不同等位突变呈现糊粉层加厚表型



DNA去甲基化过程在植物生长发育中具有重要作用

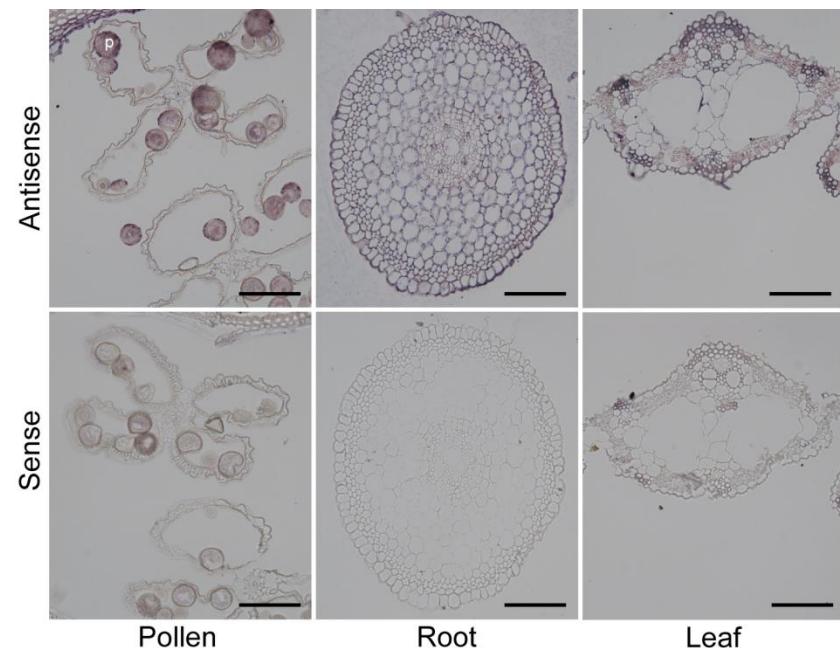
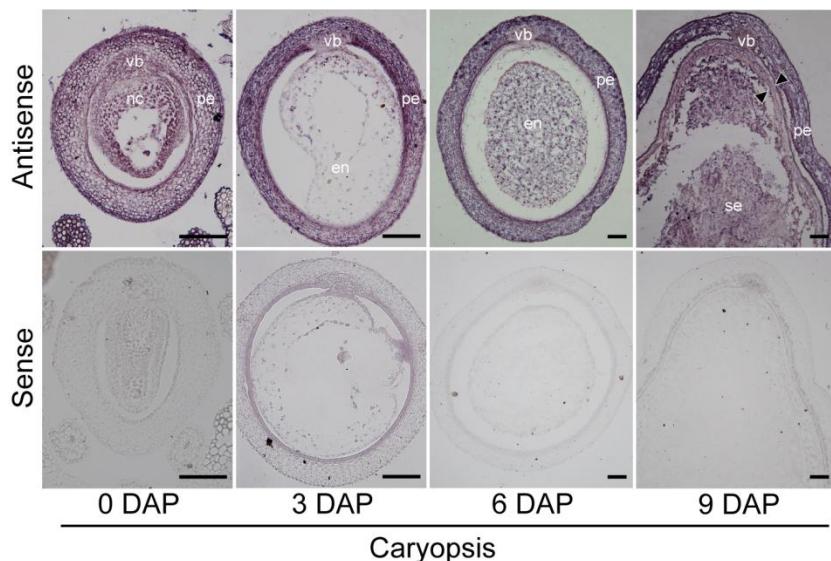
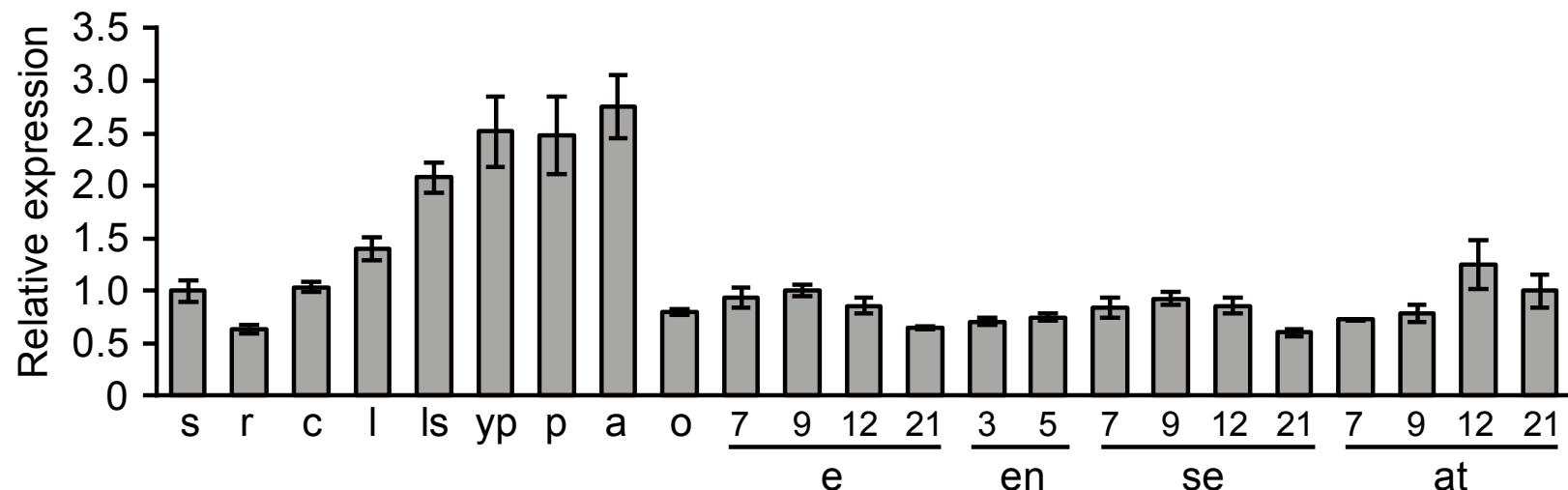


胚乳基因组DNA呈现较低的DNA甲基化水平

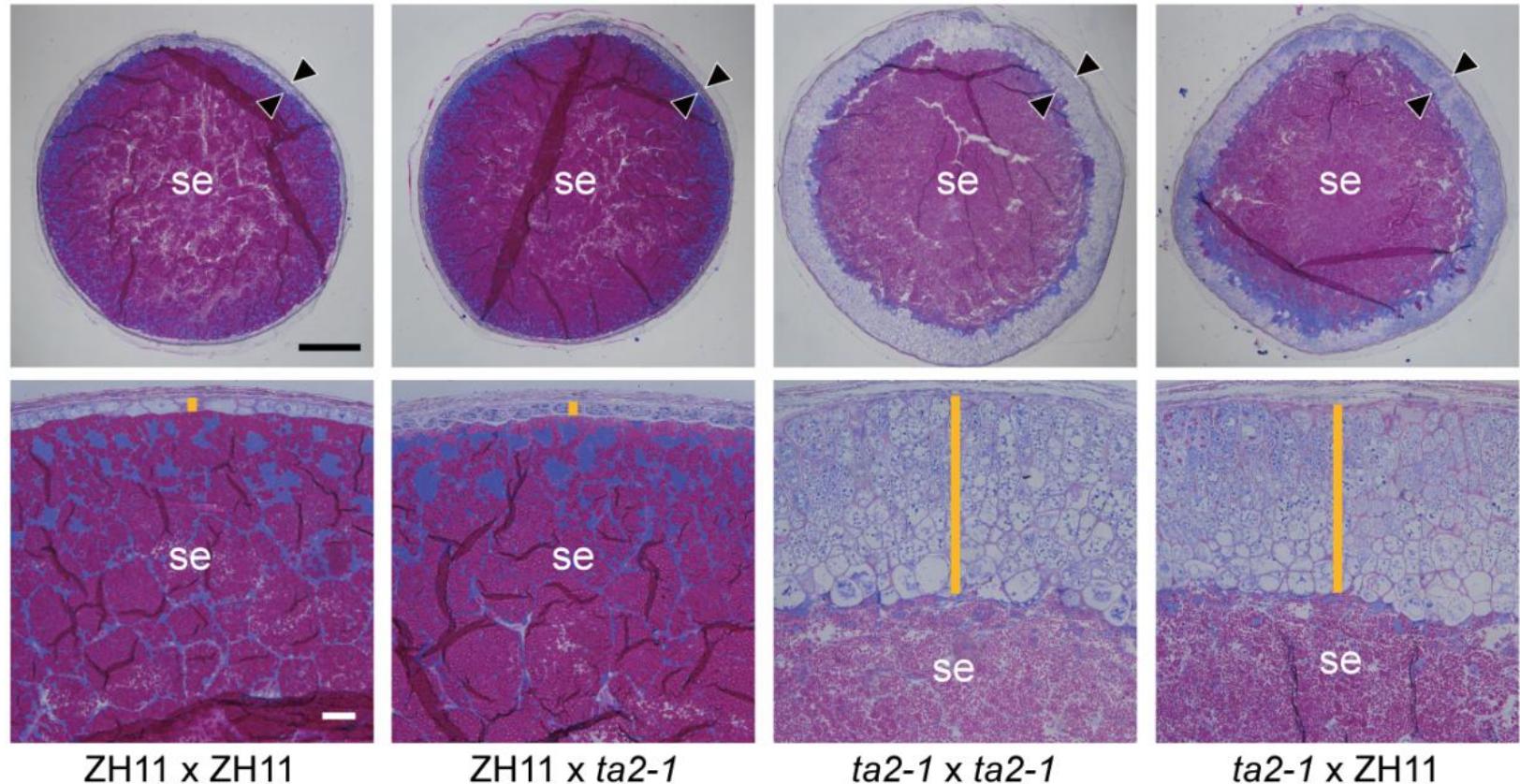


Hiseh et al, Science, 2009
Zemach et al, PNAS, 2010

*OsROS1*在营养组织、胚和胚乳中均有表达



ZH11和*ta2-1*正反交F1胚乳中糊粉层的表型

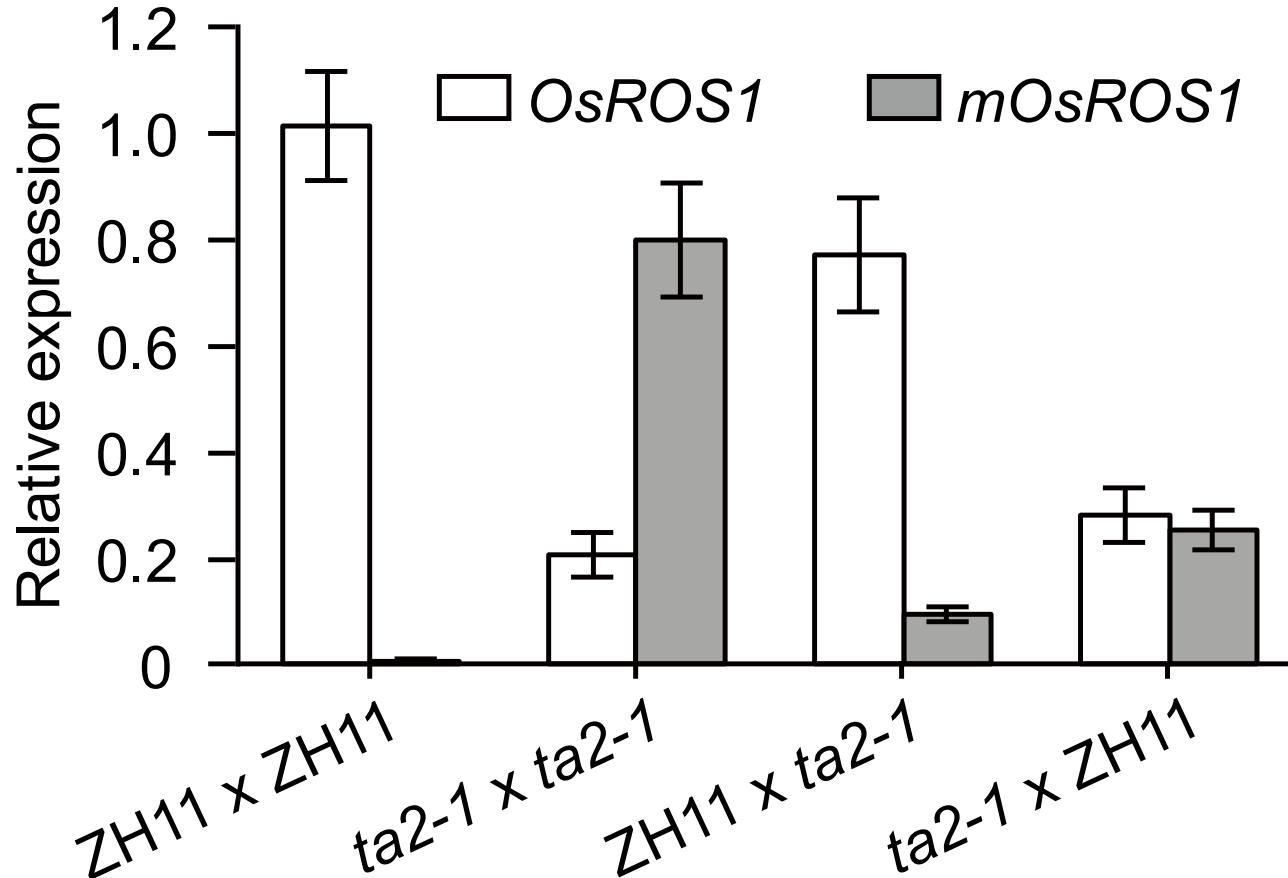


*ta2-1*糊粉层加厚表型呈现配子体母本效应

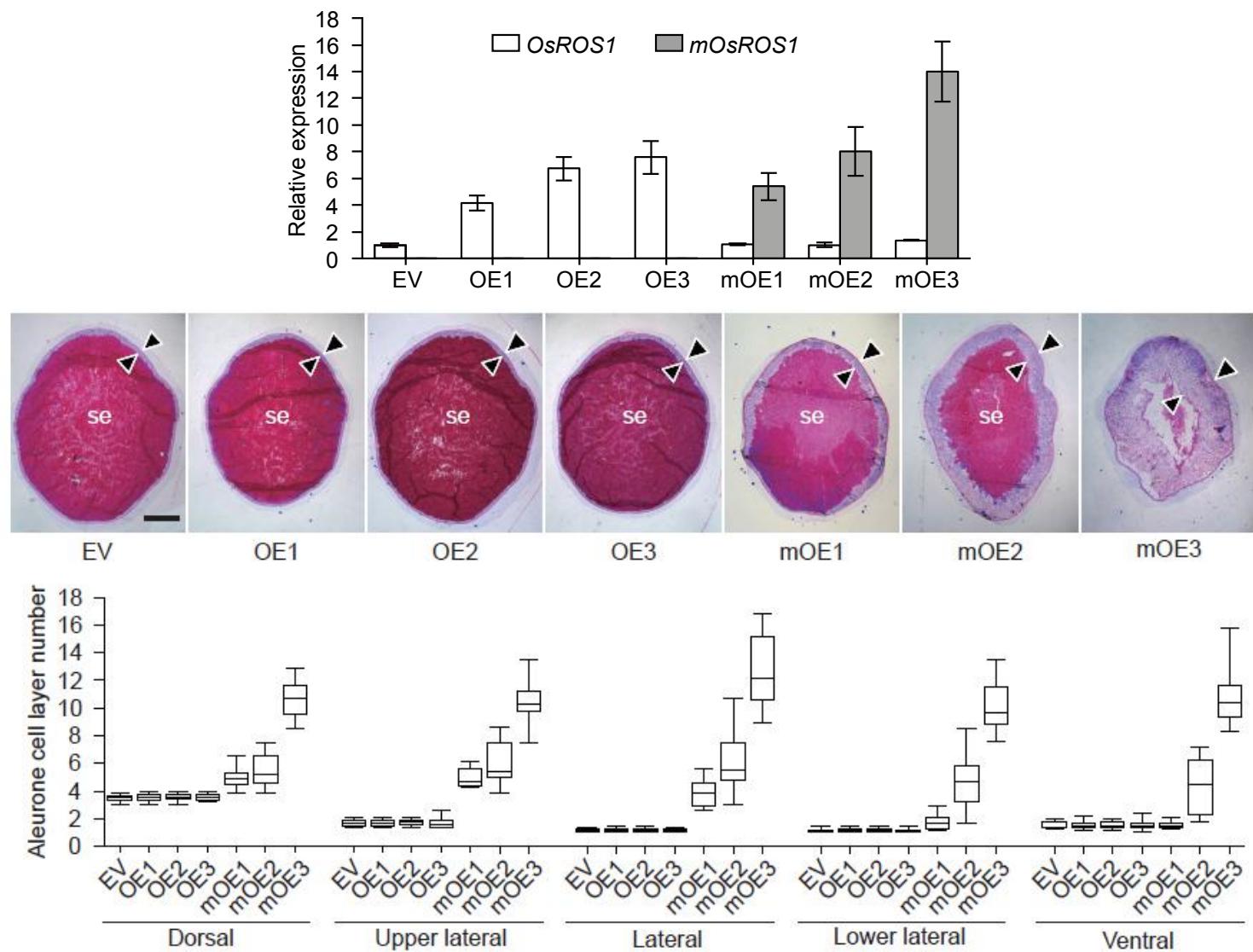
Cross combination	Endosperm phenotypes		Grains with <i>ta</i> phenotype (%)	<i>P</i> for 1:1
	Wild-type	<i>ta</i>		
<i>TA2/ta2-1</i> x <i>TA2/ta2-1</i>	321	313	49.4	0.7506 (N.S.)
ZH11 x <i>ta2-1</i>	197	0	0	N.A.
ZH11 x <i>TA2/ta2-1</i>	171	0	0	N.A.
<i>ta2-1</i> x ZH11	0	589	100	N.A.
<i>ta2-1</i> x <i>TA2/ta2-1</i>	0	422	100	N.A.
<i>TA2/ta2-1</i> x ZH11	199	193	49.2	0.7618 (N.S.)
<i>TA2/ta2-1</i> x <i>ta2-1</i>	212	214	50.2	0.9228 (N.S.)

N.S., not significant; N.A., not applicable.

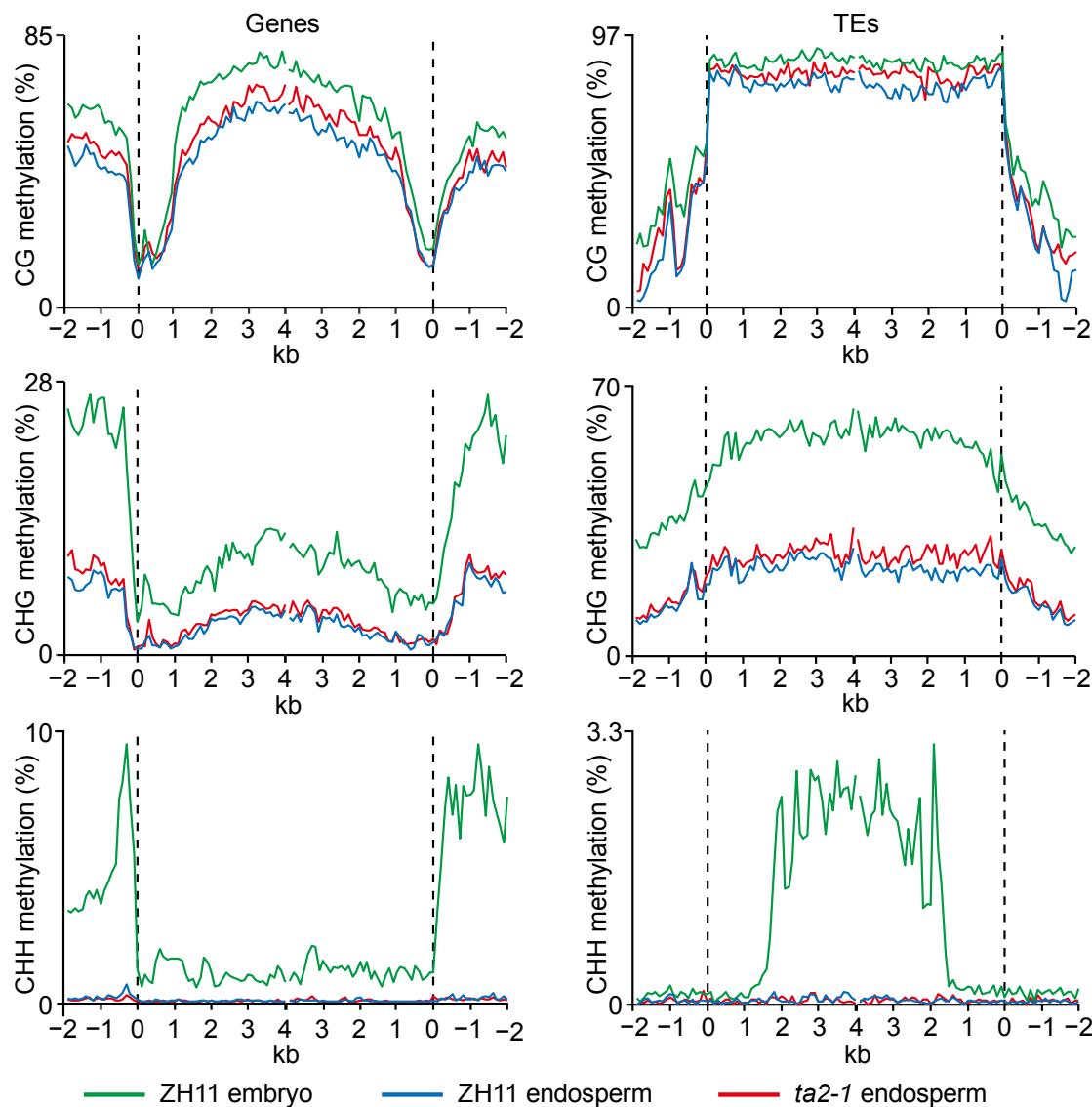
*OsROS1*和*mOsROS1*在ZH11和*ta2-1*正反交F1胚乳中的表达水平



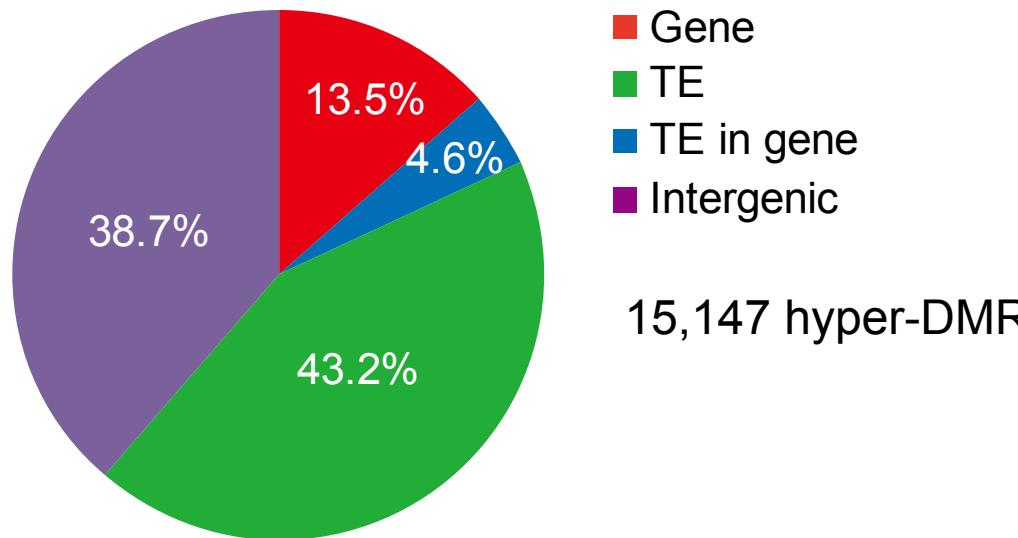
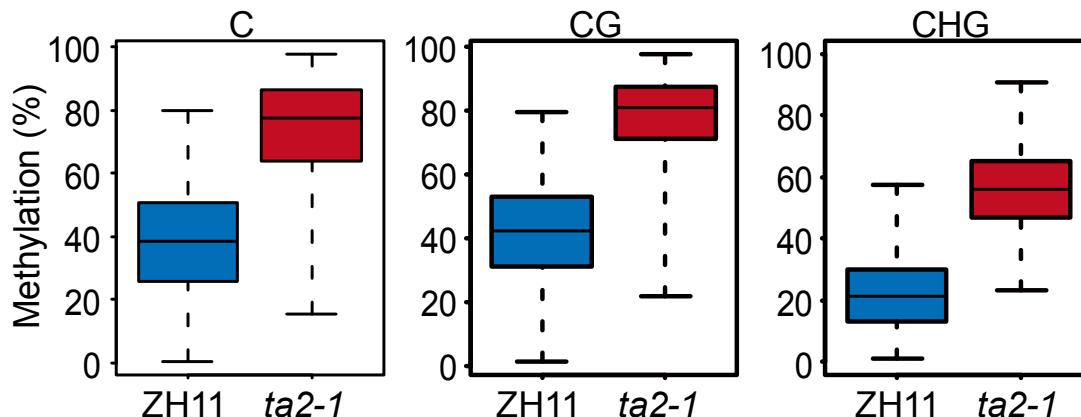
在ZH11中过表达*mOsROS1*产生糊粉层加厚表型



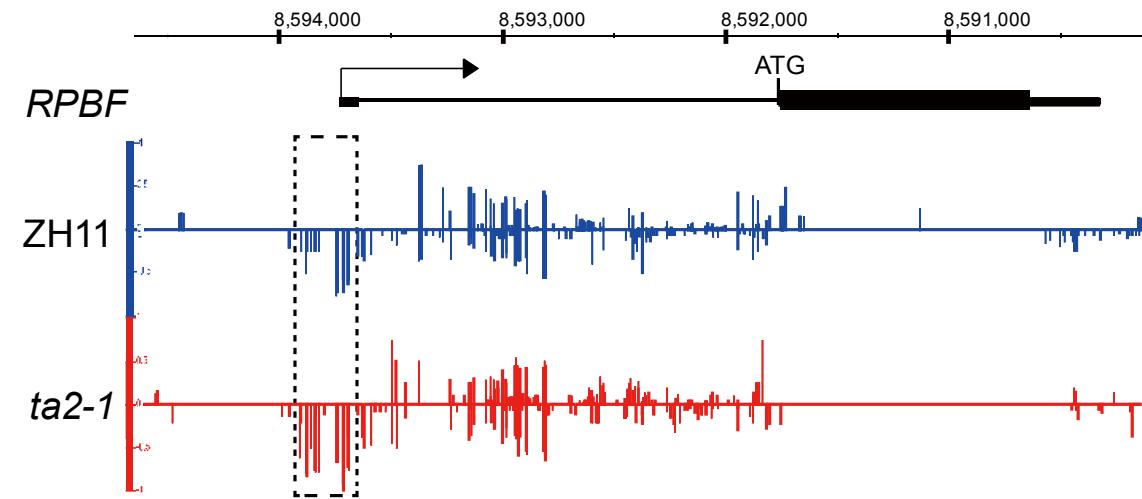
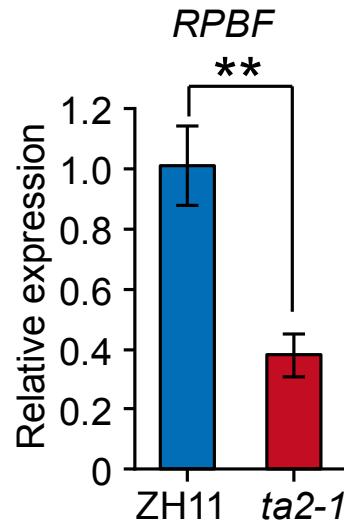
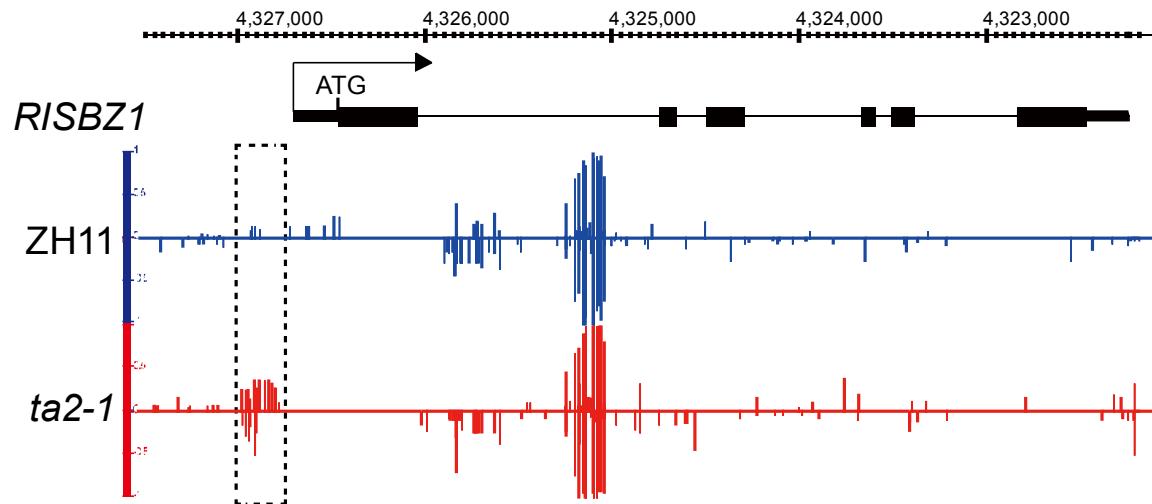
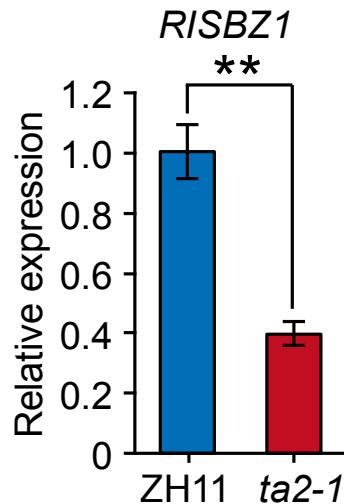
ZH11和*ta2-1*胚乳基因组中基因区域和TE区域 DNA甲基化水平分布



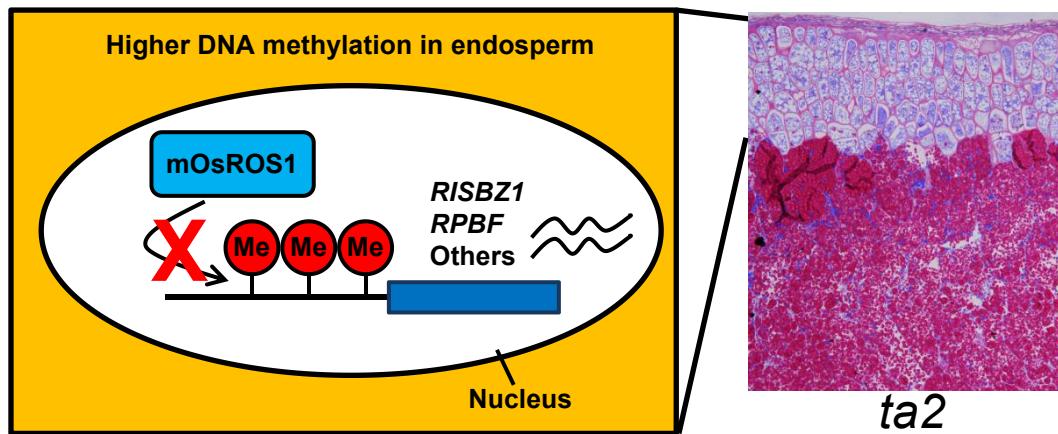
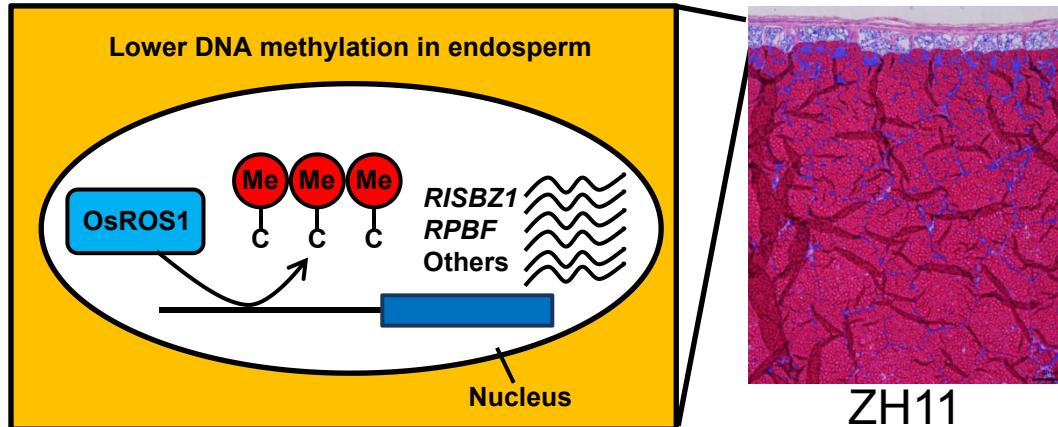
*ta2-1*胚乳基因组hyper-DMR分析



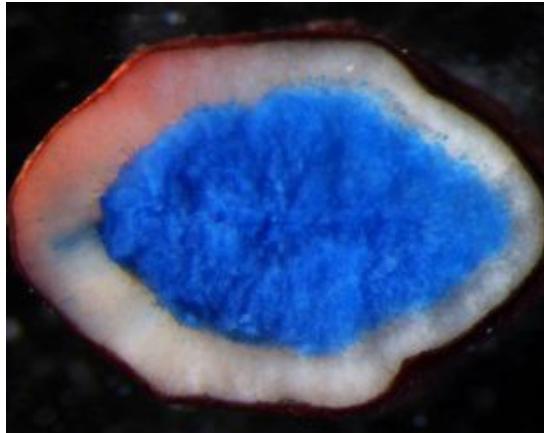
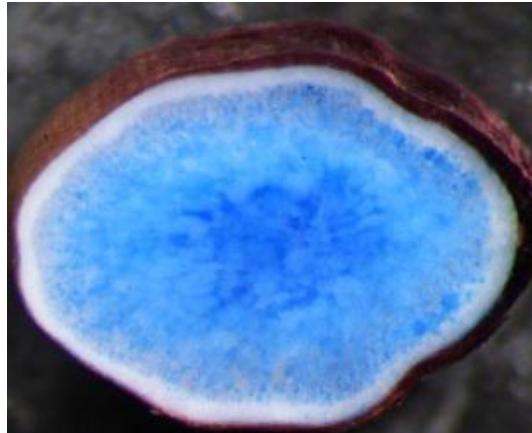
在*ta2-1*胚乳中*RISBZ1*和*RPBF*启动子区域甲基化水平升高导致表达水平显著降低



OsROS1调控胚乳糊粉层分化的分子机制



将糊粉层加厚性状导入到紫米品 种

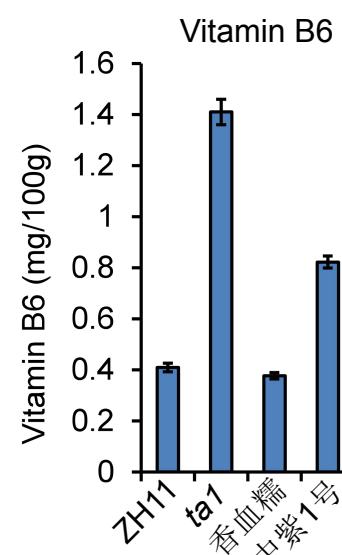
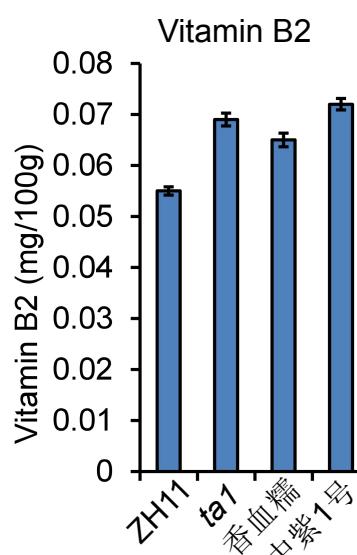
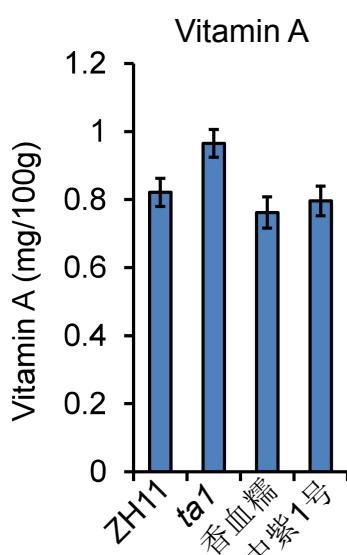
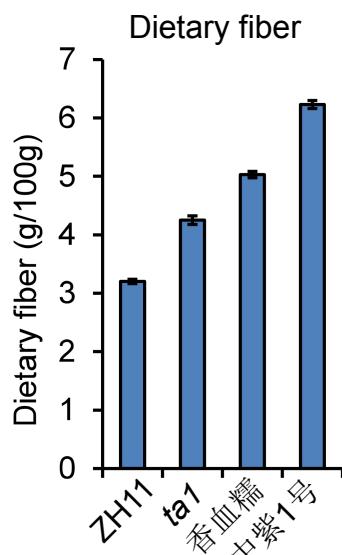
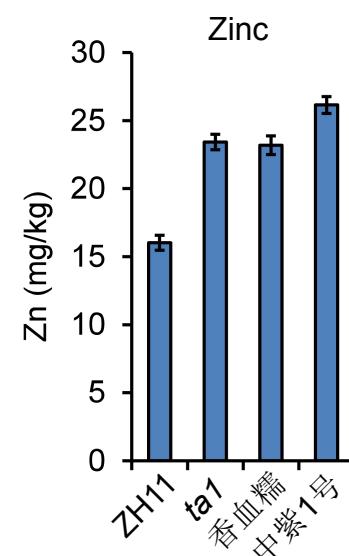
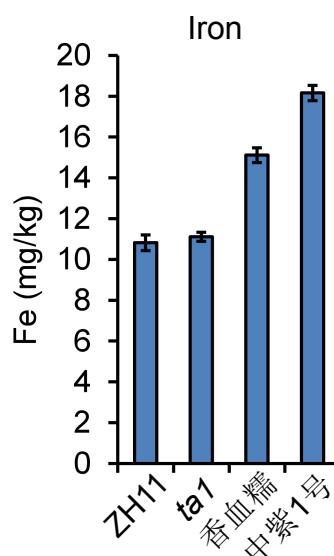
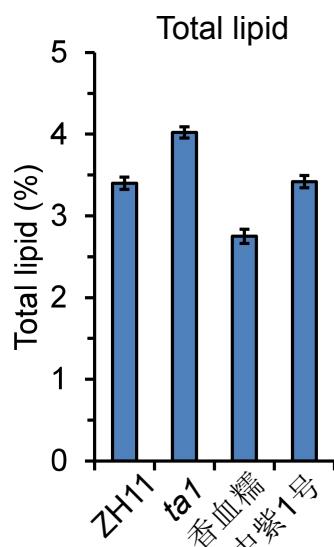
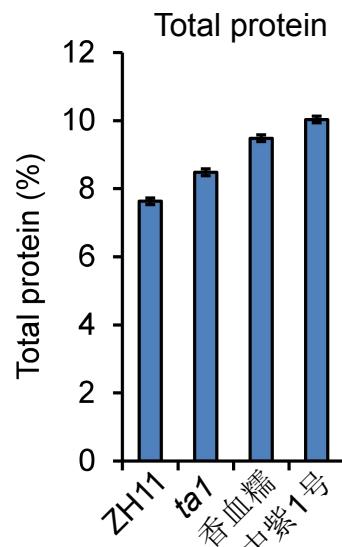


中紫1号



中紫2号

*ta1*突变导致紫米种子糊粉层加厚、营养含量提高

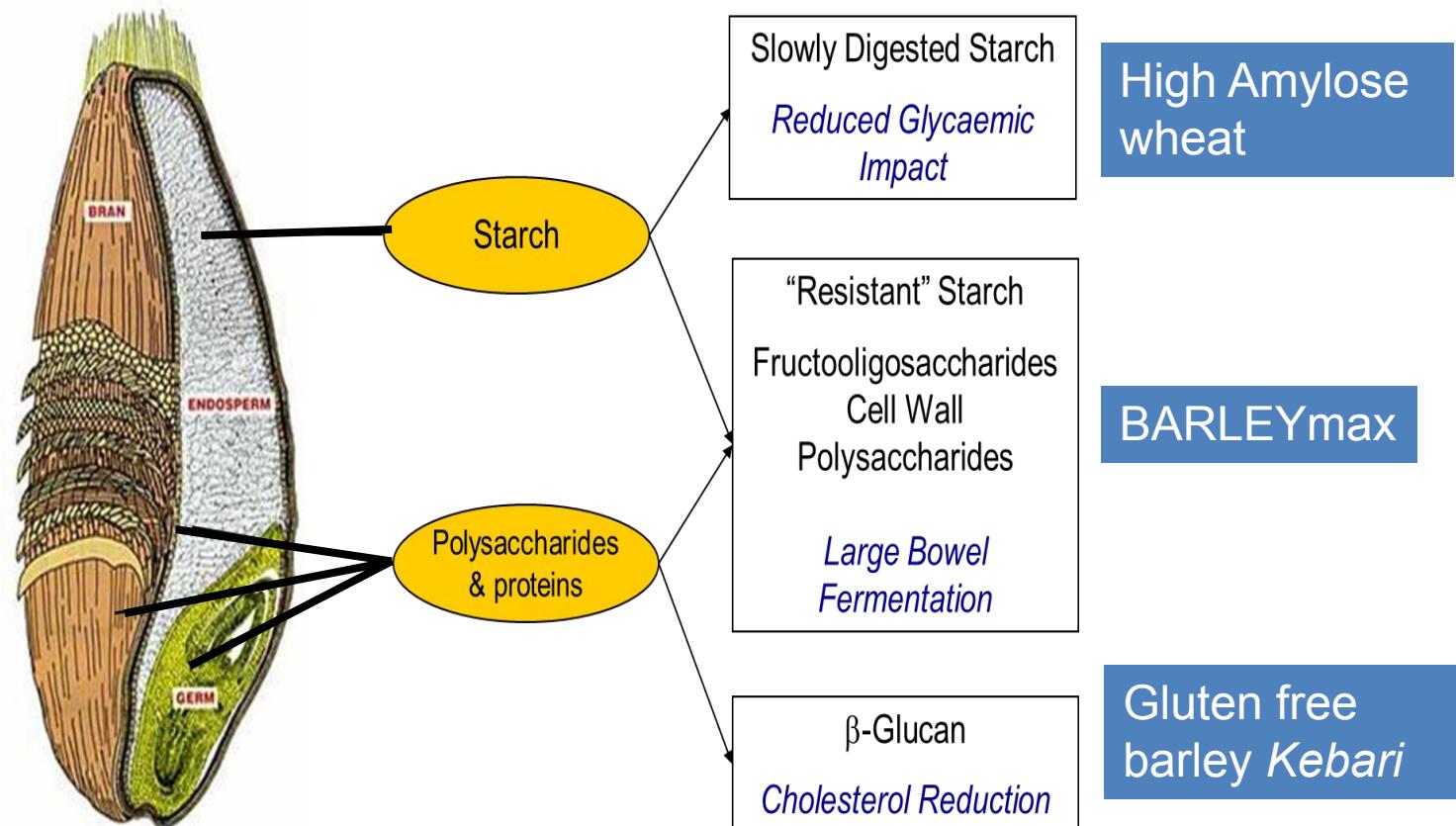


高营养水稻品种中紫1号已申请品种保护权



开发糊粉层增厚的高营养功能型禾谷类作物

Diet-related Diseases: Cardiovascular disease, Type II diabetes, Obesity and Weight Control, Gut Health, Colon Cancer.....



与澳大利亚联邦科学与工业研究组织合作

致 谢

刘春明 研究员

张士永 研究员

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Ronald Yu

Philip Larkin



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