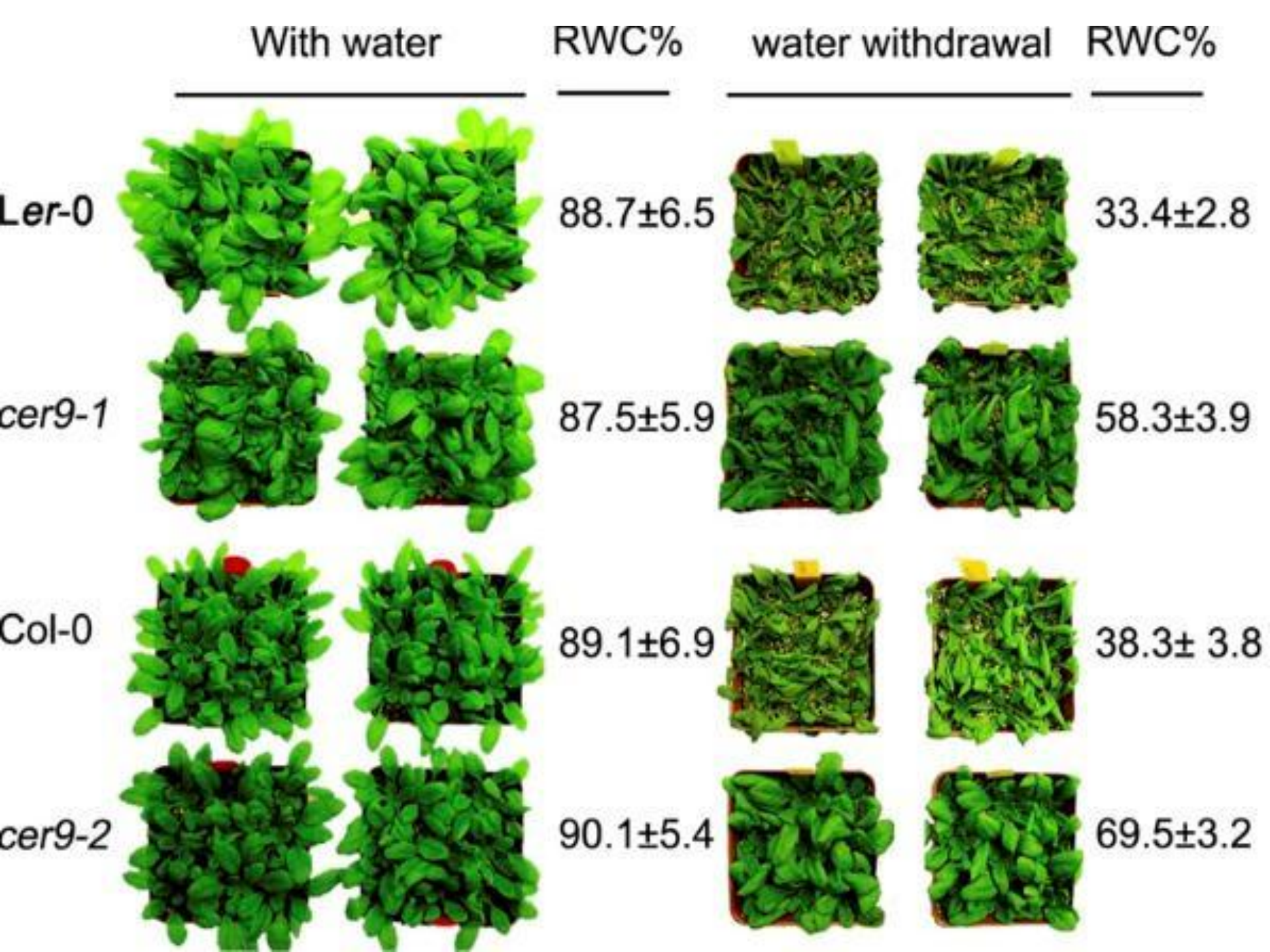
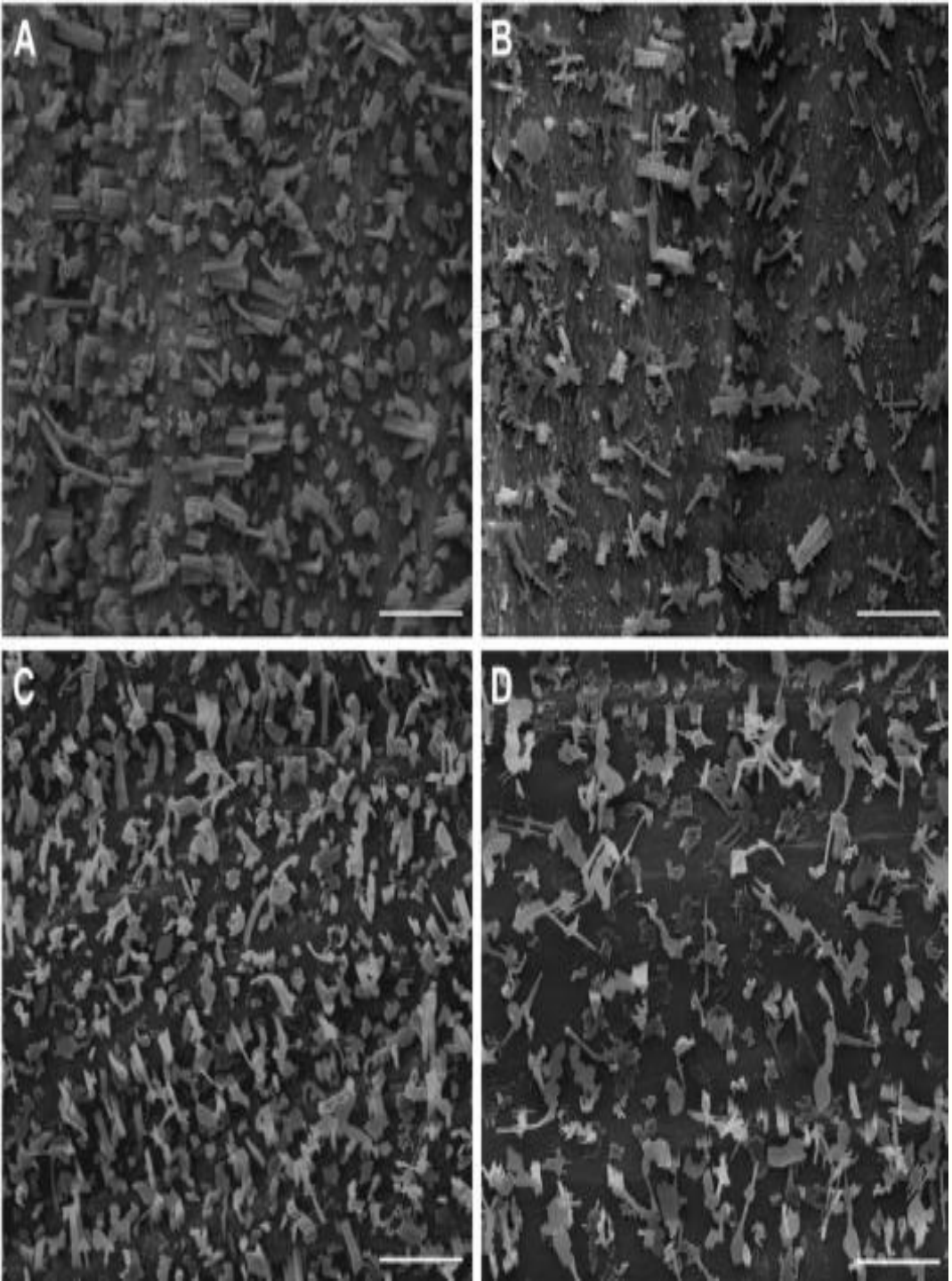
At*CER9* 的生物信息学分析

小组：G05

成员：杜依聪 范胜栩 郭宪瑞 彭鹏

- 植物主要通过蒸腾作用失水，根据路径不同可分为气孔蒸腾和角质蒸腾
- 气孔蒸腾：气孔密度、保卫细胞的活动
- 角质蒸腾：气孔脊、覆盖气孔下腔叶肉组织的角质层
- 植物表皮：脂质、不亲水
- 角质层主要脂质：非聚合表皮蜡、角质聚酯

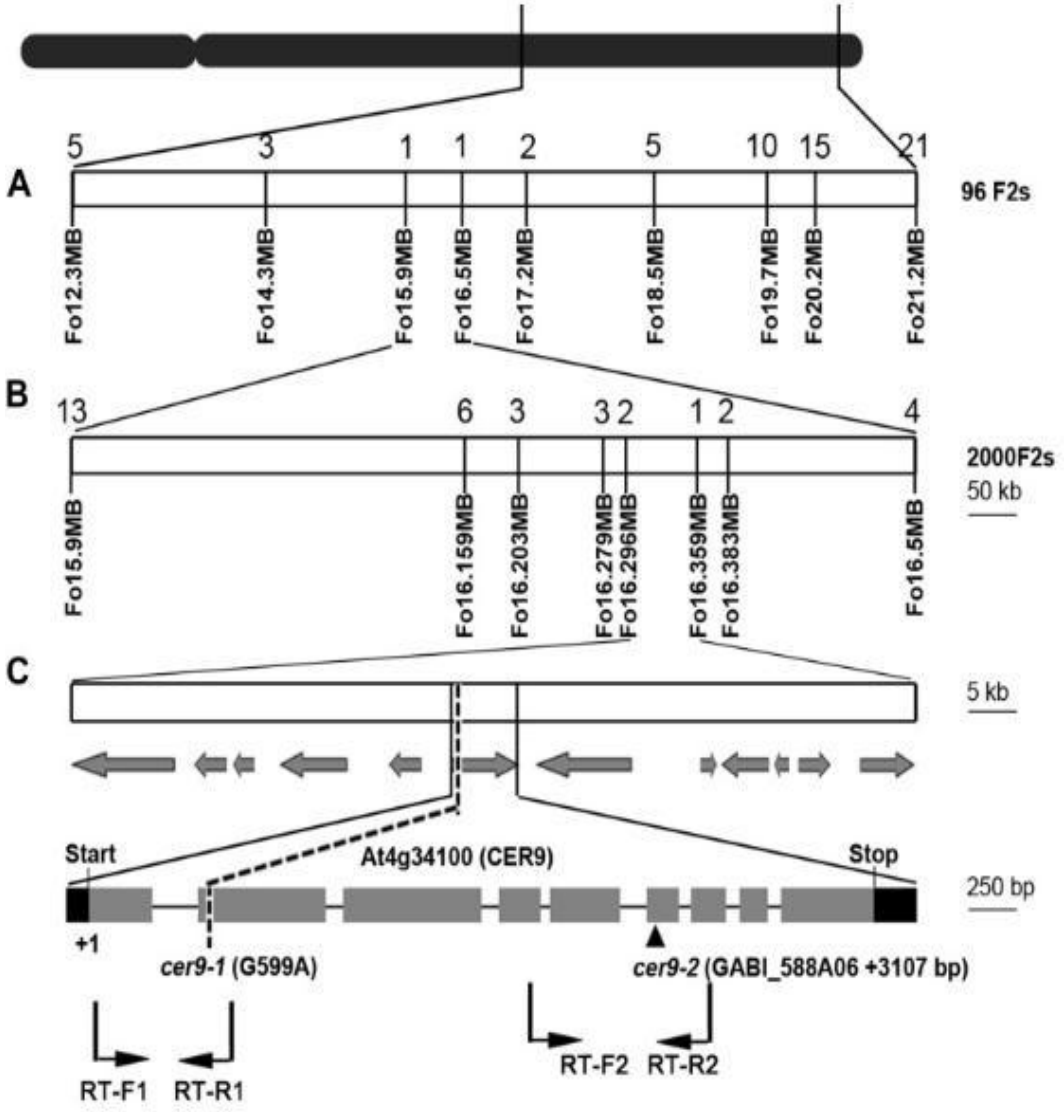

With water
RWC%
water withdrawal
RWC%
Ler-0
88.7±6.5
33.4±2.8
cer9-1
87.5±5.9
58.3±3.9
Col-0
89.1±6.9
38.3± 3.8
cer9-2
90.1±5.4
69.5±3.2



- SEM 扫描显示野生型 Ler-0 (A), cer9-1 (B), 野生型 Col-0 (C), cer9-2 (D)
- 等位测验证明cer9-2 与cer9-1 不互补，且是由同一基因引起的突变表型

总共	cer9-1(%)	cer9-2(%)
表皮蜡	27	57
	44	
角质单体	59	
	67	

Molecular Identification of *CER9*

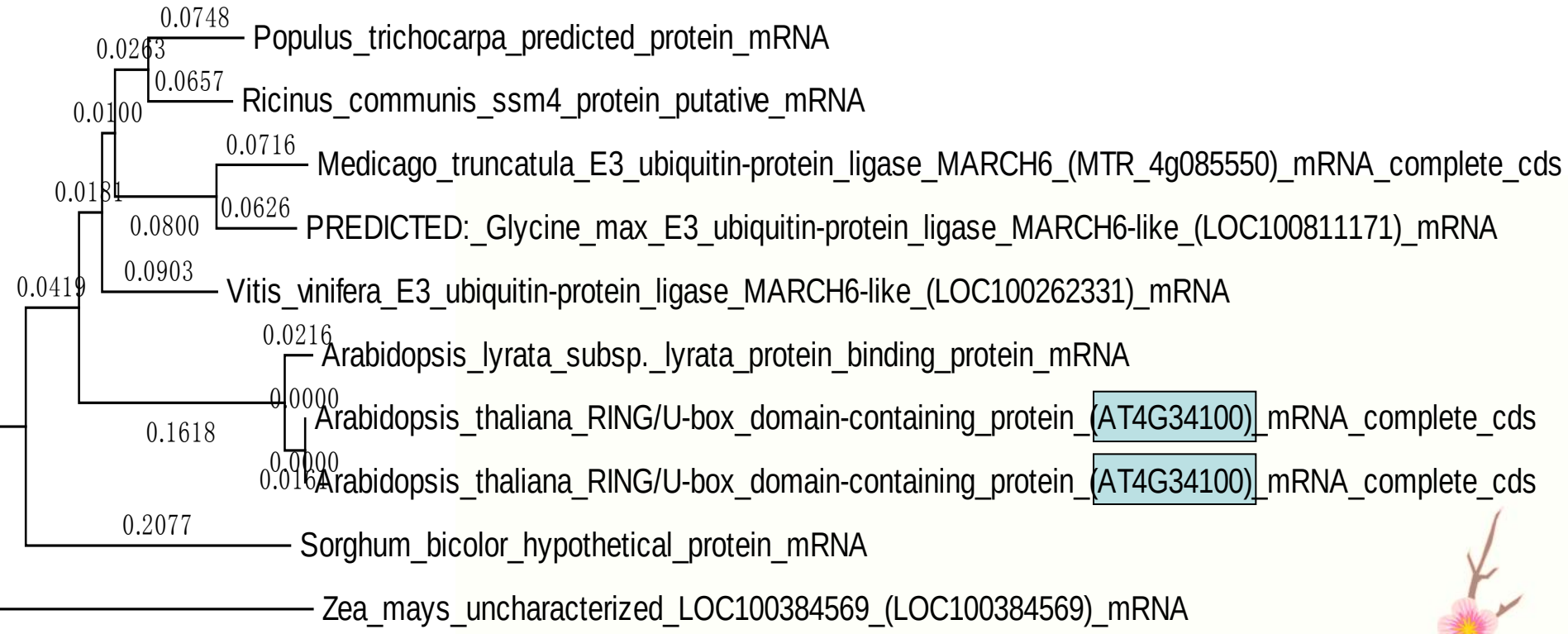


- EMS ⟶ 隐性等位突变 cer9-1 (1989)

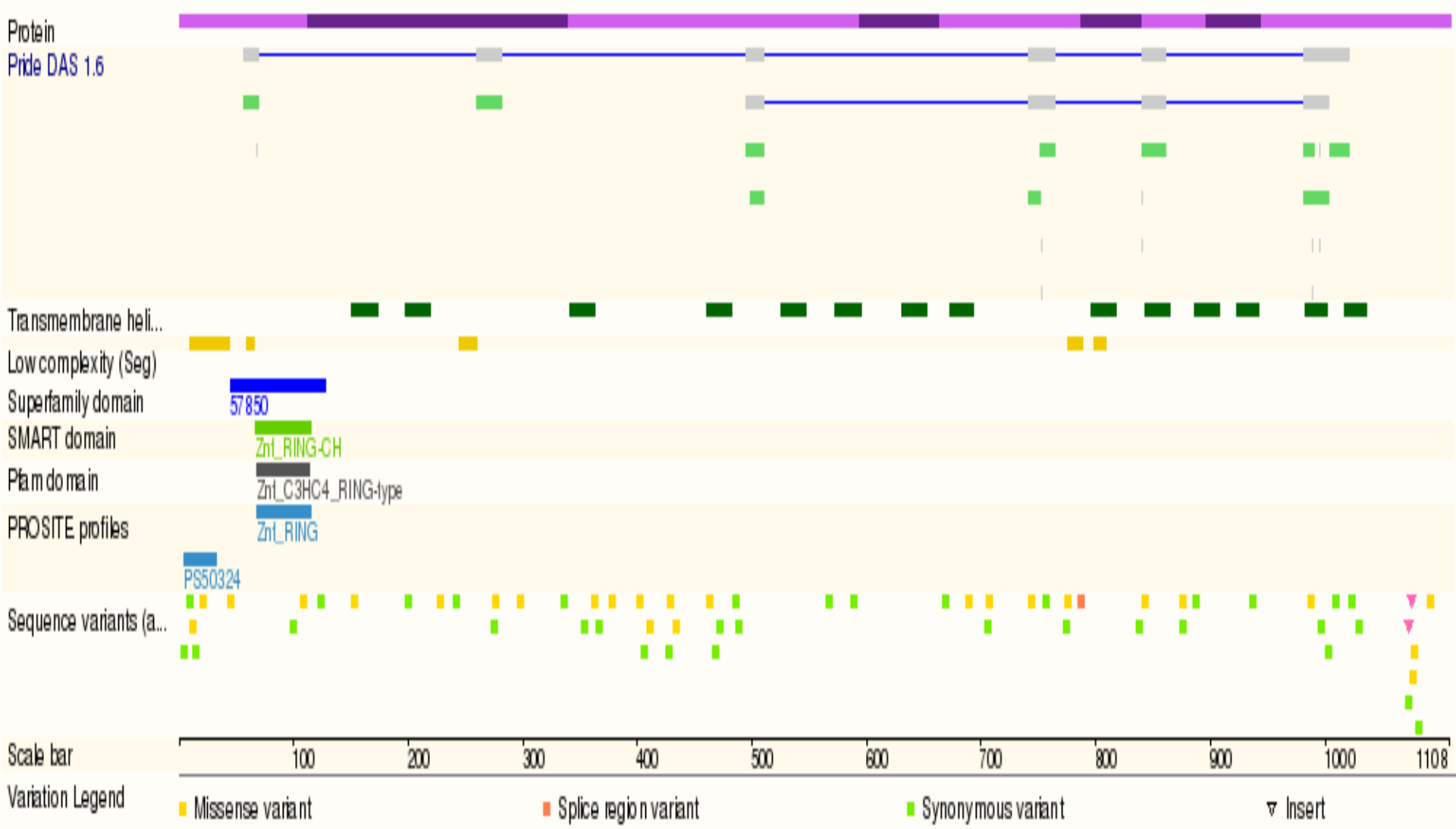
- 4号染色体(2004)
- CER9 的图位克隆

A, 用 96个 F2植株定位在一个400-kb区域

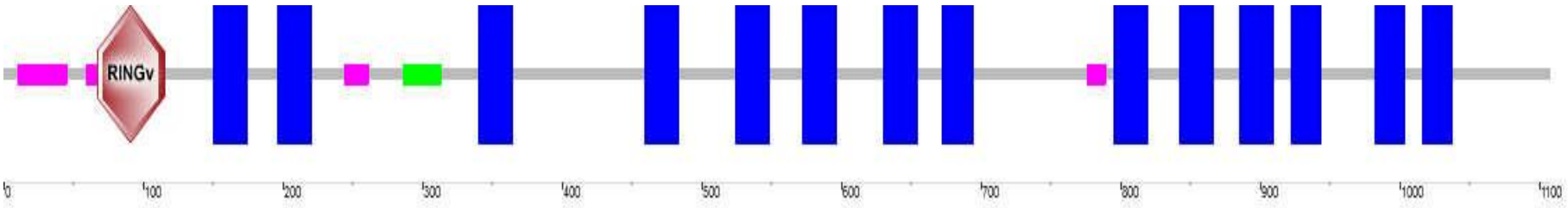
B, 进一步把 cer9-1精细定位到一个63-kb 区域 C, cer9-1测序区域和T-DNA插入突变 cer9-2.


0.0748
Populus_trichocarpa_predicted_protein_mRNA
0.0263
0.0657
Ricinus_communis_ssm4_protein_putative_mRNA
0.0100
0.0716
Medicago_truncatula_E3_ubiquitin-protein_ligase_MARCH6_(MTR_4g085550)_mRNA_complete_cds
0.0181
0.0626
0.0800
PREDICTED:_Glycine_max_E3_ubiquitin-protein_ligase_MARCH6-like_(LOC100811171)_mRNA
0.0903
0.0419
Vitis_vinifera_E3_ubiquitin-protein_ligase_MARCH6-like_(LOC100262331)_mRNA
0.0216
Arabidopsis_lyrata_subsp._lyrata_protein_binding_protein_mRNA
0.0000
Arabidopsis_thaliana_RING/U-box_domain-containing_protein_(AT4G34100)_mRNA_complete_cds
0.1618
0.0000
0.016
Arabidopsis_thaliana_RING/U-box_domain-containing_protein_(AT4G34100)_mRNA_complete_cds
0.2077
Sorghum_bicolor_hypothetical_protein_mRNA
Zea_mays_uncharacterized_LOC100384569_(LOC100384569)_mRNA

核酸序列比对分析

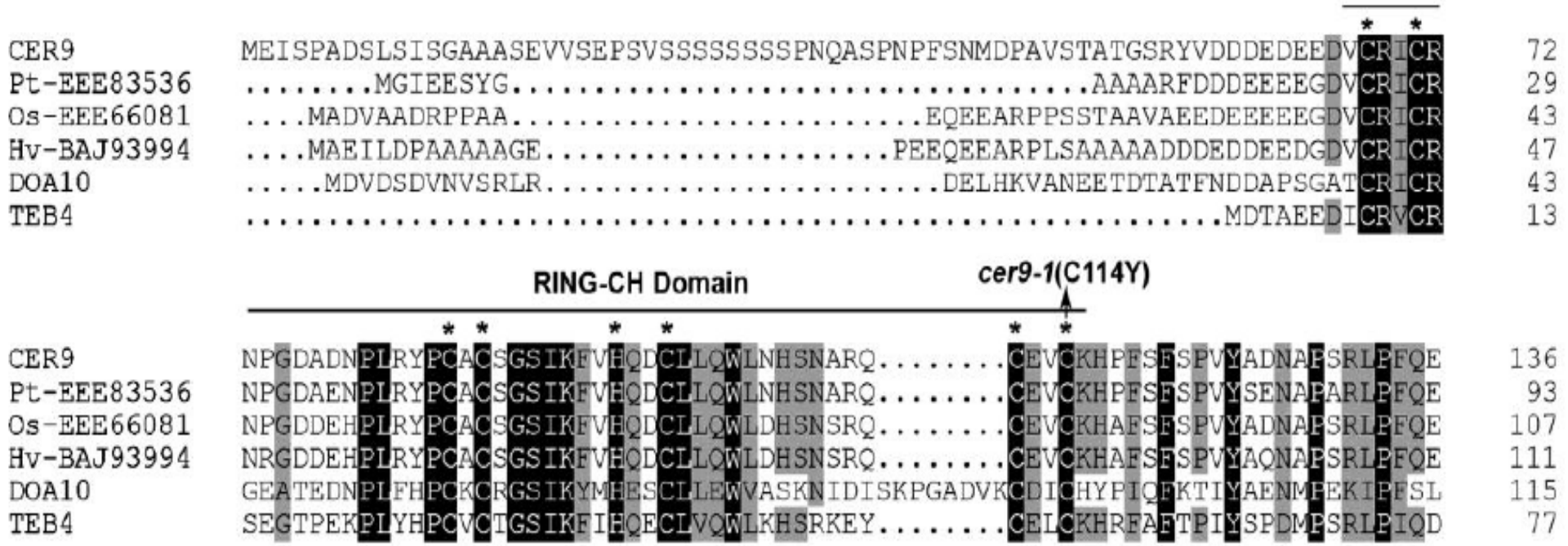

Protein
Pride DAS 1.6
Transmembrane heli...
Low complexity (Seg)
Superfamily domain
57850
SMART domain
Znf_RING-CH
Pfam domain
Znf_C3HC4_RING-type
PROSITE profiles
Znf_RING
PS50324
Sequence variants (a...
Scale bar
100
200
300
400
500
600
700
800
900
1000
1108
Variation Legend
Missense variant
Splice region variant
Synonymous variant
Insert

- SMART



67-117bp

- RING是独特的锌指节构，以交叉方式协调两个锌原子。根据Cys和His可分为三类：传统 RING finger (RING-HC)在第四协调位有一个his，第五协调位有一个cys；在 RING-H2 突变体，第4、5位都是his;RING-CH，与RING-HC非常相似，4位cys，5位his，而且在4、5位锌指协调残基间的肽段稍长，因此与 PHD 锌指结构的cys、his排列相同(C4HC3)，是一个E3连接酶，主要位于与膜有关的蛋白中；



- CER9 (At4g34100; *Arabidopsis thaliana*); Pt, *Populus trichocarpa*; Os, *Oryza sativa*; Hv, *Hordeum vulgare*; DOA10 (*Saccharomyces cerevisiae*); TEB4 (*Homo sapiens*).

```
TR|tr|F4JKK0|F4JKK0_ARATH|F4JKK0_ARATH PAVSTATGSRYVDDDEDEEDVCRICRNPGDADNPLRYPCACSGSIKFVHQDCLLQWLNHS 106
TR|tr|F4JKK1|F4JKK1_ARATH|F4JKK1_ARATH PAVSTATGSRYVDDDEDEEDVCRICRNPGDADNPLRYPCACSGSIKFVHQDCLLQWLNHS 106
TR|tr|D7MFS0|D7MFS0_ARALL|D7MFS0_ARALL PAVATATGSRYVDDDEDEEDVCRICRNPGDADNPLRYPCACSGSIKFVHQDCLLQWLNHS 106
TR|tr|B9HS29|B9HS29_POPTR|B9HS29_POPTR -KTVSLLSAARFDDDEEEEDVCRICRNPGDAENPLRYPCACSGSIKFVHQDCLLQWLNHS 102
TR|tr|I1KM21|I1KM21_SOYBN|I1KM21_SOYBN SPAPAPAPSKFDDEEEEEEDVCRICRNPGDAENPLRYPCACSGSIKFVHQDCLLQWLNHS 119
TR|tr|K4B304|K4B304_SOLLC|K4B304_SOLLC -RKDLNSLASRFDDDEEEEDVCRICRNPGEADNPLRYPCACSGSIKYVHQDCLLQWLNHS 108
TR|tr|G7JK10|G7JK10_MEDTR|G7JK10_MEDTR -ASTAPPSAKYDDDDEDEEDVCRICRNPGDADNPLRYPCACSGSIKFVHQDCLLQWLNHS 107
TR|tr|D7TPF9|D7TPF9_VITVI|D7TPF9_VITVI -PEDETRSPGKYDEEEDEGDVCRICRNPGDAENPLRYPCACSGSIKFVHQDCLLQWLNHS 98
TR|tr|B9FQ39|B9FQ39_ORYSJ|B9FQ39_ORYSJ RPPSSTAAVAEEDEEEEEGDVCRICRNPGDDEHPLRYPCACSGSIKFVHQDCLLQWLDHS 77
TR|tr|K3XUV0|K3XUV0_SETIT|K3XUV0_SETIT RPPP----GEEEDEEEEEGDVCRICRNRGDEDHPLRYPCACSGSIKFVHQDCLLQWLDHS 232
TR|tr|I1GVD2|I1GVD2_BRADI|I1GVD2_BRADI RLPPAPAGGEEDDDDEEEGDVCRICRNRGDDEHPLRYPCACSGSIKFVHQDCLLQWLDHS 87
TR|tr|B9RYE5|B9RYE5_RICCO|B9RYE5_RICCO -ATAATSAARYEDDDEEEEDVCRICRNPGDAENPLRYPCACSGSIKFVHQDCLLQWLNHS 103
                                          *::*:* ******** *: :.**************:***********:**

TR|tr|F4JKK0|F4JKK0_ARATH|F4JKK0_ARATH NARQCEVCKHPFSFSPVYADNAPSRLPFQEFVVGIAMKACHVLQFFLRLSFVLSVWLLTI 166
TR|tr|F4JKK1|F4JKK1_ARATH|F4JKK1_ARATH NARQCEVCKHPFSFSPVYADNAPSRLPFQEFVVGIAMKACHVLQFFLRLSFVLSVWLLTI 166
TR|tr|D7MFS0|D7MFS0_ARALL|D7MFS0_ARALL NARQCEVCKHPFSFSPVYADNAPSRLPFQEFVVGIAMKACHVLQFFLRLSFVLSVWLLTI 166
TR|tr|B9HS29|B9HS29_POPTR|B9HS29_POPTR NARQCEVCKHPFSFSPVYAENAPARLPFQEFVVGMTMKTCHVLQFFLRLSFVLSVWLLII 162
TR|tr|I1KM21|I1KM21_SOYBN|I1KM21_SOYBN NARQCEVCKHAFSFSPVYAENAPARLPFQEFVVGMAMKACHVLQFFLRLSFVLSVWLLII 179
TR|tr|K4B304|K4B304_SOLLC|K4B304_SOLLC NARQCEVCKHAFSFSPVYAENAPARLPFQEFVVGMAMKACHVLQFFLRLSFVLSVWLLII 168
TR|tr|G7JK10|G7JK10_MEDTR|G7JK10_MEDTR NARQCEVCKHPFSFSPVYAENAPARLPFQEFVVGMAMKACHVLQFFVRLSFVLSVWLLII 167
TR|tr|D7TPF9|D7TPF9_VITVI|D7TPF9_VITVI NARQCEVCKYAFSFSPVYAENAPARLPFQEFVVGMAMKACHVLQFFLRLSFVLSVWLLII 158
TR|tr|B9FQ39|B9FQ39_ORYSJ|B9FQ39_ORYSJ NSRQCEVCKHAFSFSPVYADNAPSRLPFQELIVGVGMKACHVLQFVLRLAFVLSVWLMII 137
TR|tr|K3XUV0|K3XUV0_SETIT|K3XUV0_SETIT NSRQCEVCKHAFSFSPVYADNAPTRLPFQELIVGVGMKACHVFQFILRLAFVLSVWLMII 292
TR|tr|I1GVD2|I1GVD2_BRADI|I1GVD2_BRADI NSRQCEVCKHAFSFSPVYAQNAPTRLPFQELVFGVGMKACHVFQFVLRLAFVLSVWLMII 147
TR|tr|B9RYE5|B9RYE5_RICCO|B9RYE5_RICCO NARQCEVCKHAFSFSPVYAENAPTRLPFQEFVVGMAMKACHVLQFFLRLSFVLSVWLLII 163
                                         *:********: *********:***:*******::.*: **:***:**.:**:********: *
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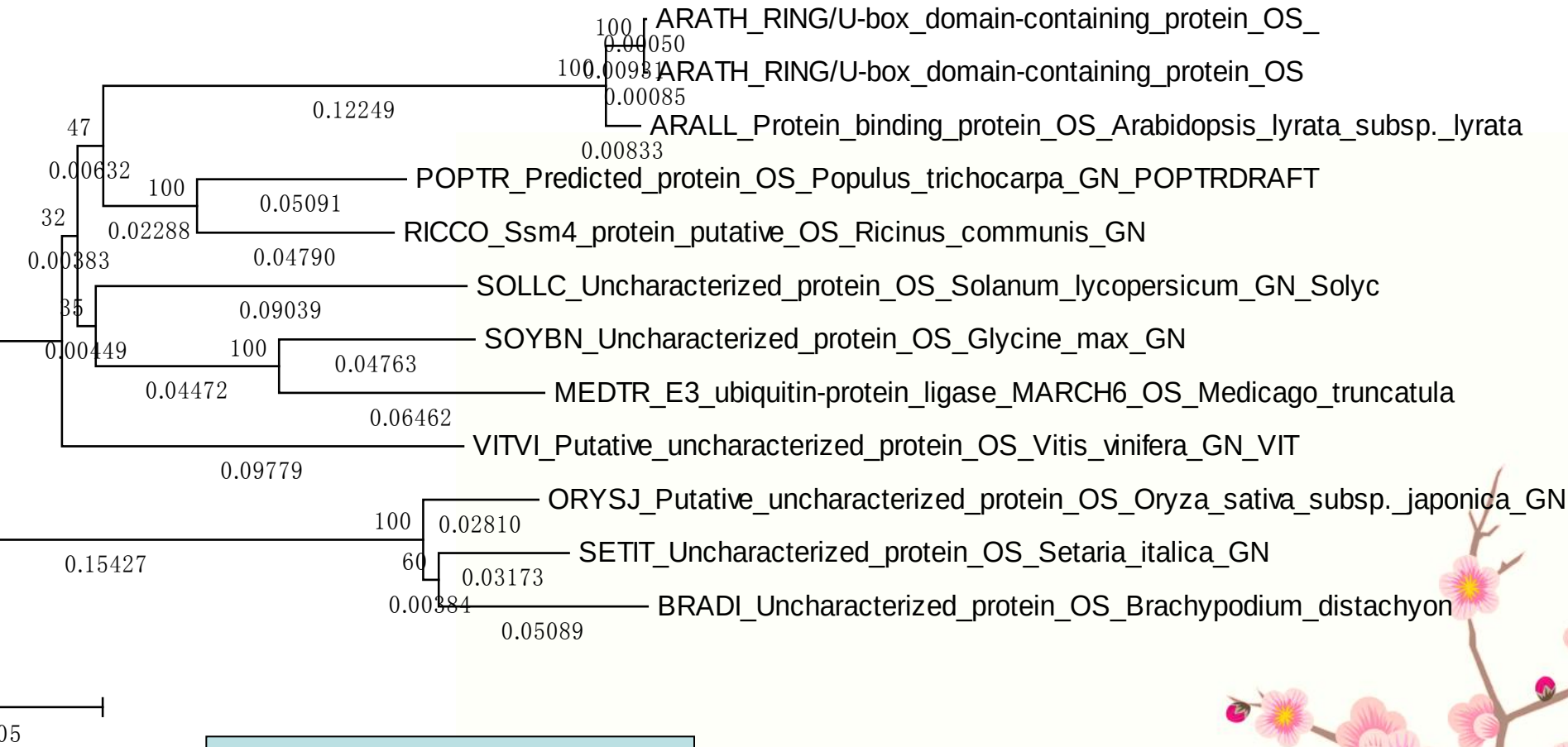
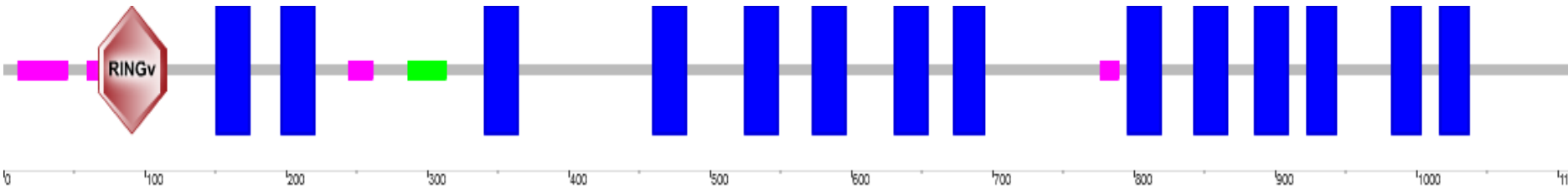
- RING-variant 域:67-117 bp


ARATH_RING/U-box_domain-containing_protein_OS_
ARATH_RING/U-box_domain-containing_protein_OS
ARALL_Protein_binding_protein_OS_Arabidopsis_lyrata_subsp._lyrata
POPTR_Predicted_protein_OS_Populus_trichocarpa_GN_POPTRDRAFT
RICCO_Ssm4_protein_putative_OS_Ricinus_communis_GN
SOLLC_Uncharacterized_protein_OS_Solanum_lycopersicum_GN_Solyc
SOYBN_Uncharacterized_protein_OS_Glycine_max_GN
MEDTR_E3_ubiquitin-protein_ligase_MARCH6_OS_Medicago_truncatula
VITVI_Putative_uncharacterized_protein_OS_Vitis_vinifera_GN_VIT
ORYSJ_Putative_uncharacterized_protein_OS_Oryza_sativa_subsp._japonica_GN
SETIT_Uncharacterized_protein_OS_Setaria_italica_GN
BRADI_Uncharacterized_protein_OS_Brachypodium_distachyon
0.12249
0.00833
0.00085
0.00050
0.00632
0.05091
0.02288
0.04790
0.00383
0.09039
0.00449
0.04763
0.04472
0.06462
0.09779
0.02810
0.15427
0.03173
0.00384
0.05089
100
47
32
35
60

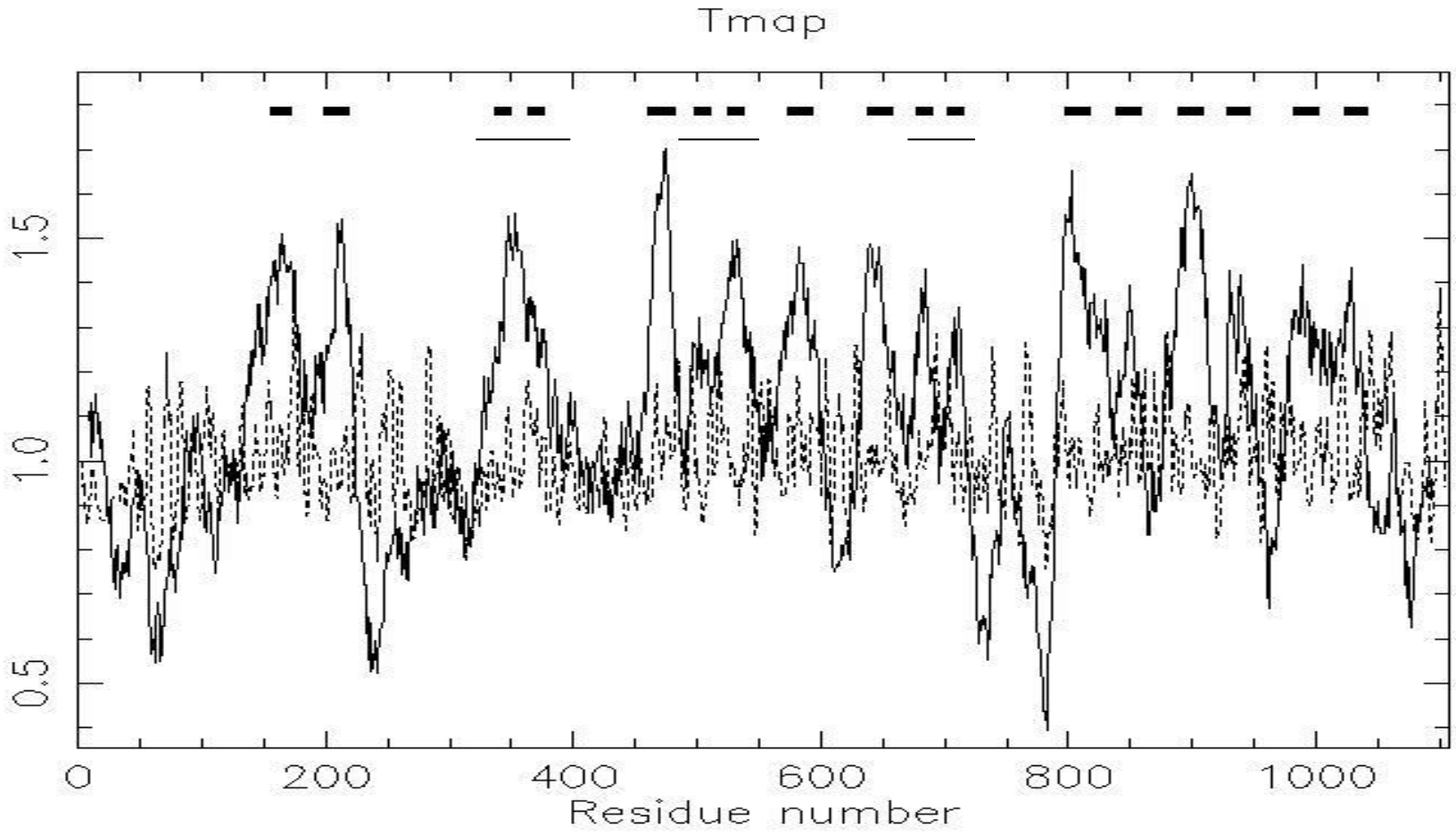
氨基酸序列比对



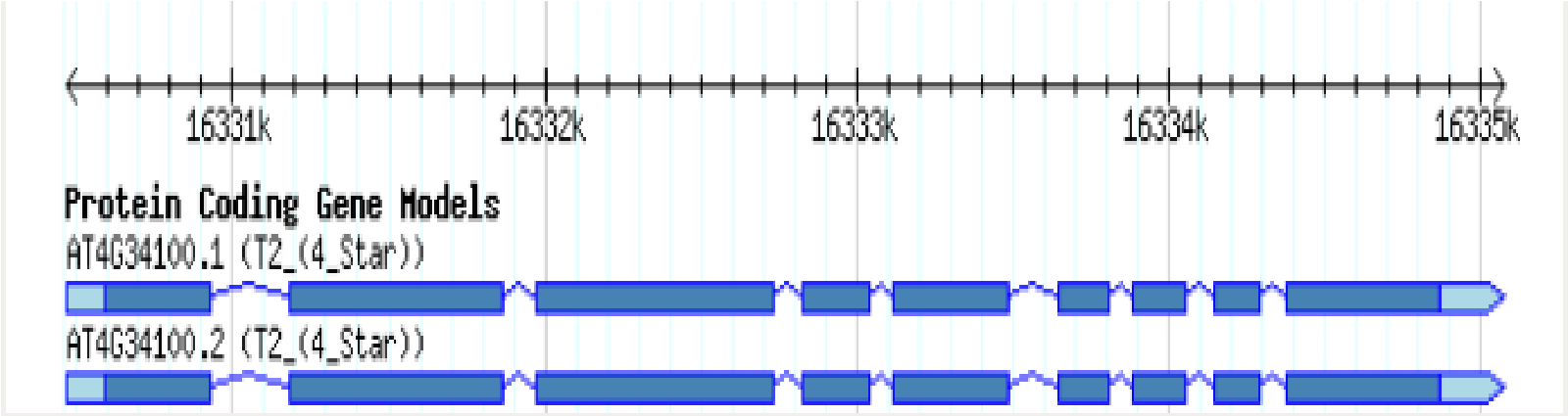
67-117bp

- RING variant domain: C-x (2) -C-x(10-45)-C-x (1) -C-x (7) -H-x(2)-C-x(11-25)-C-x(2)-C As opposed to a PHD: C-x(1-2) -C-x (7-13)-C-x(2-4)-C-x(4-5)-H-x(2)-C-x(10-21)-C-x(2)-C Classical RING domain: C-x (2) -C-x (9-39)-C-x(1-3)-H-x(2-3)-C-x(2)-C-x(4-48) -C-x(2)-C

JemBoss显示17个跨膜螺旋

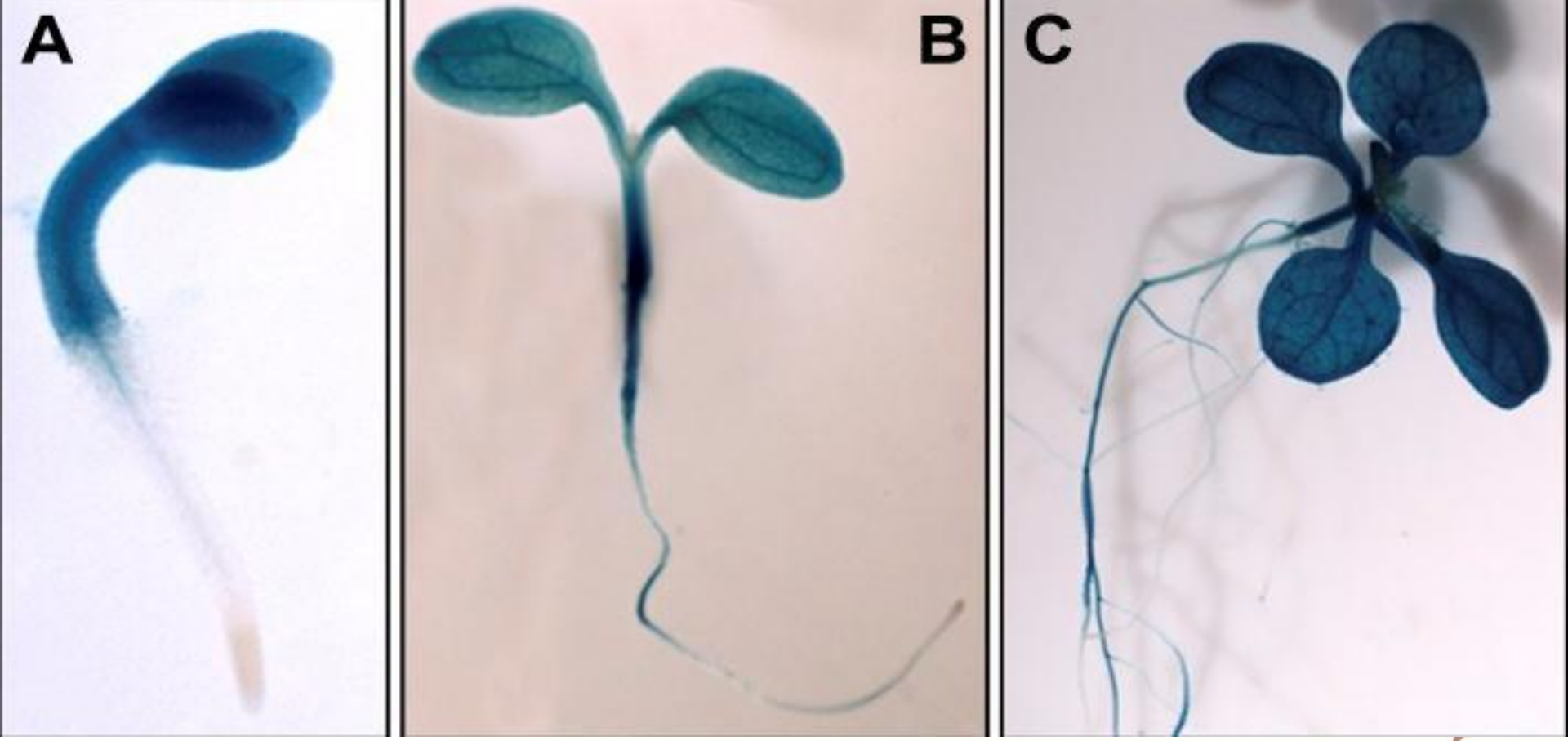


有两个转录本


16331k
16332k
16333k
16334k
16335k
Protein Coding Gene Models
AT4G34100.1 (T2_(4_Star))
AT4G34100.2 (T2_(4_Star))

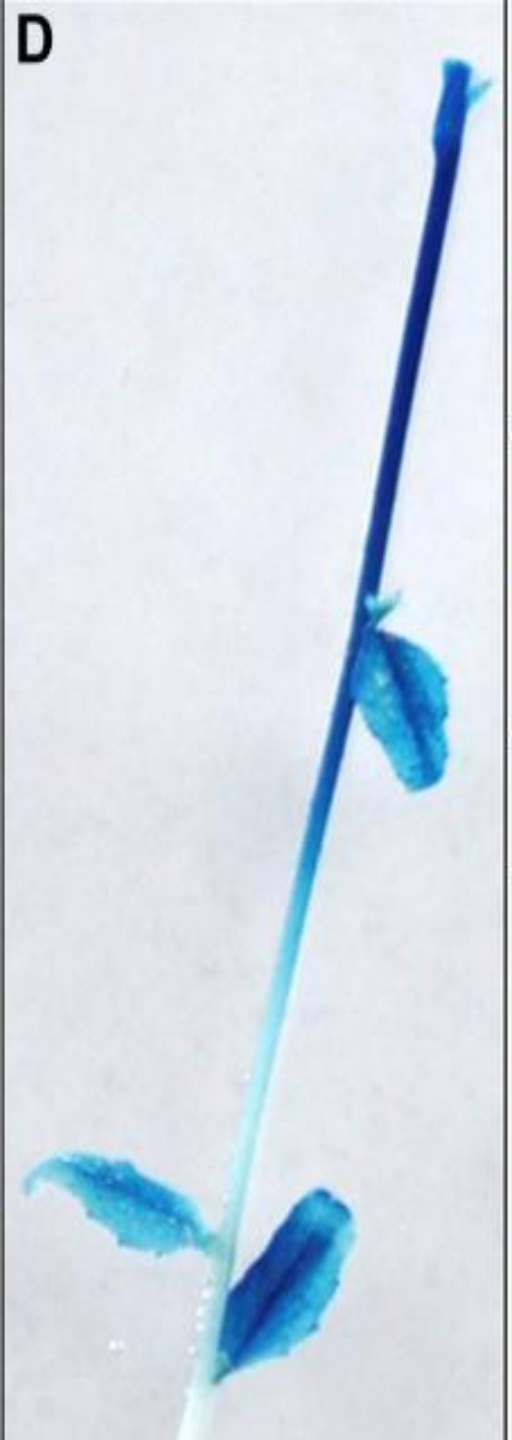
TAIR

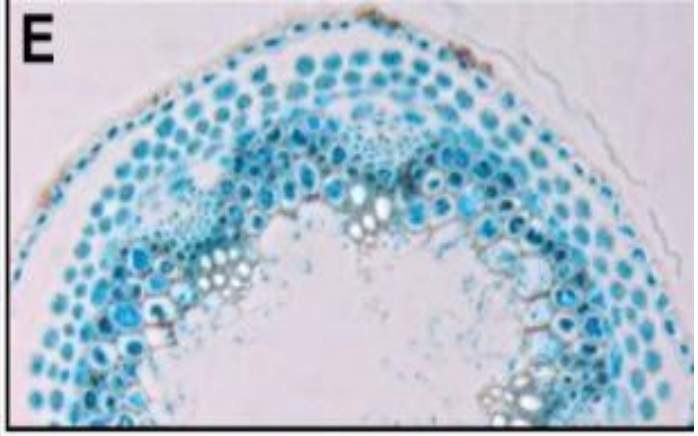
CER9 器官、组织表达特异性?



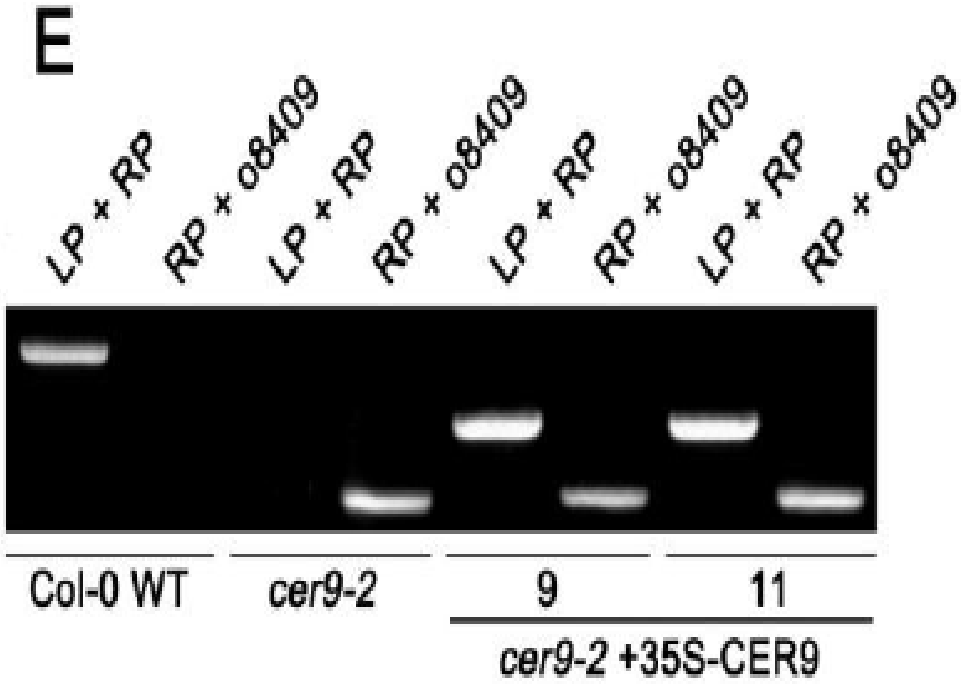
2d 5d 10d

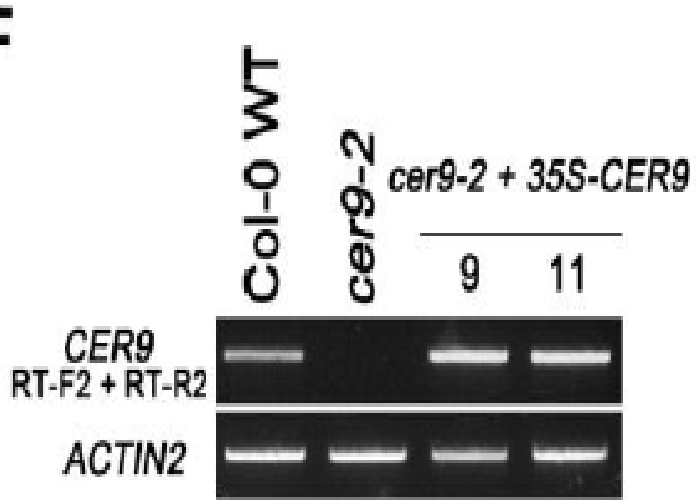
CER9pro::GUS报告基因检测





- 成熟植株的表达量检测





分子水平互补功能验证：

cer9-2+35S-CER9可以恢复野生型表型

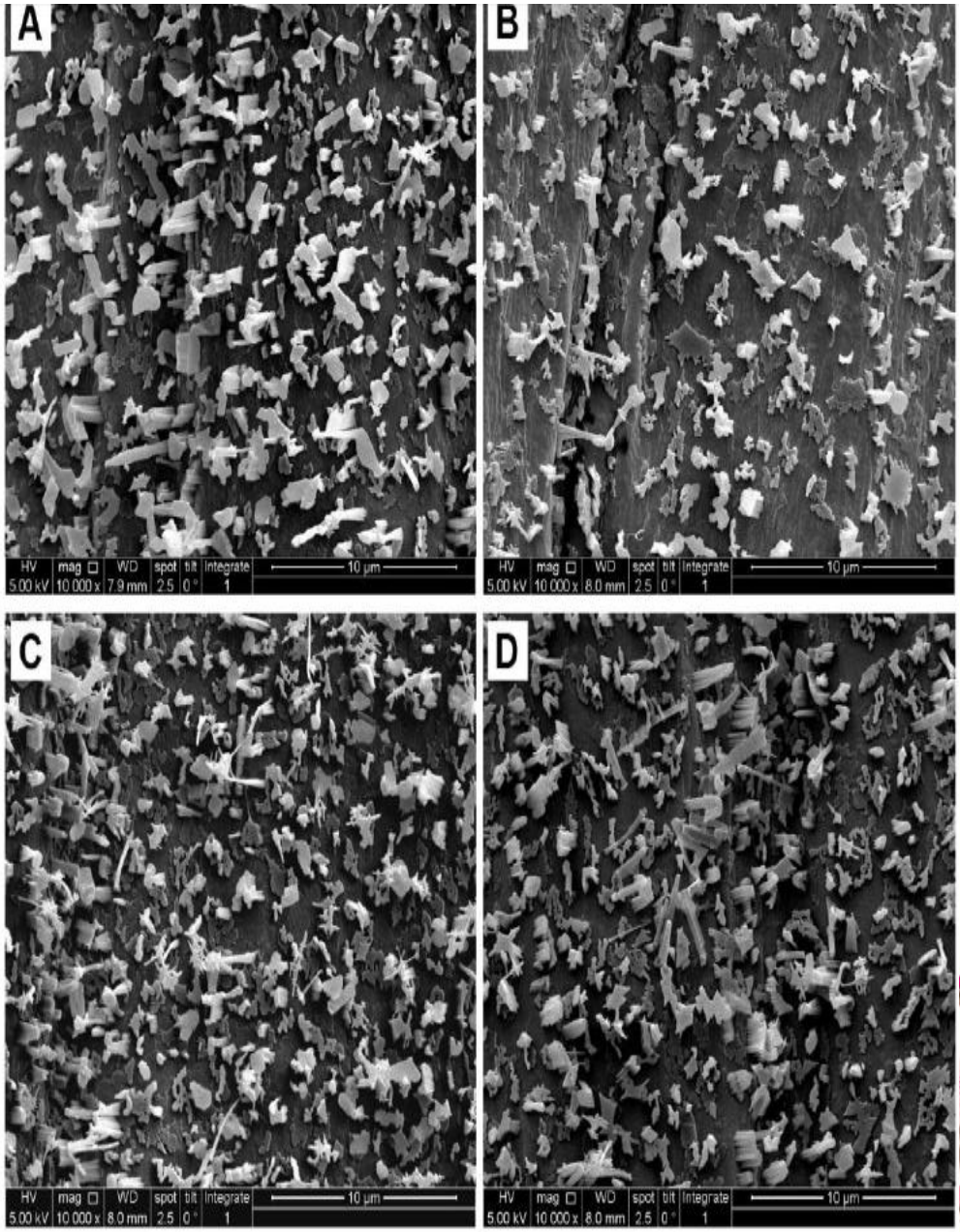
- SEM 扫描显示结果

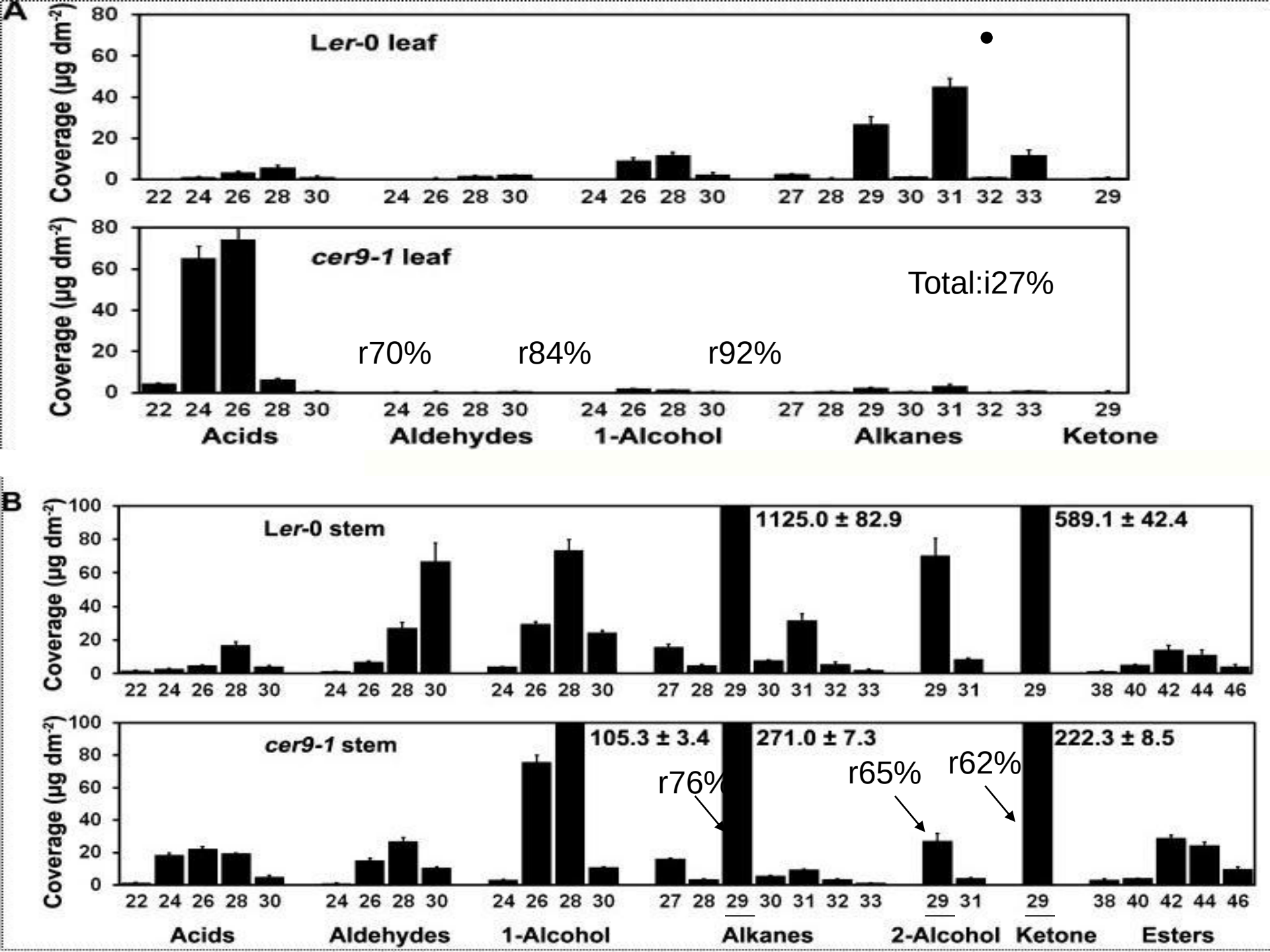
  (A) wild type Col-0;

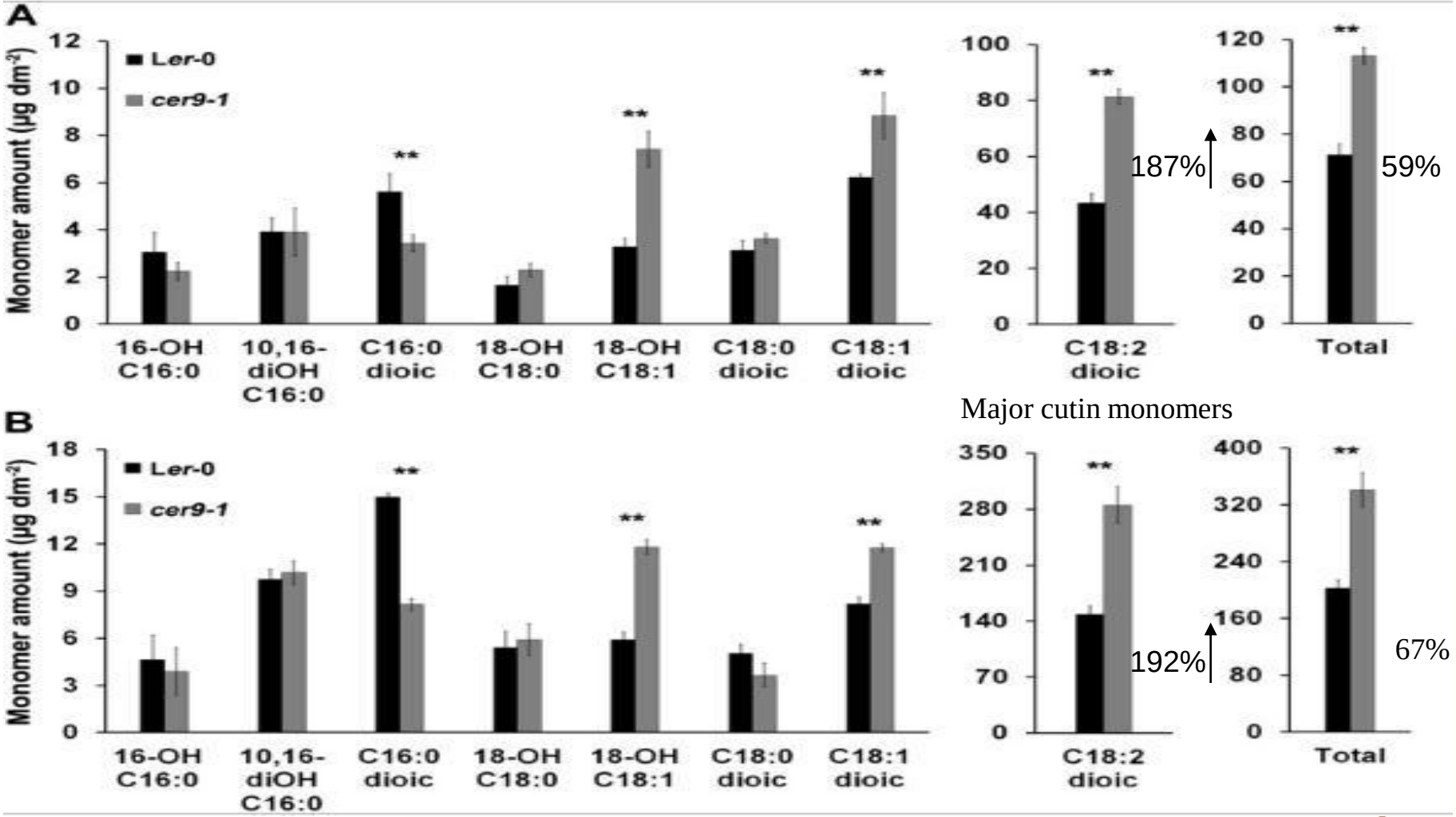
  (B) *cer9-2*;

  (C) *cer9-2* 含 *35S-CER9*的转基因株系9;

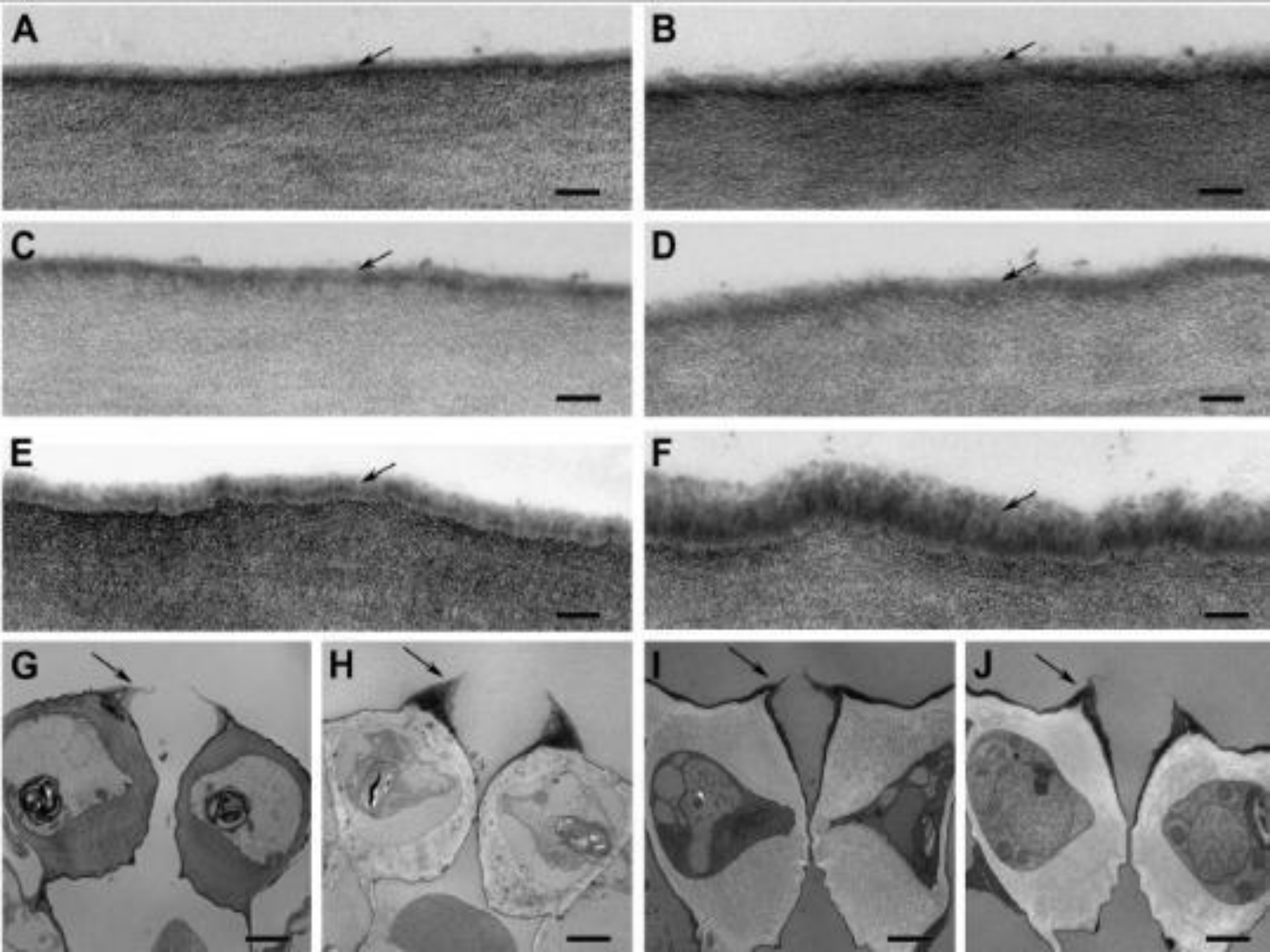
  (D) *cer9-2* 含 *35S-CER9*的转基因株系11;



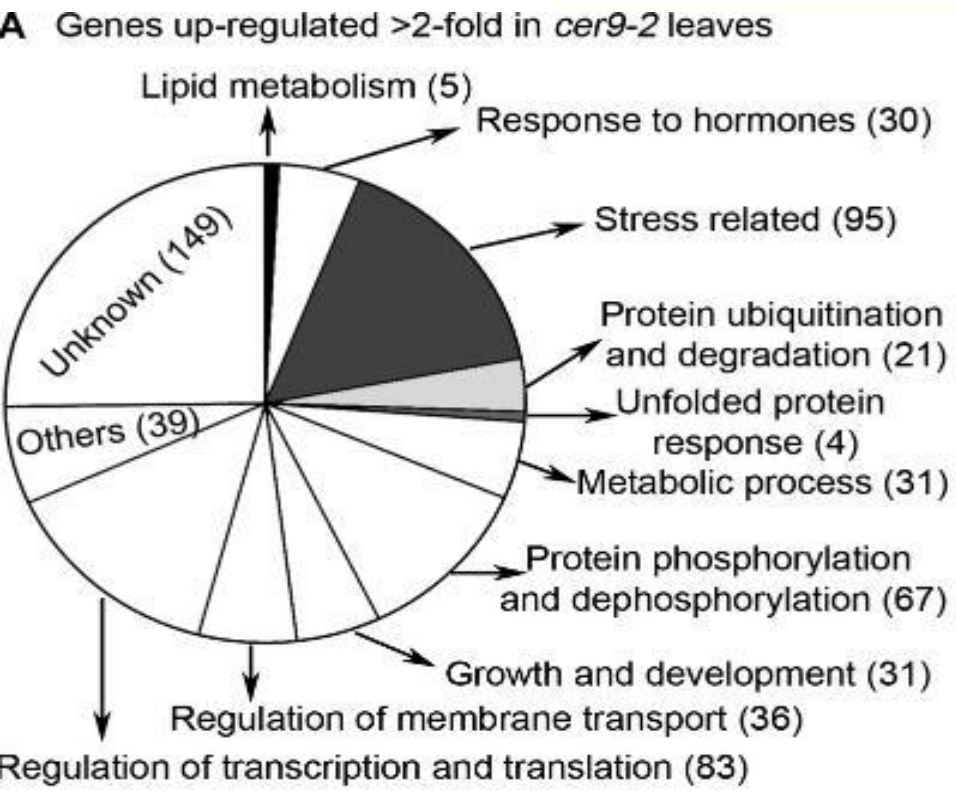

A
Ler-0 leaf
cer9-1 leaf
Coverage (µg dm-2)
Total:i27%
r70%
r84%
r92%
Acids
Aldehydes
1-Alcohol
Alkanes
Ketone
B
Ler-0 stem
1125.0 ± 82.9
589.1 ± 42.4
cer9-1 stem
105.3 ± 3.4
271.0 ± 7.3
222.3 ± 8.5
r76%
r65%
r62%
Acids
Aldehydes
1-Alcohol
Alkanes
2-Alcohol
Ketone
Esters

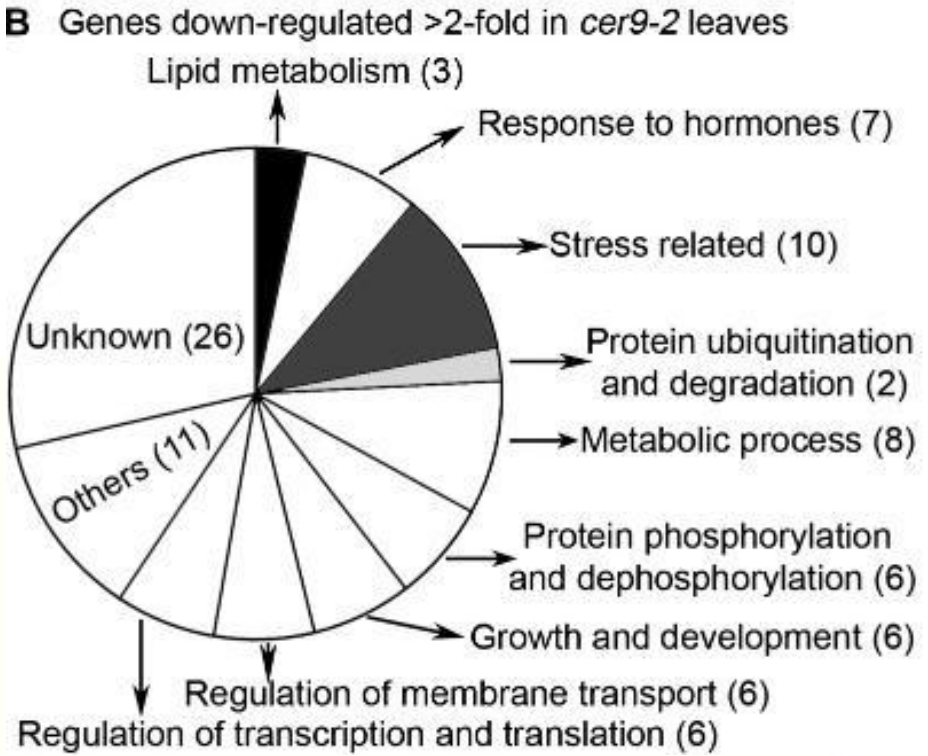

A
Monomer amount (µg dm-2)
Ler-0
cer9-1
16-OH C16:0
10,16-diOH C16:0
C16:0 dioic
18-OH C18:0
18-OH C18:1
C18:0 dioic
C18:1 dioic
C18:2 dioic
Total
187%
59%
Major cutin monomers
B
Monomer amount (µg dm-2)
Ler-0
cer9-1
16-OH C16:0
10,16-diOH C16:0
C16:0 dioic
18-OH C18:0
18-OH C18:1
C18:0 dioic
C18:1 dioic
C18:2 dioic
Total
192%
67%

化学构成分析


A
B
C
D
E
F
G
H
I
J

DNA芯片检测基因在突变体中表达量的变化



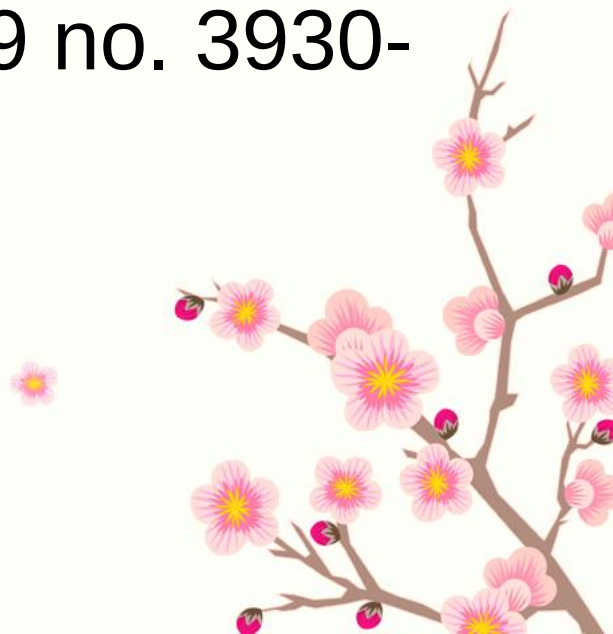


结论：

- cer9是第一个降低蒸腾速率、提高水分利用率、延迟萎蔫的有关角质合成的基因；能促进表皮蜡长链饱和脂肪酸增多，角质单体增多，角质膜亚显微结构变化，但都不足以解释表皮层通透性的变化。
- CER9 是表皮蜡和角质单质合成的一个负调控因子，*CER9* 蛋白与酵母的Doa10 蛋白高度相似，推测其作为泛素连接酶参与错误折叠和非聚合蛋白在内质网上降解，参与脂质合成过程。

主要参考文献：

- 1、Arabidopsis ECERIFERUM9 Involvement in Cuticle Formation and Maintenance of Plant Water Status. Plant Physiology, July 2012 vol. 159 no. 3930-944



Thank you for your attention !