



乙酰乳酸合成酶(ALS)的序列结构与功能分析

Sequence, structure and function analysis of acetylactate synthase

报告人：3G07A曹艺

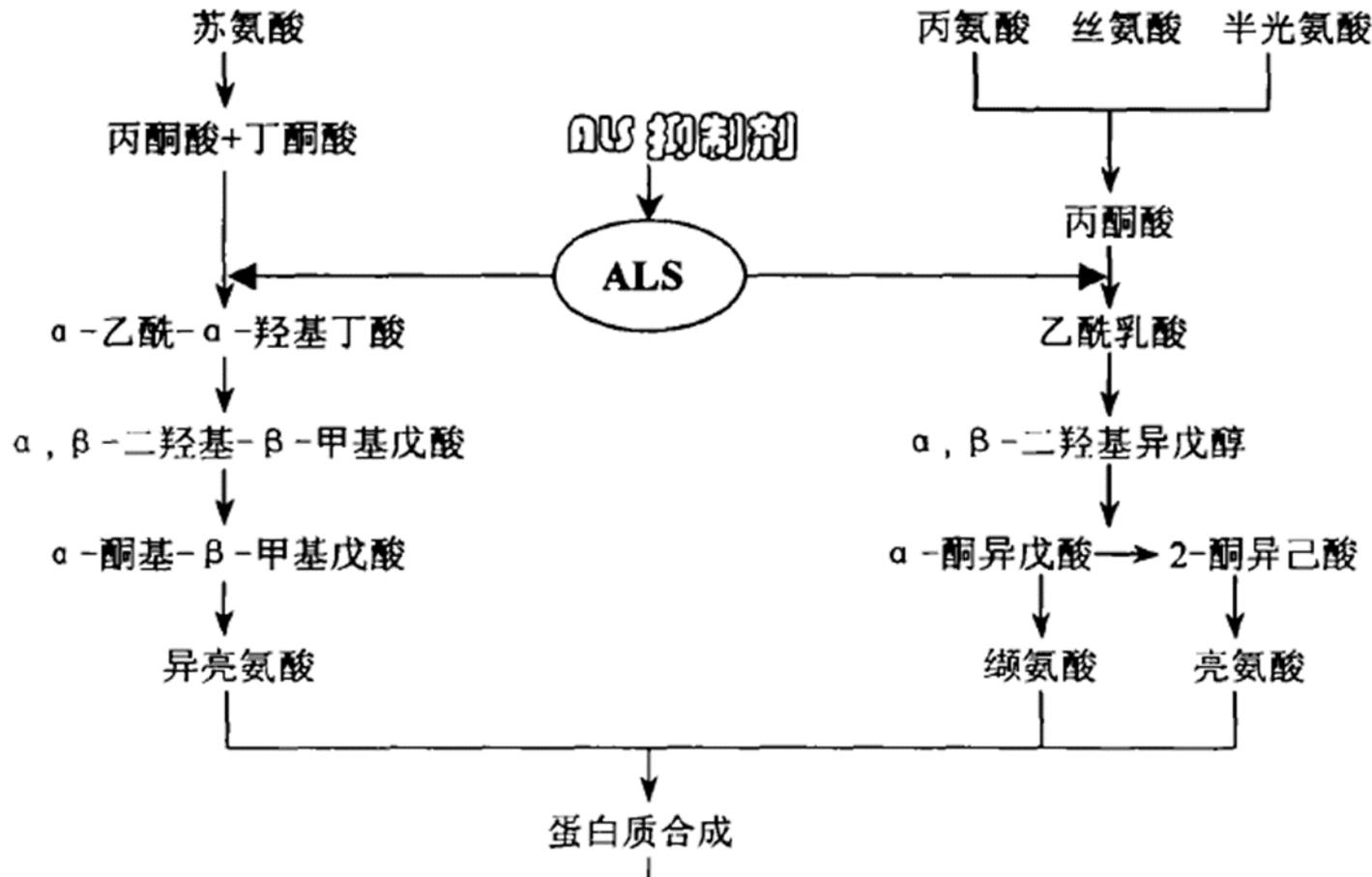
2021年5月10日

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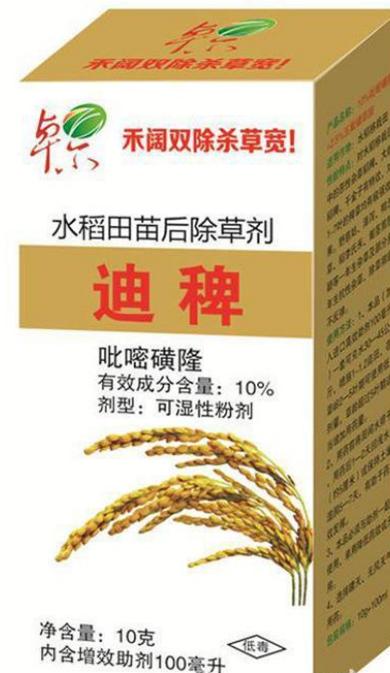
主要内容

1. 背景介绍
2. ALS序列分析
3. ALS结构分析
4. ALS功能分析
5. 课题相关



➤ ALS即乙酰乳酸合成酶,又称AHAS,乙酰羟基酸合成酶。

➤ 可以催化乙酰羟基丁酸和乙酰乳酸的形成。是支链氨基酸缬氨酸、亮氨酸和异亮氨酸生物合成途径中的第一个酶。

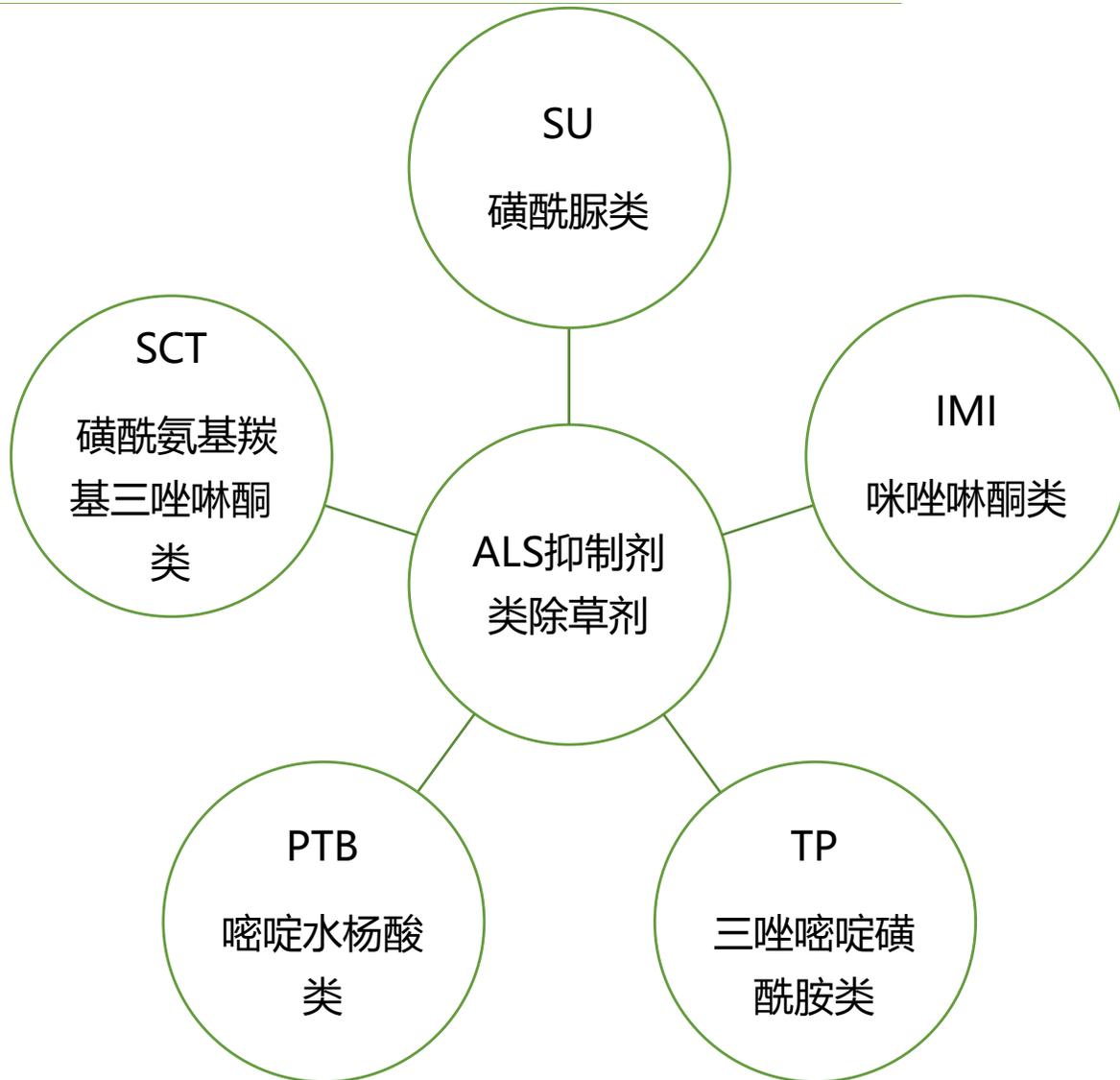


哈尔滨市亿亩粮田生物科技有限公司
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➤ **ALS**仅存在植物和微生物中,对人畜安全,自1982年,氯磺隆生产以来,ALS抑制剂类除草剂就成为除草剂产品开发的主流。

背景介绍



- **ALS抑制剂类除草剂**在除草时就是与靶标乙酰乳酸合成酶结合而抑制其活性，从而抑制植物体内合成三种支链氨基酸，影响蛋白质和DNA的合成，从而导致植物有丝分裂不能正常完成，导致植物停止生长直至死亡。

背景介绍

INTERNATIONAL HERBICIDE-RESISTANT WEED DATABASE

Herbicide-Resistant Weeds by Site of Action

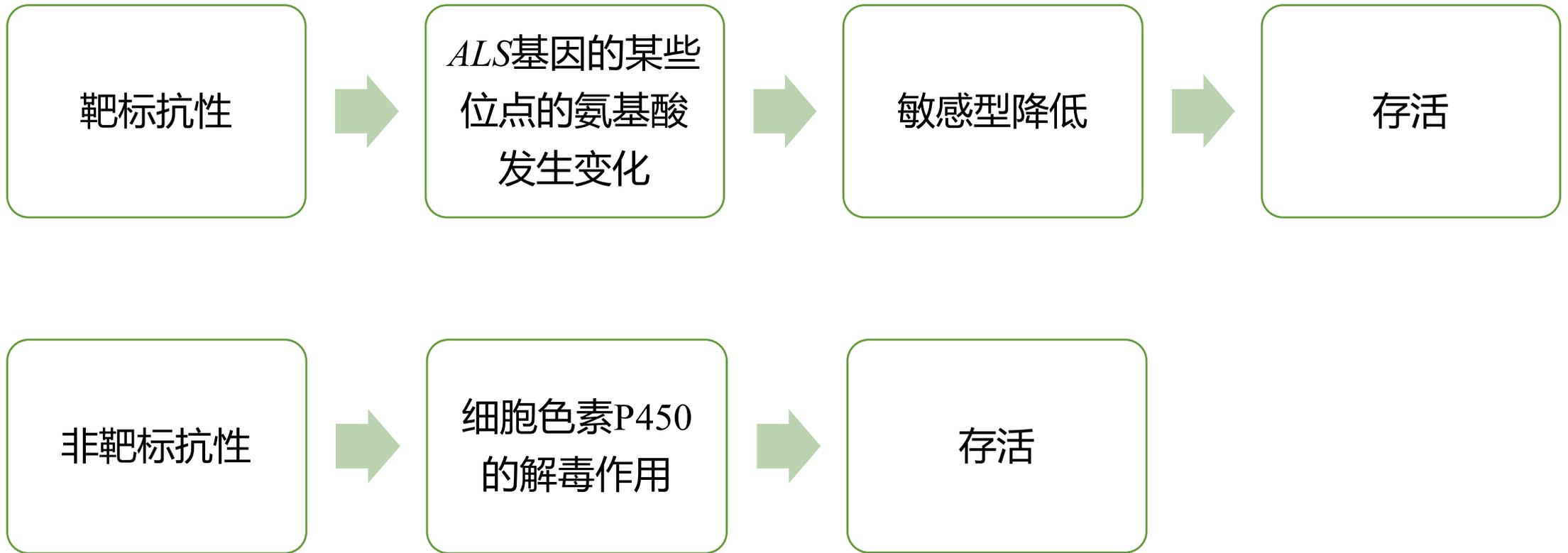
This table lists the number of species resistant to each site of action. Please note that **many species have evolved resistance to more than one site of action**, so the grand total represents unique cases of resistance, not the number of species.
(click on a column header to sort, hover mouse over links for more information or click on a links for details)

HRAC Group	Legacy HRAC Group	Site of Action	Example Herbicide	Dicots	Monocots	Total
2	B	ALS inhibitors	Chlorsulfuron	102	65	167
5	C1	Photosystem II- Serine 264 Binders	Atrazine	51	23	74
9	G	EPSP synthase inhibitors	Glyphosate	26	27	53
1	A	ACCase inhibitors	Sethoxydim	0	49	49
4	O	Auxin Mimics	2,4-D	33	8	41
22	D	PSI Electron Diverter	Paraquat	22	10	32
5	C2	PSII inhibitors - Serine 264 Binders	Chlorotoluron	11	18	29
14	E	PPO inhibitors	Oxyfluorfen	10	3	13
3	K1	Microtubule Assembly inhibitors	Trifluralin	2	10	12
15	N	Lipid Inhibitors	Triallate	0	10	10
15	K3	Very Long-Chain Fatty Acid Synthesis inhibitors	Butachlor	3	5	8
34	F3	Lycopene Cyclase inhibitors	Amitrole	1	5	6
12	F1	Phytoene Desaturase inhibitors	Diflufenican	4	1	5

- **ALS抑制剂类除草剂**在杂草的防控中占有很高的比例,目前,全世界已经有167种杂草,其中102种双子叶植物,65种单子叶植物对ALS抑制剂类除草剂产生了抗药性。

背景介绍

杂草抗药性机理



ALS序列分析

UniProtKB - Q9FFF4 (ILVH1_ARATH)

Display

Help video

BLAST

Align

Format

Add to basket

History

Entry

Publications

Feature viewer

Feature table

Protein | **Acetolactate synthase small subunit 1, chloroplastic**

Gene | **VAT1**

Organism | *Arabidopsis thaliana (Mouse-ear cress)*

Status |  Reviewed - Annotation score: ●●●●● - Experimental evidence at protein levelⁱ

UniProtKB - Q93YZ7 (ILVH2_ARATH)

Display

Help video

BLAST

Align

Format

Add to basket

History

Entry

Publications

Feature viewer

Feature table

Protein | **Acetolactate synthase small subunit 2, chloroplastic**

Gene | **At2g31810**

Organism | *Arabidopsis thaliana (Mouse-ear cress)*

Status |  Reviewed - Annotation score: ●●●●● - Experimental evidence at protein levelⁱ

UniProt数据库,拟南芥有两条经过人工审阅的ALS序列

Q9FFF4(ILVH1_ARATH)

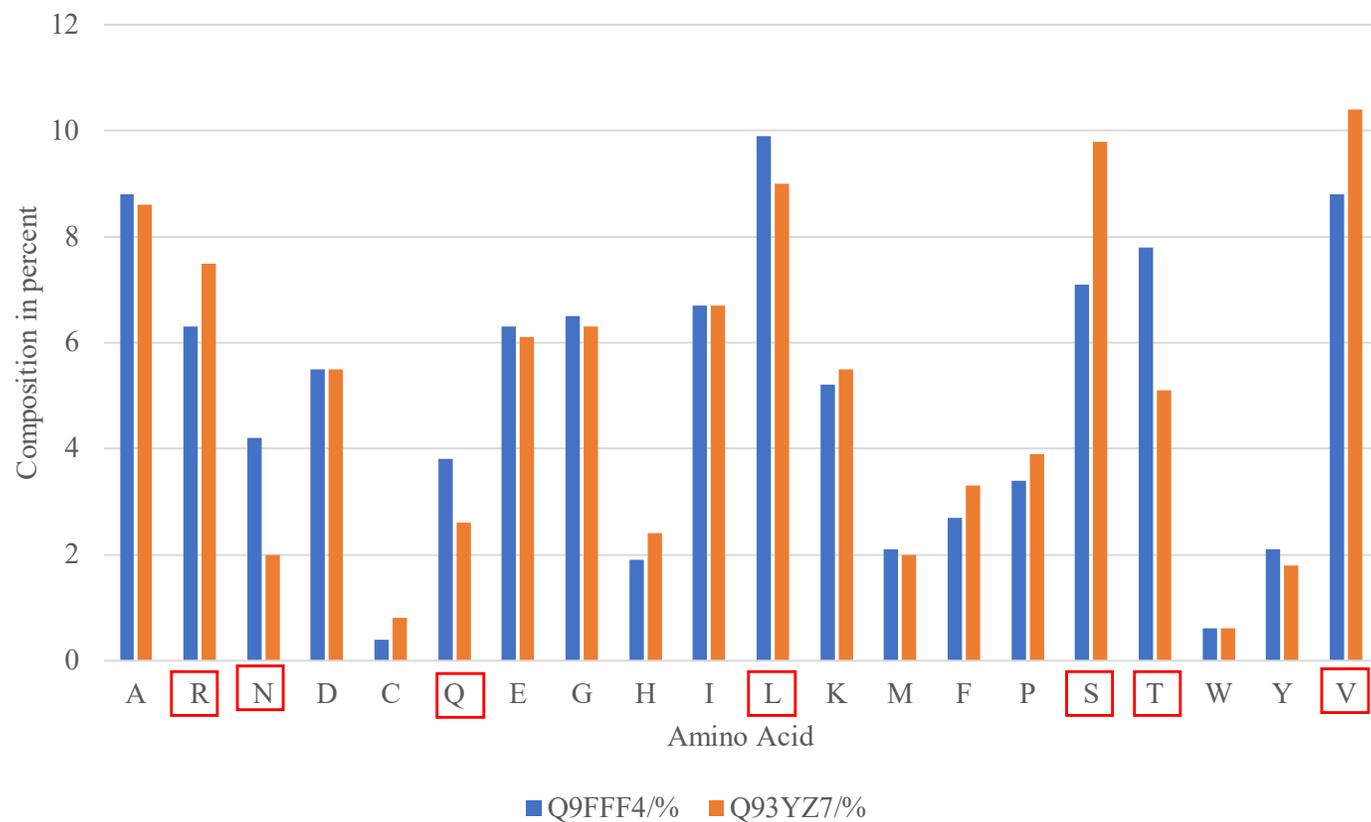
Q93YZ7(ILVH2_ARATH)

两者是位于叶绿体上的ALS的不同亚基。两个亚基均属于乙酰羟氨酸合酶的调节亚基。参与支链氨基酸的反馈抑制。

ALS序列分析

AA	Q9FFF4/%	Q93YZ7/%	Database%
A	8.8	8.6	8.25
R	6.3	7.5	5.53
N	4.2	2	4.06
D	5.5	5.5	5.46
C	0.4	0.8	1.38
Q	3.8	2.6	3.93
E	6.3	6.1	6.72
G	6.5	6.3	7.07
H	1.9	2.4	2.27
I	6.7	6.7	5.91
L	9.9	9	9.65
K	5.2	5.5	5.8
M	2.1	2	2.41
F	2.7	3.3	3.86
P	3.4	3.9	4.73
S	7.1	9.8	6.63
T	7.8	5.1	5.35
W	0.6	0.6	1.1
Y	2.1	1.8	2.92
V	8.8	10.4	6.86

氨基酸组成比较



ALS序列分析

将这两条序列分别与实验室在藜上扩增出来的序列进行Water比对

```
#
# Aligned_sequences: 2
# 1: ILVH1_ARATH
# 2: li
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 170
# Identity:      36/170 (21.2%)
# Similarity:   58/170 (34.1%)
# Gaps:         52/170 (30.6%)
# Score: 61.0
#
#
#-----
```

```
..
#
# Aligned_sequences: 2
# 1: ILVH2_ARATH
# 2: li
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 461
# Identity:      88/461 (19.1%)
# Similarity:   146/461 (31.7%)
# Gaps:         178/461 (38.6%)
# Score: 58.5
#
#
#-----
```

ALS序列分析

通过查阅文献得到PDB编号为1YBH(拟南芥乙酰羟羧酸合酶与磺酰脲类除草剂氯吡脲乙酯的配合物的晶体结构), 进而找到了其蛋白质登录号: P17597(ILVB_ARATH)

UniProtKB - P17597 (ILVB_ARATH)

Display

[Help video](#)

[BLAST](#)

[Align](#)

[Format](#)

[Add to basket](#)

[History](#)

Entry

[Publications](#)

[Feature viewer](#)

[Feature table](#)

Protein | **Acetolactate synthase, chloroplastic**

Gene | **ALS**

Organism | *Arabidopsis thaliana* (Mouse-ear cress)

Status |  Reviewed - Annotation score:  - Experimental evidence at protein levelⁱ

赵奇,邓培渊,郭运宏等.乙酰乳酸合成酶抗性突变的分子动力学模拟[J].南方农业学报,2020,51(09):2167-2173.)

ALS序列分析

Water比对

```
#=====
#
# Aligned_sequences: 2
# 1: li
# 2: ILVB_ARATH
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 551
# Identity: 452/551 (82.0%)
# Similarity: 506/551 (91.8%)
# Gaps: 4/551 ( 0.7%)
# Score: 2408.5
#
#=====
```

```
li 1 MEIHQALTRSGSIRNVLP RHEQGGVFAAEGYARATGRVGVCIATSGPGAT 50
ILVB_ARATH 124 MEIHQALTRSSSIRNVLP RHEQGGVFAAEGYARSSGKPGIC IATSGPGAT 173

li 51 NLVSLADALLDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNY 100
ILVB_ARATH 174 NLVSLADALLDSVPLVAITGQVPRRMIGTDAFQETPIVEVTRSITKHNY 223

li 101 LVL DVEDIPRVVKEAFY LANSRPGPVLIDIPKDIQQQLVVPNWDQPIKL 150
ILVB_ARATH 224 LVMDVEDIPRIIEEAFFLATSGRPGPVLVDVPKDIQQQLAIPNWEQAMRL 273

li 151 GGYVSRLPKSKFSANEGLLEQIVRLMSEAKKPVLYVGGGCLN STEELRK 200
ILVB_ARATH 274 PGYMSRMPKPP----EDSHLEQIVRLISESKKPVLYVGGGCLN SSDLGR 319

li 201 FVELTGIPVASTLMGLGAYPCNDEL SLHMLGMHGT VYANYAVDKADLLA 250
ILVB_ARATH 320 FVELTGIPVASTLMGLGSYPCDEL SLHMLGMHGT VYANYAVEHSDLLA 369

li 251 FGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGKNKQPHV SICADV K LAL 300
ILVB_ARATH 370 FGVRFDDRVTGKLEAFASRAKIVHIDIDSAEIGKNKTPHVSVC GDV K LAL 419

li 301 KGMNKILESRKGLNLDYSSWREELGEQKKKFP LSFKTFGEAIP P QYAIQ 350
ILVB_ARATH 420 QGMNKVLENRAEELKLD FGVWRNELNVQKQKFP LSFKTFGEAIP P QYAIK 469

li 351 MLDELTDGNAVI STGVGQHQM WAAQHYKYRNP RQWL TSGGLGAMGFGLPA 400
ILVB_ARATH 470 VLDELTDGKAI I STGVGQHQM WAAQFYNYKKP RQWL SSGGLGAMGFGLPA 519

li 401 AIGAAVARPESVVDIDGDG SFIMNVQELATIRVENLPVKIMLLNNQH LG 450
ILVB_ARATH 520 AIGASVANPDAI VVDIDGDG SFIMNVQELATIRVENLPVKVLLNNQH LG 569

li 451 MVMQWEDRFYKANRAHTY LGNPAKEAEIFPDMLKFAEACDIPAARVTKVS 500
ILVB_ARATH 570 MVMQWEDRFYKANRAHTFLGDP AQEDEIFPNMLLFAAACGIPAARVTKKA 619

li 501 ELRAAMQKMLDTPGPYLLDVI VPHQEHVLP MIPSGAAFKDI INEGDGR TS 550
ILVB_ARATH 620 DLREAIQTMLDTPGPYLLDVI CPHQEHVLP MIPSGGTFNDVIT EGDGR IK 669

li 551 Y 551
|
ILVB_ARATH 670 Y 670
```

ALS序列分析

P17597进行Blastp的结果

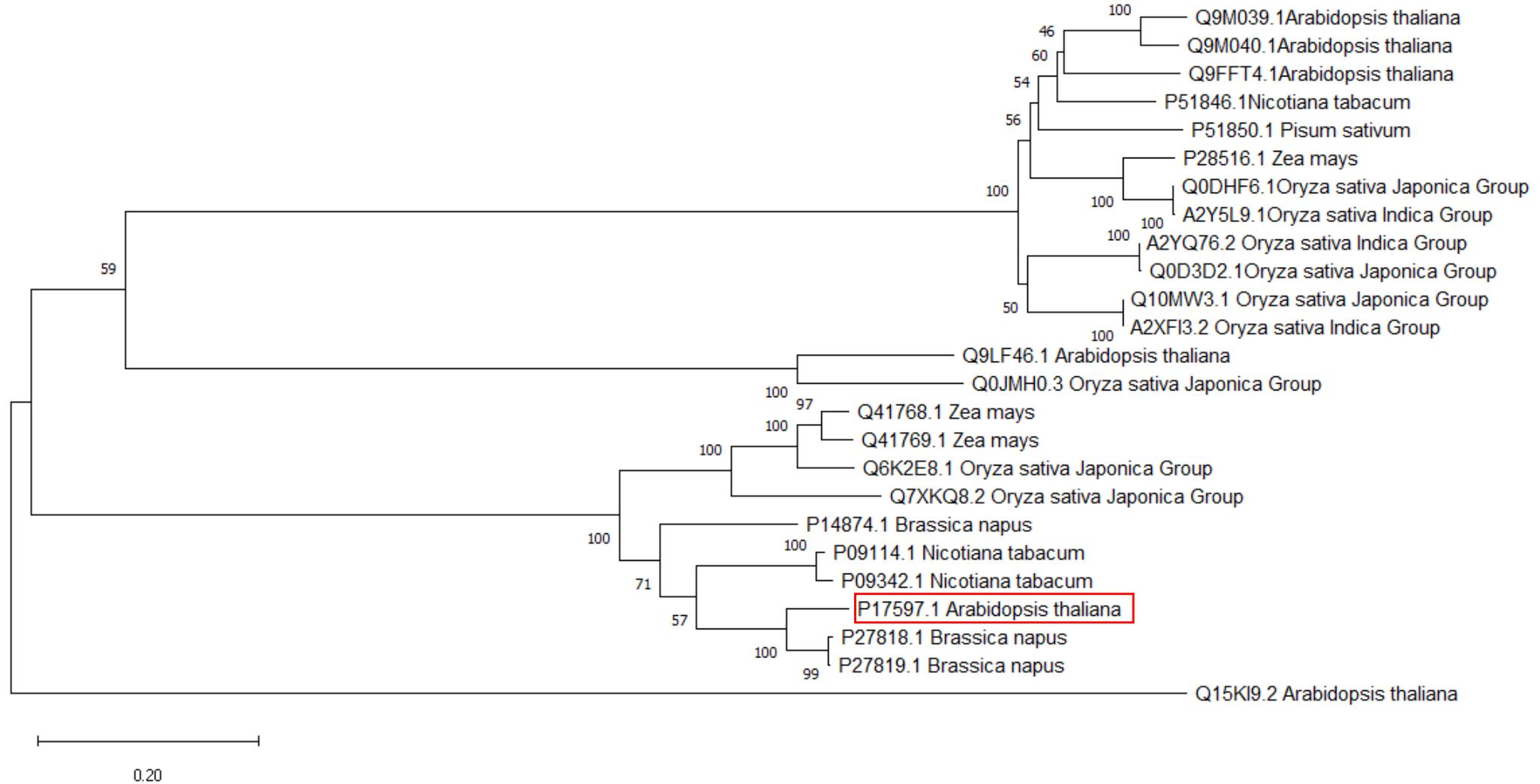
Sequences producing significant alignments

Download New Select columns Show 1000

select all 25 sequences selected [GenPept](#) [Graphics](#) [Distance tree of results](#) [Multiple alignment](#) New [MSA Viewer](#)

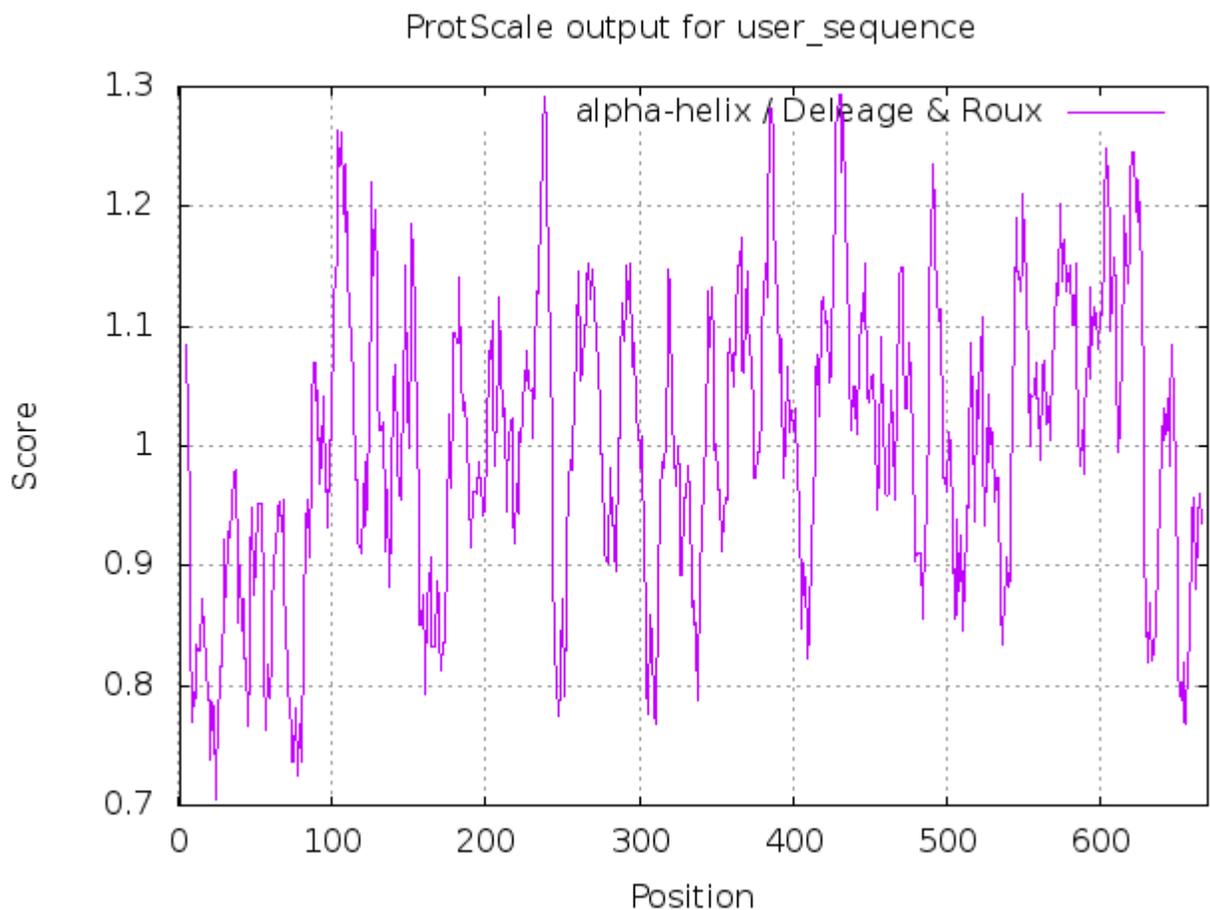
	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=ALS I; AltName: Full=Acetohydroxy-acid synt...	Brassica napus	1000	1000	99%	0.0	88.57%	655	P27818.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase, chloroplastic; Short=AtALS; AltName: Full=Acetohydroxy-acid synthase; Alt...	Arabidopsis thalia...	997	997	95%	0.0	100.00%	670	P17597.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 3, chloroplastic; AltName: Full=ALS III; AltName: Full=Acetohydroxy-acid sy...	Brassica napus	995	995	99%	0.0	88.57%	652	P27819.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 2, chloroplastic; AltName: Full=ALS II; AltName: Full=Acetohydroxy-acid syn...	Nicotiana tabacum	892	892	95%	0.0	78.56%	664	P09114.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=ALS I; AltName: Full=Acetohydroxy-acid synt...	Nicotiana tabacum	887	887	95%	0.0	78.06%	667	P09342.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 2, chloroplastic; AltName: Full=ALS II; AltName: Full=Acetohydroxy-acid syn...	Brassica napus	862	862	97%	0.0	76.99%	637	P14874.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 1; Flags: Precur...	Oryza sativa Japo...	789	789	87%	0.0	75.30%	644	Q6K2E8.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 1; Flags: Precur...	Zea mays	788	788	93%	0.0	71.09%	638	Q41768.1
<input checked="" type="checkbox"/>	RecName: Full=Acetolactate synthase 2, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 2; Flags: Precur...	Zea mays	778	778	86%	0.0	74.96%	638	Q41769.1
<input checked="" type="checkbox"/>	RecName: Full=Probable acetolactate synthase 2, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 2; Flag...	Oryza sativa Japo...	751	751	86%	0.0	71.75%	663	Q7XKQ8.2
<input checked="" type="checkbox"/>	RecName: Full=2-hydroxyacyl-CoA lyase; AltName: Full=2-hydroxyphytanoyl-CoA lyase; Short=2-HPCL; AltName: ...	Arabidopsis thalia...	102	102	70%	9e-25	27.68%	572	Q9LF46.1
<input checked="" type="checkbox"/>	RecName: Full=2-hydroxyacyl-CoA lyase; AltName: Full=2-hydroxyphytanoyl-CoA lyase; Short=2-HPCL; AltName: ...	Oryza sativa Japo...	76.3	76.3	61%	2e-15	25.17%	577	Q0JMH0.3
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 3; Short=PDC [Oryza sativa Indica Group]	Oryza sativa Indic...	48.2	48.2	34%	7e-06	24.80%	587	A2YQ76.2
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 3; Short=PDC [Oryza sativa Japonica Group]	Oryza sativa Japo...	48.2	48.2	34%	7e-06	24.80%	587	Q0D3D2.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 2; Short=PDC [Oryza sativa Japonica Group]	Oryza sativa Japo...	47.7	47.7	33%	1e-05	26.27%	605	Q10MW3.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 2; Short=PDC [Oryza sativa Indica Group]	Oryza sativa Indic...	47.7	47.7	33%	1e-05	26.27%	606	A2XF13.2
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 2; Short=AtPDC2 [Arabidopsis thaliana]	Arabidopsis thalia...	46.6	46.6	23%	2e-05	25.62%	607	Q9FFT4.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 3; Short=AtPDC3 [Arabidopsis thaliana]	Arabidopsis thalia...	46.1	46.1	15%	3e-05	28.43%	592	Q9M039.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 4; Short=AtPDC4 [Arabidopsis thaliana]	Arabidopsis thalia...	45.6	45.6	21%	5e-05	24.83%	603	Q9M040.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 2; Short=NtPDC2 [Nicotiana tabacum]	Nicotiana tabacum	45.0	45.0	13%	7e-05	28.26%	614	P51846.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 1; Short=PDC [Pisum sativum]	Pisum sativum	42.4	42.4	20%	5e-04	24.46%	593	P51850.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 1; Short=PDC [Oryza sativa Japonica Group]	Oryza sativa Japo...	39.8	39.8	15%	0.004	27.45%	605	Q0DHF6.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 1; Short=PDC [Oryza sativa Indica Group]	Oryza sativa Indic...	39.8	39.8	15%	0.004	27.45%	605	A2Y5L9.1
<input checked="" type="checkbox"/>	RecName: Full=Pyruvate decarboxylase 1; Short=PDC [Zea mays]	Zea mays	39.0	39.0	15%	0.006	30.39%	610	P28516.1
<input checked="" type="checkbox"/>	RecName: Full=Protein PHYLLQ, chloroplastic; Includes: RecName: Full=Inactive isochorismate synthase; AltName...	Arabidopsis thalia...	38.7	38.7	26%	0.011	23.47%	1715	Q15K19.2

ALS序列分析

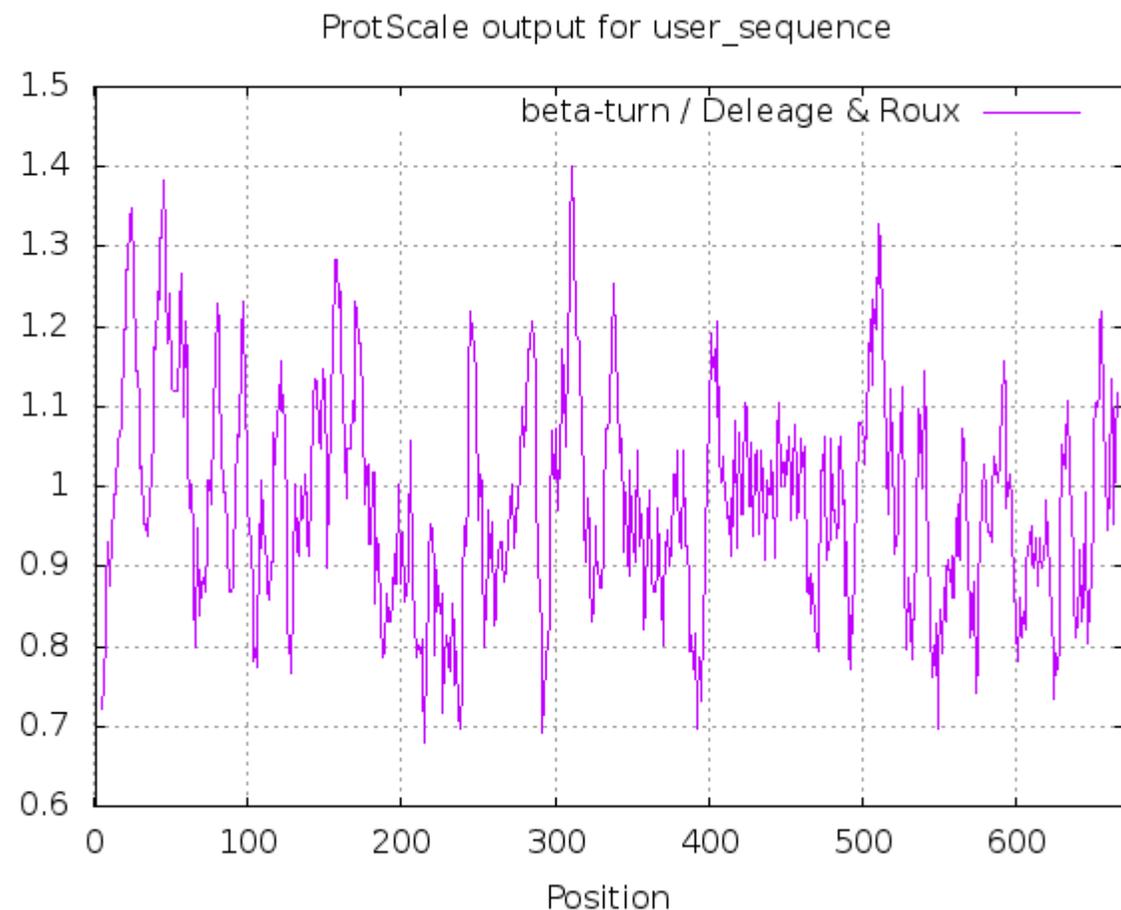


phylogenetic tree construction of ALS

ALS结构分析

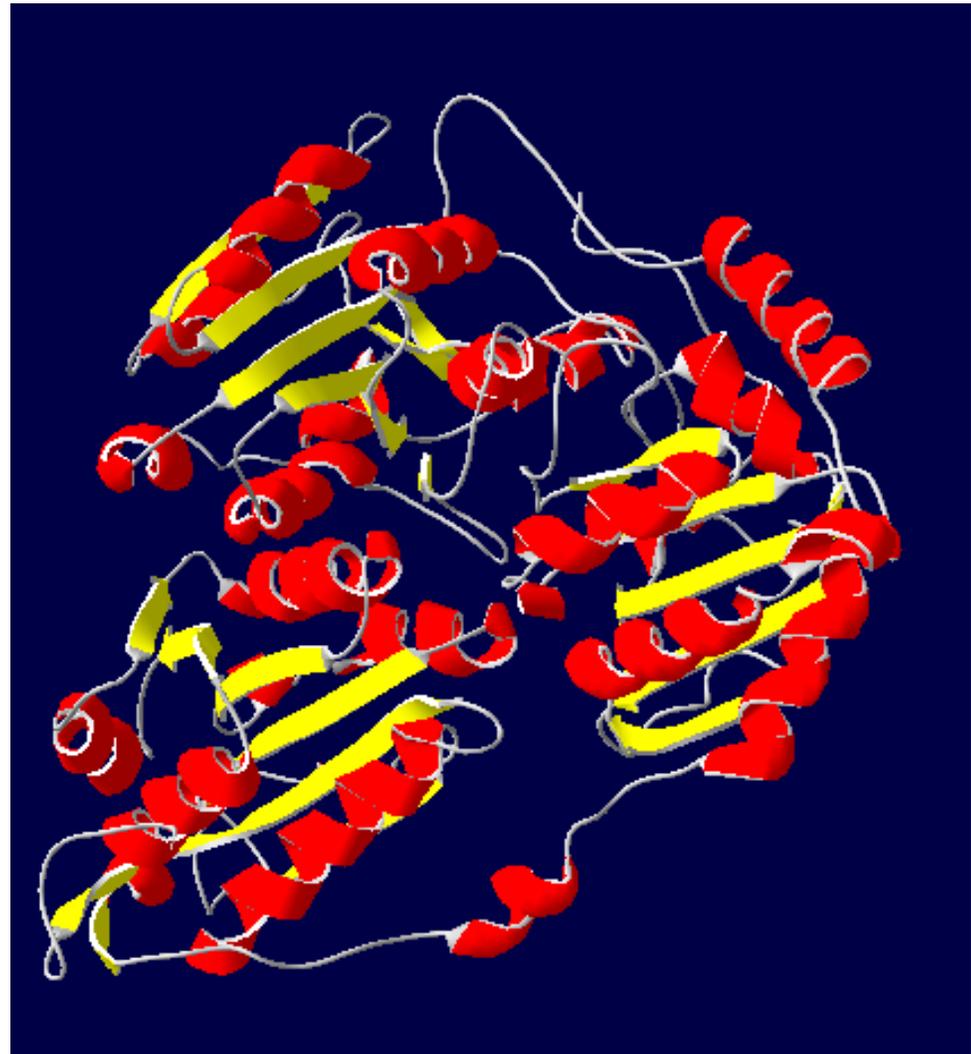
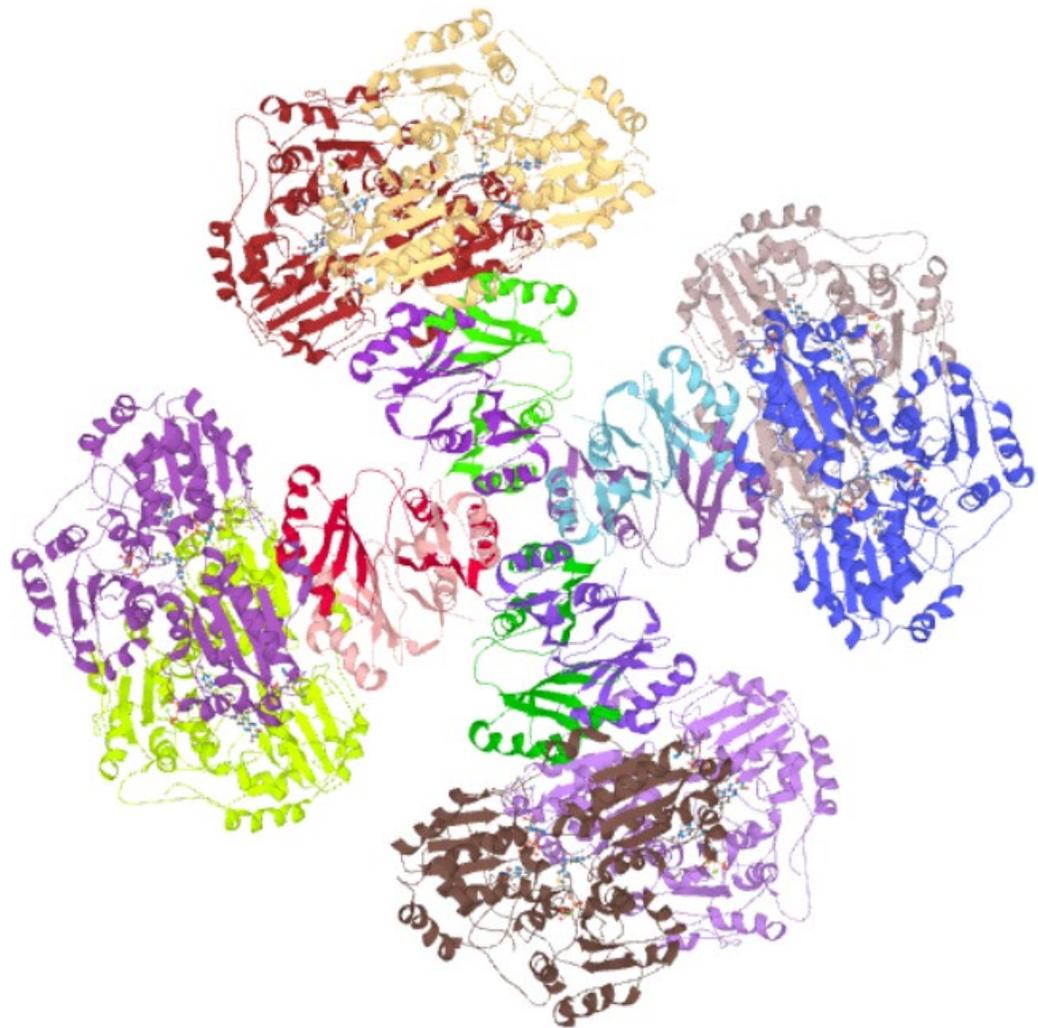


33个alpha螺旋



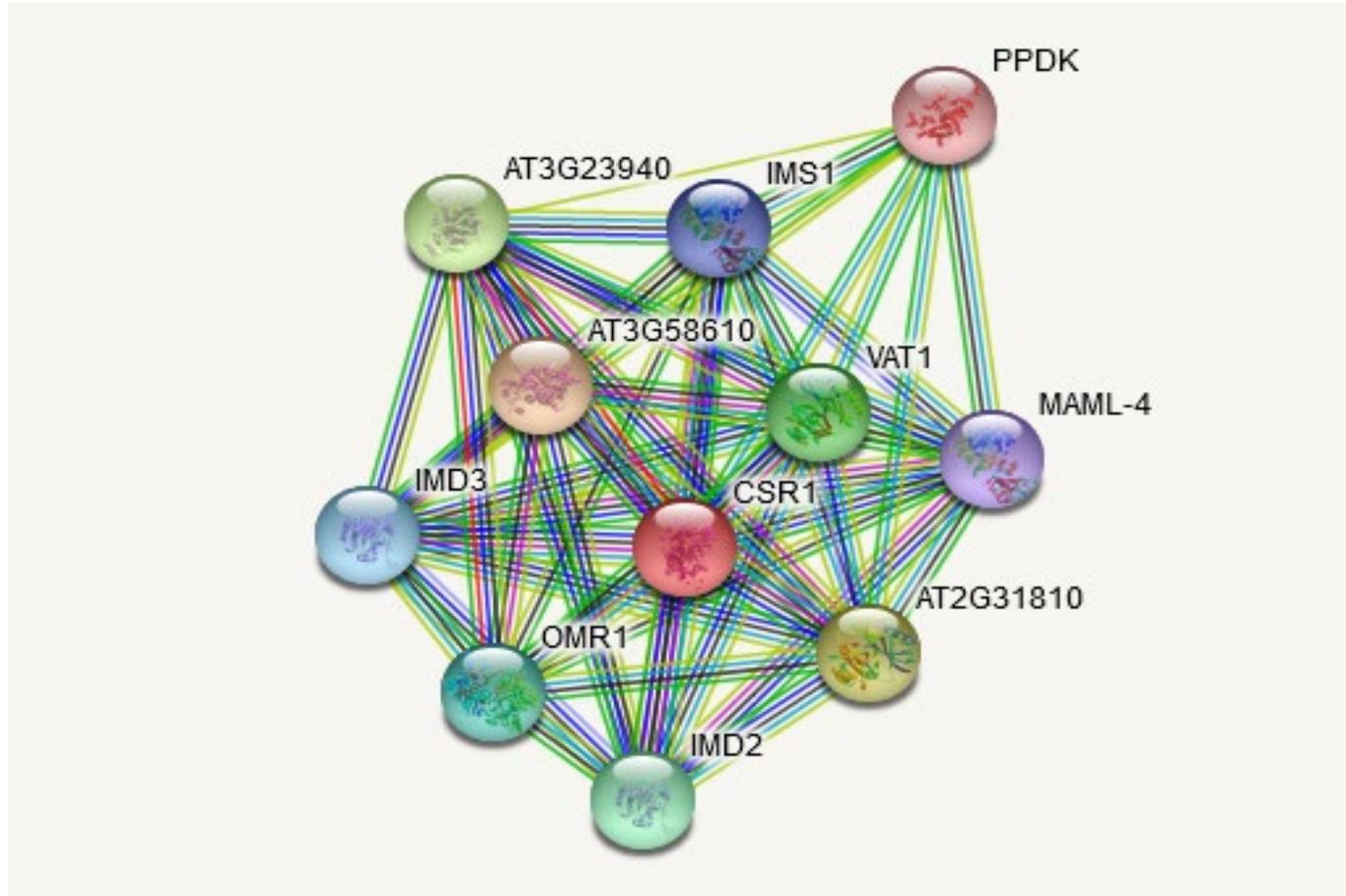
43个beta折叠

ALS结构分析



ALS功能分析

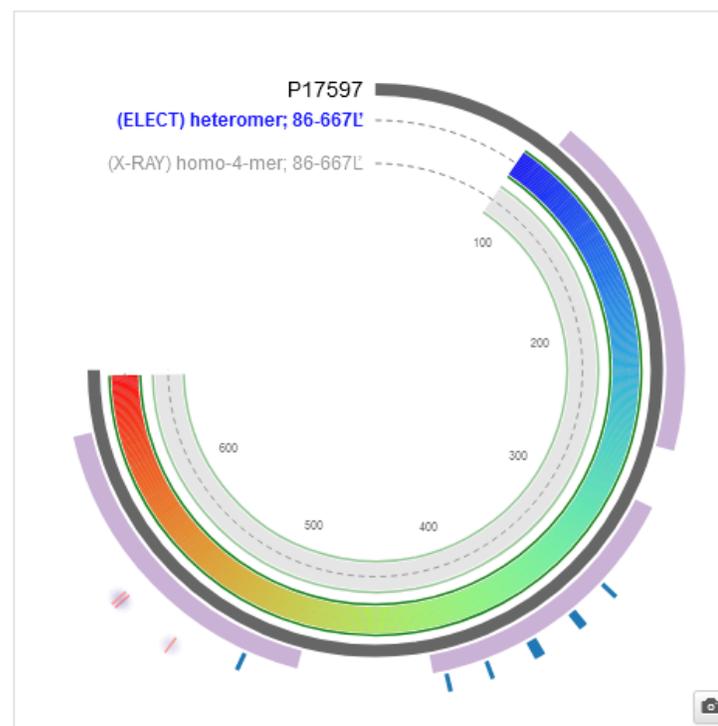
CSR1的互作蛋白，这些蛋白基本都是参与氨基酸合成的蛋白



ALS上存在FAD（黄素腺嘌呤二核苷酸）、TPP（焦磷酸硫胺素）、Mg²⁺等辅因子。

Feature key	Position(s)	Description	Actions	Graphical view	Length
Binding site ⁱ	144	Thiamine pyrophosphate 1 Publication			
Binding site ⁱ	207	Thiamine pyrophosphate 1 Publication			
Binding site ⁱ	220	Imidazolinone herbicides			
Binding site ⁱ	246	FAD 1 Publication			
Binding site ⁱ	246	Imidazolinone herbicides; via amide nitrogen			
Binding site ⁱ	256	Sulfonylurea herbicides 1 Publication			
Binding site ⁱ	308	FAD; via amide nitrogen 1 Publication			
Binding site ⁱ	377	Imidazolinone and sulfonylurea herbicides			
Metal binding ⁱ	538	Magnesium 2 Publications			
Metal binding ⁱ	565	Magnesium 2 Publications			
Metal binding ⁱ	567	Magnesium; via carbonyl oxygen 2 Publications			
Binding site ⁱ	574	Sulfonylurea herbicides 1 Publication			
Binding site ⁱ	653	Sulfonylurea herbicides 1 Publication			

Domains



Molecule Processing

538	Magnesium
565	Magnesium
567	Magnesium; via carbonyl oxygen
331-332	FAD
349-352	FAD
371-375	FAD
395-396	FAD

Electron Microscopy

6U9H "*Arabidopsis thaliana* acetohydroxyacid synthase complex"

Released: 2020-07-15

Coordinates: [↓](#)

8 x MAGNESIUM ION [📄](#)

8 x FLAVIN-ADENINE DINUCLEOTIDE [📄](#)

8 x THIAMINE DIPHOSPHATE [📄](#)

[RCSB](#) [PDB](#) [PDB-KB](#) [PDBj](#) [PDBsum](#)

Sequence Features [▼](#)

Metal binding

Nucleotide binding

InterPro

[+ Add](#)

98-261	Thiamine pyrophosphate enzyme, N-terminal TPP-binding domain IPR012001 PF02776
290-420	Thiamine pyrophosphate enzyme, central domain IPR012000 PF00205
484-639	Thiamine pyrophosphate enzyme, C-terminal TPP-binding IPR011766 PF02775

课题相关

Ala-122, Pro-197, Ala-205, Asp-376, Arg-377, Trp-574, Ser-653, and Gly-654

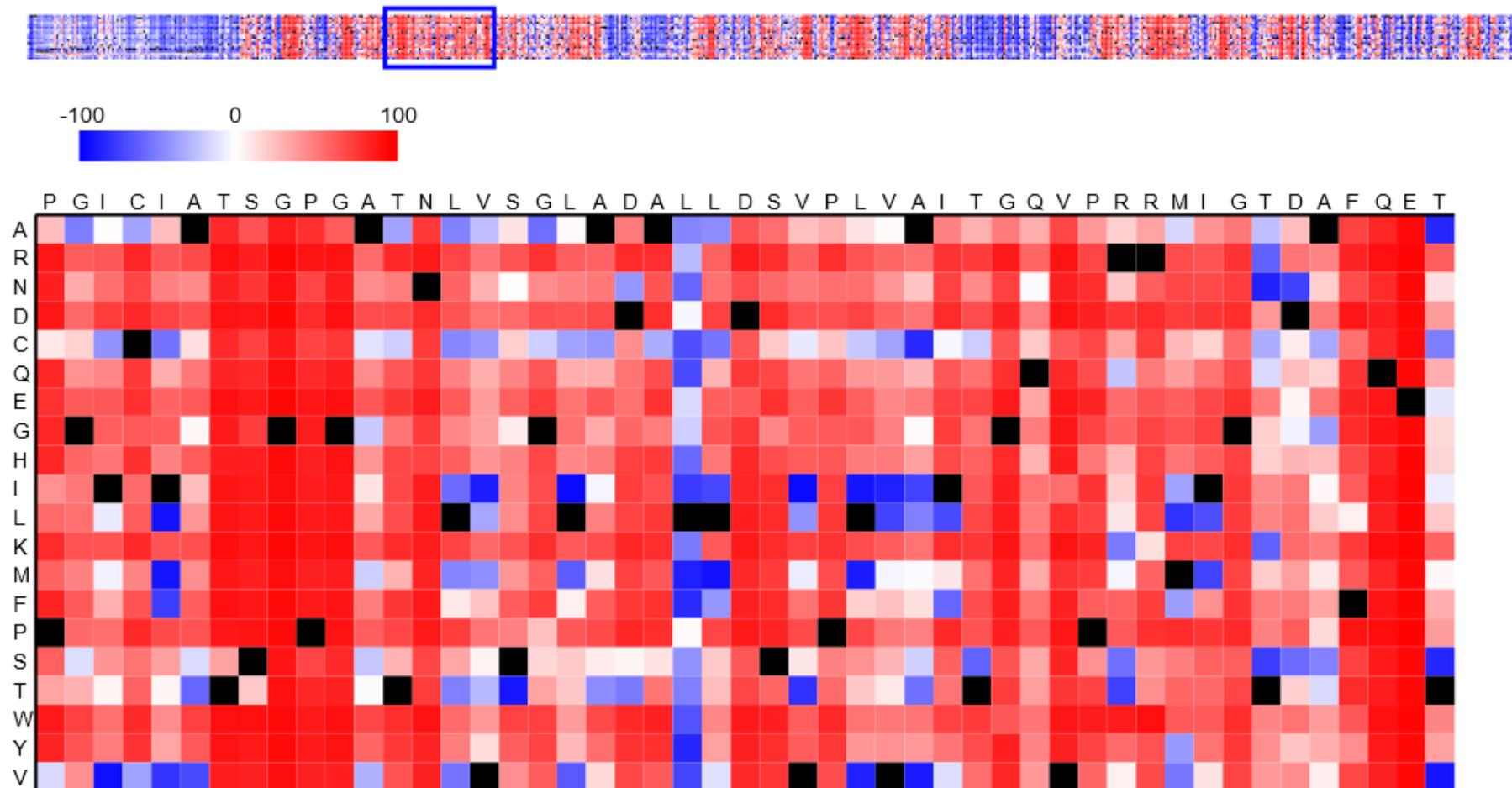
Mutagenesis

Feature key	Position(s)	Description	Actions	Graphical view	Length
Mutagenesis ⁱ	122	A → V: Reduced catalytic activity. Resistant to imidazolinone herbicides but not to sulfonylurea herbicides. 1 Publication			1
Mutagenesis ⁱ	124	M → E: Reduced catalytic activity. Resistant to imidazolinone herbicides and reduced sensitivity to sulfonylurea herbicides. 1 Publication			1
Mutagenesis ⁱ	124	M → I: No effect on catalytic activity. Increased resistance to imidazolinone herbicides. 1 Publication			1
Mutagenesis ⁱ	197	P → S in csr1-1/GH50; resistant to sulfonylurea but not to imidazolinone herbicides. 1 Publication			1
Mutagenesis ⁱ	199	R → A or E: No effect on catalytic activity. Resistant to imidazolinone herbicides but not to sulfonylurea herbicides. 1 Publication			1
Mutagenesis ⁱ	574	W → L: Increased catalytic activity. Resistant to imidazolinone and sulfonylurea herbicides. 1 Publication			1
Mutagenesis ⁱ	574	W → S: Slightly decreased catalytic activity. Resistant to imidazolinone and sulfonylurea herbicides. 1 Publication			1
Mutagenesis ⁱ	653	S → A: No effect on catalytic activity or sensitivity to herbicides. 4 Publications			1
Mutagenesis ⁱ	653	S → F: No effect on catalytic activity. Resistant to imidazolinone herbicides and also slightly sulfonylurea-resistant. 4 Publications			1
Mutagenesis ⁱ	653	S → N in csr1-2/GH90; no effect on catalytic activity. Resistant to imidazolinone but not to sulfonylurea herbicides. 4 Publications			1
Mutagenesis ⁱ	653	S → T: No effect on catalytic activity. Resistant to imidazolinone herbicides but not to sulfonylurea herbicides. 4 Publications			1

课题相关

突变热图分析

红色区域为以后
重点关注位点



Thanks!