黑麦草ALS基因抗性分析与结构预测

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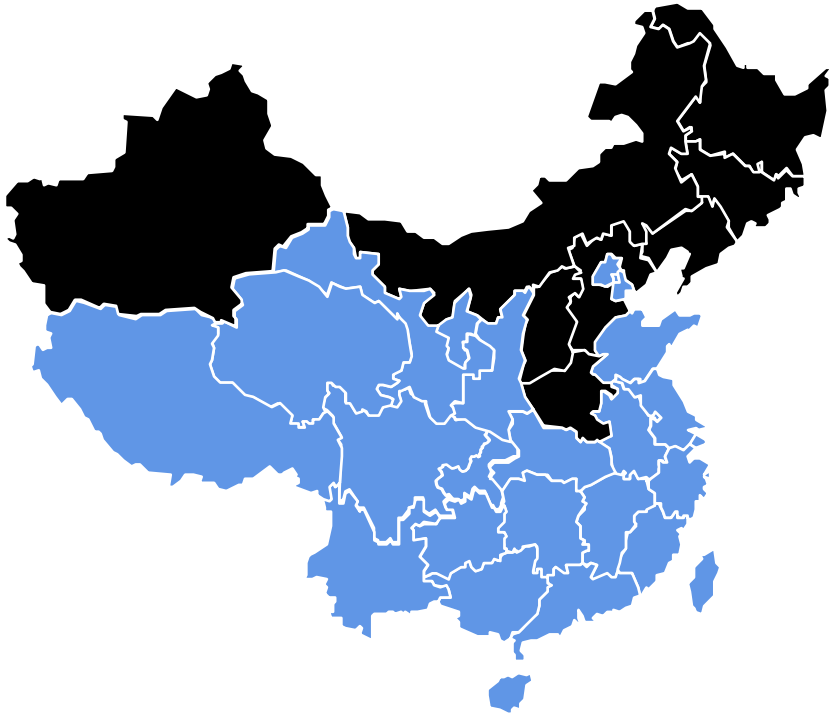
Contents

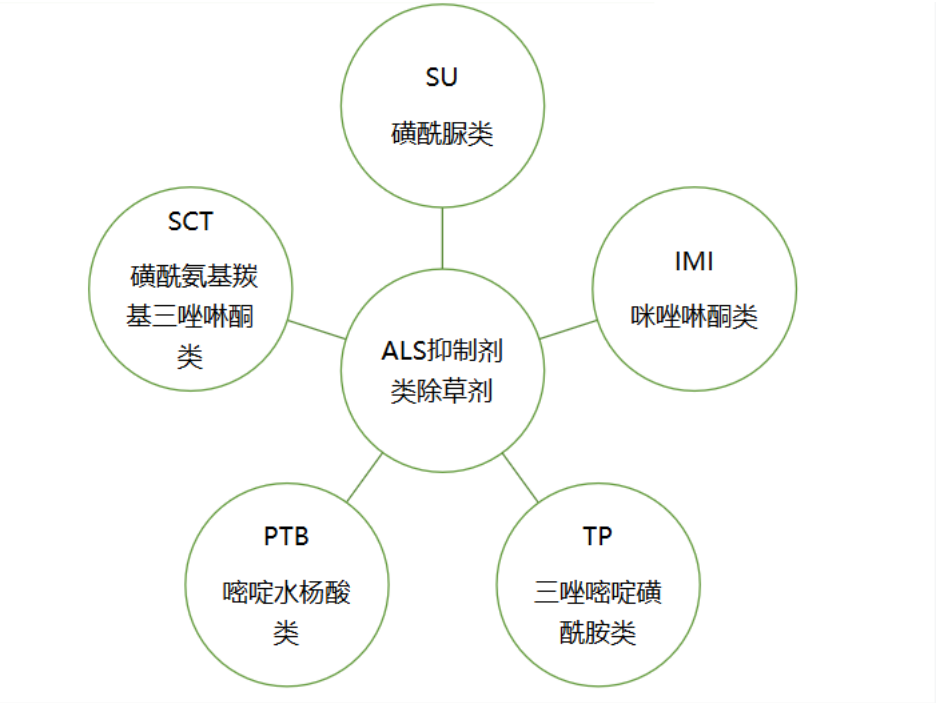
多年生黑麦草（Lolium perenne）是一种原产于欧洲、中亚、中东和北非的杂草，在08世纪初作为草坪草引入中国，随后扩散进入麦田成为影响冬小麦产量的主要杂草，在河南等地区，农民长期使用ALS抑制剂类除草剂（如甲基二磺隆）防治多年生黑麦草，但由于多年连续使用，杂草已产生显著抗药性，导致防治效果急剧下降。



黑麦草在我国的主要分布地区（黑色区域）

黑麦草

ALS（乙酰乳酸合酶）抑制剂是一类高效、低毒的除草剂，通过抑制植物体内支链氨基酸（缬氨酸、亮氨酸、异亮氨酸）合成途径中的关键酶——ALS酶来发挥作用。



该类农药主要包括：磺酰脲类（Sulfonylureas）、咪唑啉酮类（Imidazolinones）、三唑并嘧啶类（Triazolopyrimidines）、嘧啶水杨酸类（Pyrimidinylthiobenzoates）和磺酰氨基羰基三唑啉酮类。

ALS酶在植物体内催化两个重要反应：

- 丙酮酸→乙酰乳酸（缬氨酸和亮氨酸合成前体）
- 丙酮酸→乙酰羟基丁酸（异亮氨酸合成前体）

当ALS抑制剂进入植物体后：

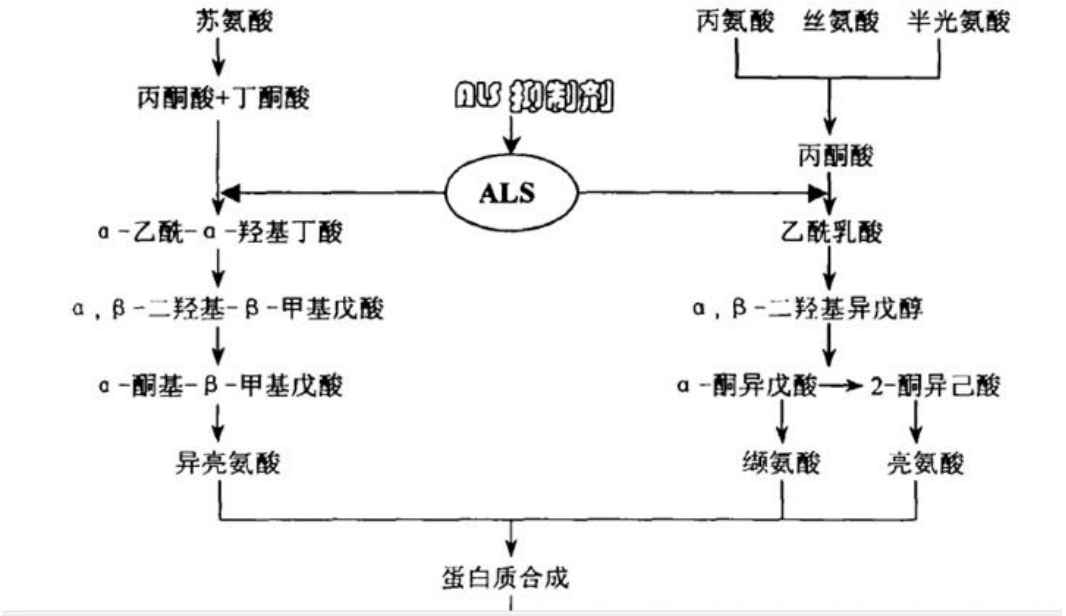
与ALS酶活性位点特异性结合

阻断支链氨基酸的生物合成

导致蛋白质合成受阻

细胞分裂停止，生长受抑制

最终使杂草死亡

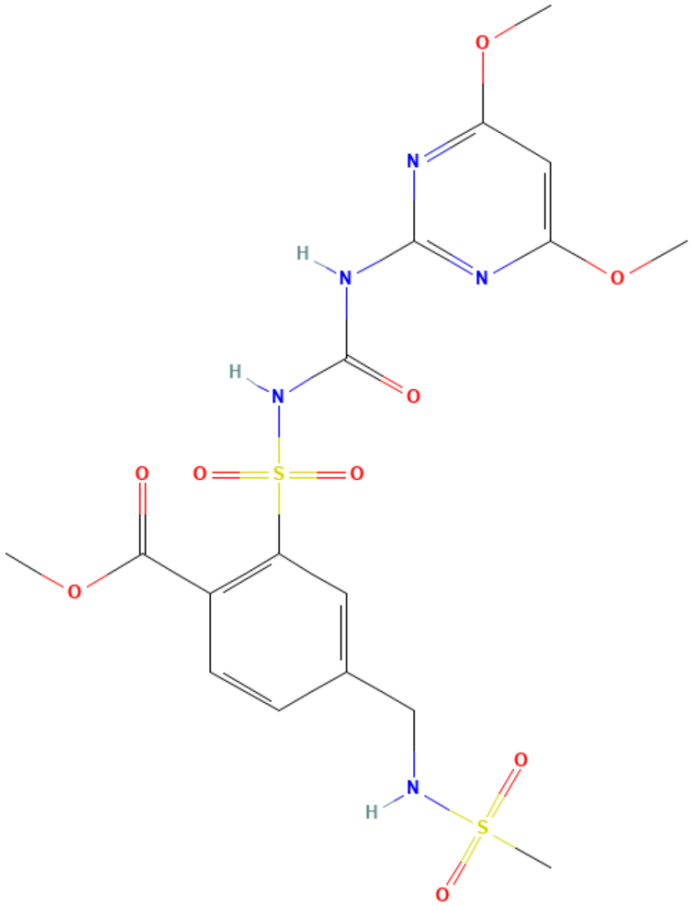


ALS类除草剂的作用机制

- 甲基二磺隆：全称甲基2-[(4,6-二甲氧基嘧啶-2-基)氨基甲酰氨基磺酰基]-4-甲磺酰氨基甲基苯甲酸酯，分子式为$C_{17}H_{21}N_5O_9S_2$。

- 甲基二磺隆通过特异性抑制乙酰乳酸合酶（ALS），阻断支链氨基酸（缬氨酸、亮氨酸、异亮氨酸）的生物合成途径，最终导致杂草死亡。

- 对作物安全的原因在于作物能够快速代谢分解该除草剂，而敏感杂草缺乏这种代谢能力。因而作为除草剂被广泛使用。



PubChem CID:11409499

1. 黑麦草ALS序列查找

Lolium rigidum clone 6c chloroplast acetolactate synthase gene, partial cds; chloroplast

GenBank: KM000068.1

FASTA Graphics

Go to:

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LOCUS       KM000068               1866 bp    DNA     linear   PLN 20-SEP-2014
DEFINITION  Lolium rigidum clone 6c chloroplast acetolactate synthase gene,
            partial cds; chloroplast.
ACCESSION   KM000068
VERSION     KM000068.1
KEYWORDS    .
SOURCE      chloroplast Lolium rigidum
  ORGANISM  Lolium rigidum
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; Liliopsida; Poales; Poaceae; BOP
            clade; Pooideae; Poodae; Poeae; Poeae Chloroplast Group 2 (Poeae
            type); Loliodinae; Loliinae; Lolium.
REFERENCE   1  (bases 1 to 1866)
  AUTHORS   Al-Doss,A.A., Al-Faifi,S.A., Ghazy,A.I., Salem,A.K., Ammar,M.H.,
            Migdadi,H.M. and Althamra,M.I.
  TITLE     Molecular evidence for new ALS-resistance alleles in Saudi Lolium
            rigidum populations
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1866)
  AUTHORS   Al-Doss,A.A., Al-Faifi,S.A., Ghazy,A.I., Salem,A.K., Ammar,M.H.,
            Migdadi,H.M. and Althamra,M.I.
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ALS基因与其对应的蛋白

Lolium multiflorum acetolactate synthase (ALS) gene, partial cds

GenBank: MK922479.1

FASTA Graphics

Go to:

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DEFINITION  Lolium multiflorum acetolactate synthase (ALS) gene, partial cds.
ACCESSION   MK922479
VERSION     MK922479.1
KEYWORDS    .
SOURCE      Lolium multiflorum (Italian ryegrass)
  ORGANISM  Lolium multiflorum
            Eukaryota; Viridiplantae; Streptophyta; Embryophyta; Tracheophyta;
            Spermatophyta; Magnoliopsida; Liliopsida; Poales; Poaceae; BOP
            clade; Pooideae; Poodae; Poeae; Poeae Chloroplast Group 2 (Poeae
            type); Loliodinae; Loliinae; Lolium.
REFERENCE   1  (bases 1 to 1355)
  AUTHORS   Kaya Altop,E. and Erken,S.
  TITLE     Determination of ALS and accase herbicides resistance of Lolium
            spp. in wheat fields in Samsun Province
```

chloroplast acetolactate synthase

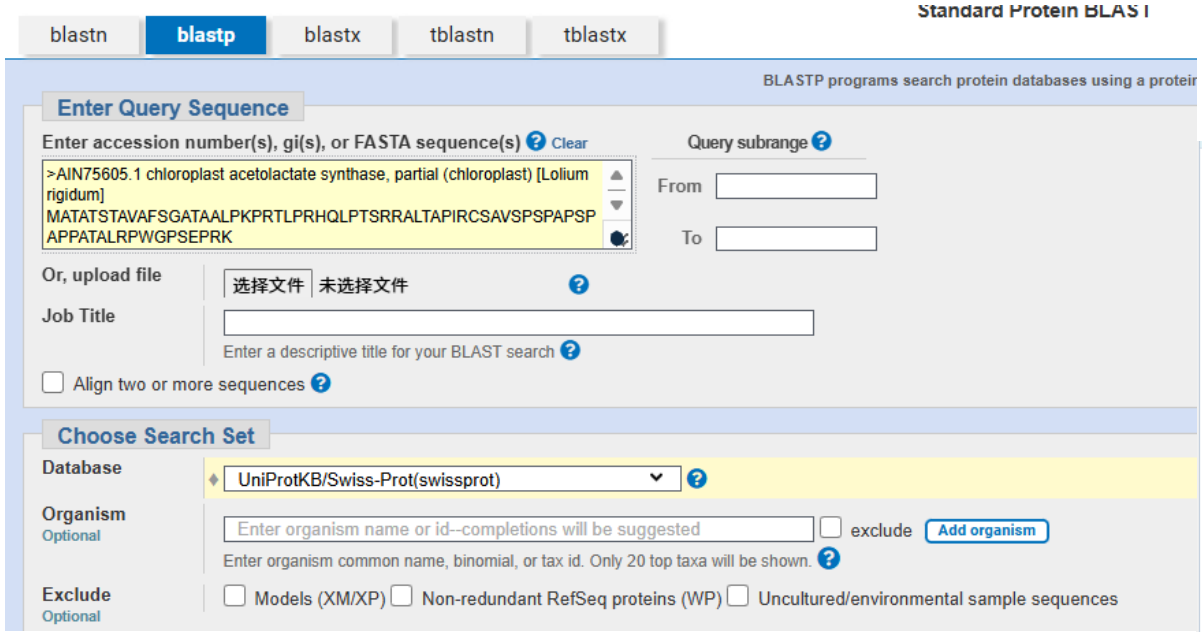
GenPept FASTA Graphics BLAST

Name: chloroplast acetolactate synthase
RefSeq Selected Product: AIN75605.1, 622 amino acids
Taxonomic Group: monocots
Assembly Accessions: 0
Protein Accessions: 1
CDS Regions: 1
Total Rows: 1

Source	CDS Region in Nucleotide	Protein	Name	Organism	Strain	Assembly
INSDC	KM000068.1 1-1866 (+)	AIN75605.1	chloroplast acetolactate synthase	Lolium rigidum		

2. 查找近缘物种

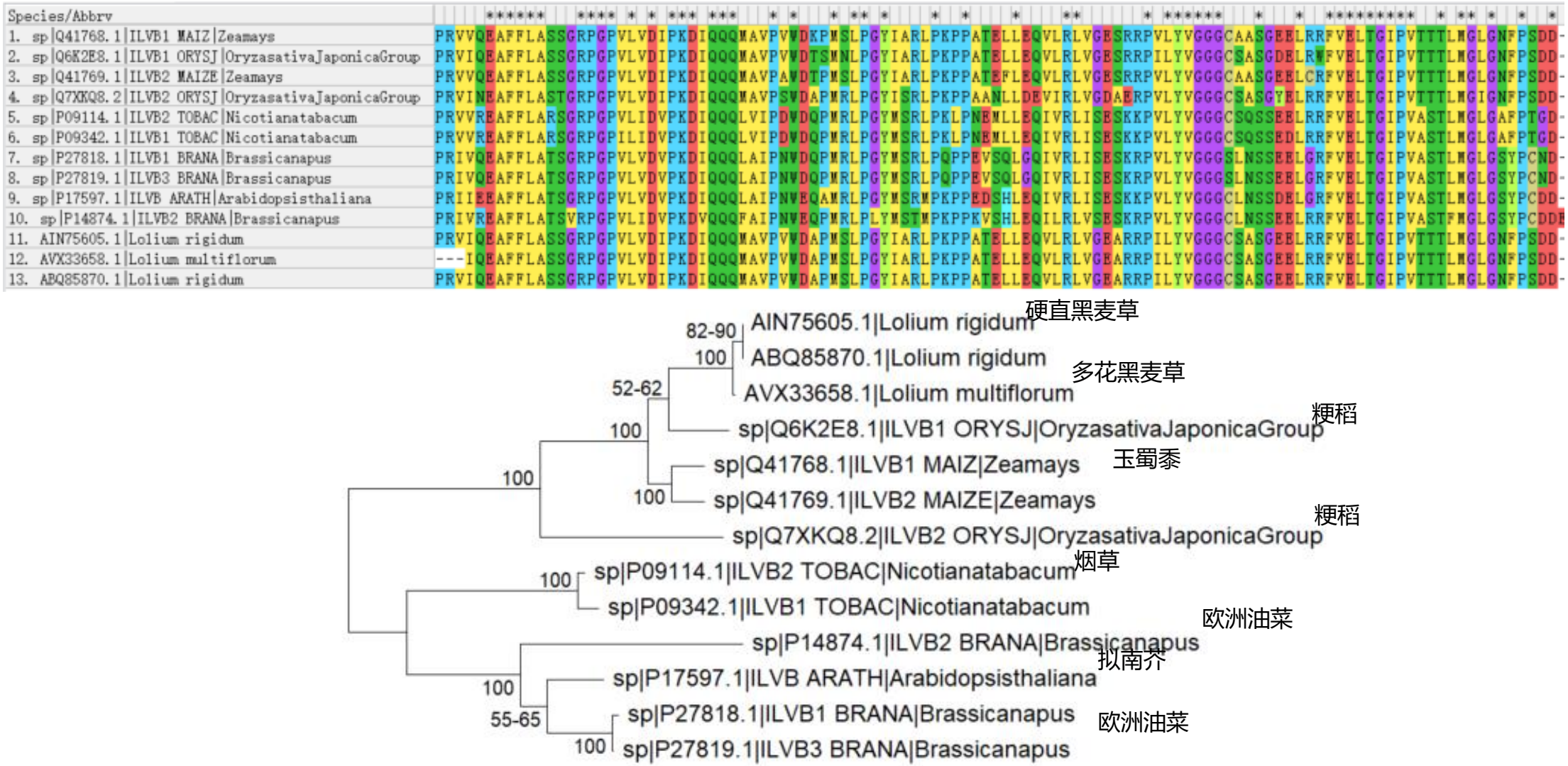
通过蛋白序列，利用NCBI的Blast找到序列相似的物种的蛋白序列



RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 1; Flags: Precu...	Zea mays	1134	1134	97%	0.0	90.37%	638	Q41768.1
RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 1; Flags: Precu...	Oryza sativa Jap...	1122	1122	96%	0.0	92.95%	644	Q6K2E8.1
RecName: Full=Acetolactate synthase 2, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 2; Flags: Precu...	Zea mays	1095	1095	97%	0.0	89.20%	638	Q41769.1
RecName: Full=Probable acetolactate synthase 2, chloroplastic; AltName: Full=Acetohydroxy-acid synthase 2; Fla...	Oryza sativa Jap...	960	960	91%	0.0	82.04%	663	Q7XKQ8.2
RecName: Full=Acetolactate synthase 2, chloroplastic; AltName: Full=ALS II; AltName: Full=Acetohydroxy-acid sy...	Nicotiana tabacum	926	926	91%	0.0	76.64%	664	P09114.1
RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=ALS I; AltName: Full=Acetohydroxy-acid sy...	Nicotiana tabacum	925	925	91%	0.0	76.28%	667	P09342.1
RecName: Full=Acetolactate synthase 1, chloroplastic; AltName: Full=ALS I; AltName: Full=Acetohydroxy-acid sy...	Brassica napus	920	920	100%	0.0	69.50%	655	P27818.1
RecName: Full=Acetolactate synthase 3, chloroplastic; AltName: Full=ALS III; AltName: Full=Acetohydroxy-acid s...	Brassica napus	919	919	100%	0.0	69.94%	652	P27819.1
RecName: Full=Acetolactate synthase, chloroplastic; Short=AtALS; AltName: Full=Acetohydroxy-acid synthase; Al...	Arabidopsis thali...	905	905	91%	0.0	74.51%	670	P17597.1
RecName: Full=Acetolactate synthase 2, chloroplastic; AltName: Full=ALS II; AltName: Full=Acetohydroxy-acid sy...	Brassica napus	859	859	91%	0.0	70.25%	637	P14874.1
RecName: Full=Acetolactate synthase large subunit; Short=AHAS; AltName: Full=Acetohydroxy-acid synthase lar...	Corynebacterium...	518	518	93%	1e-176	48.63%	626	P42463.1

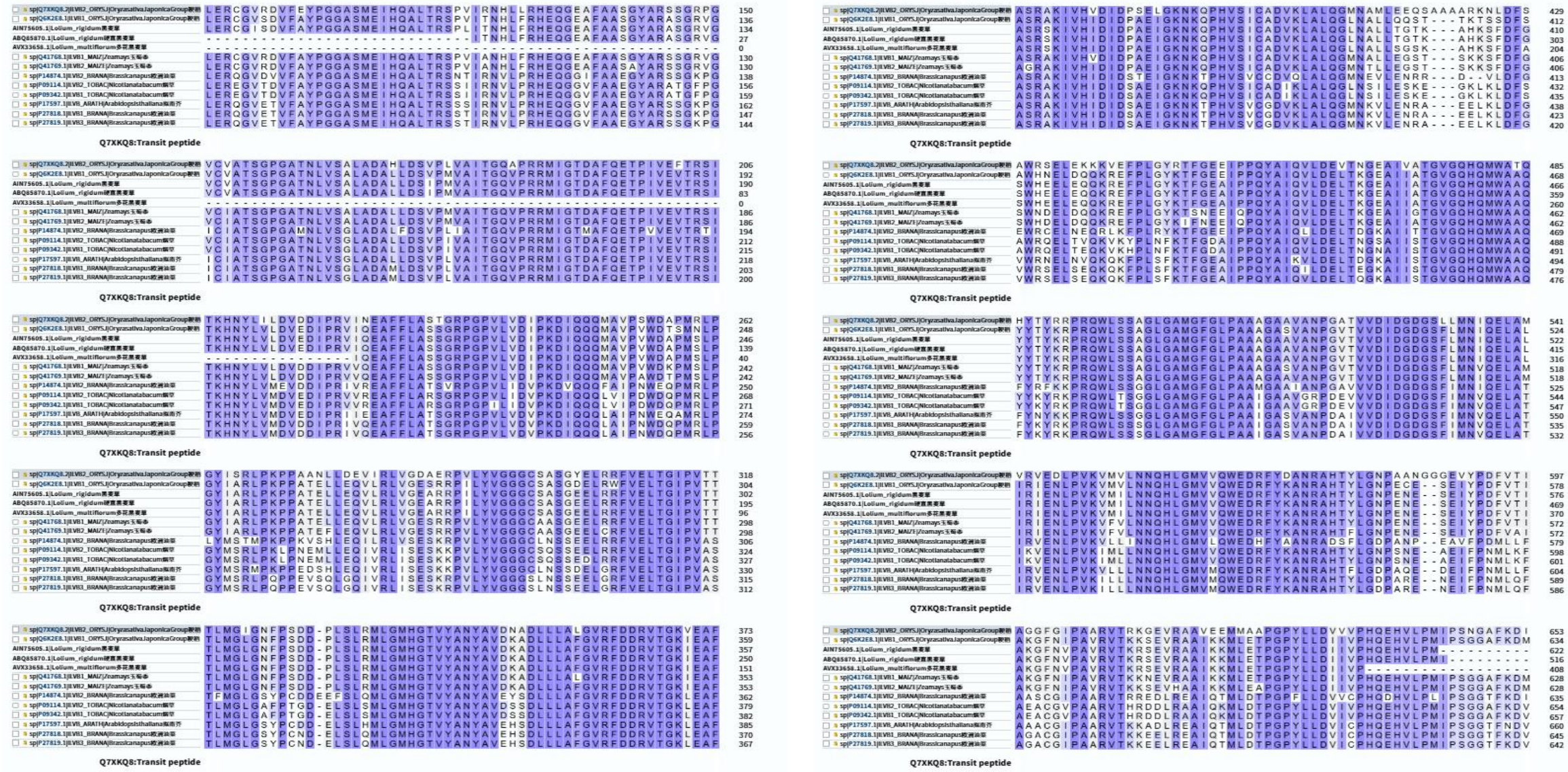
3. 构建系统发育树

通过蛋白序列，利用Blast找到其近缘物种，建立发育树



4. 序列保守性分析

通过蛋白序列利用uniprot分析是否保守

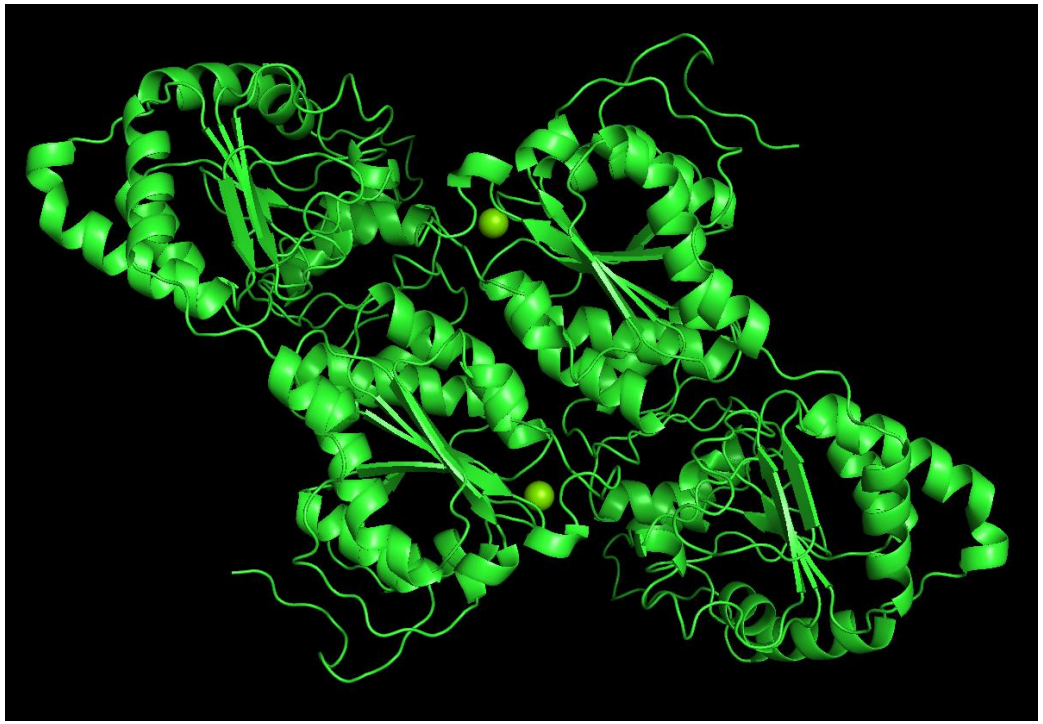


5. 序列比对

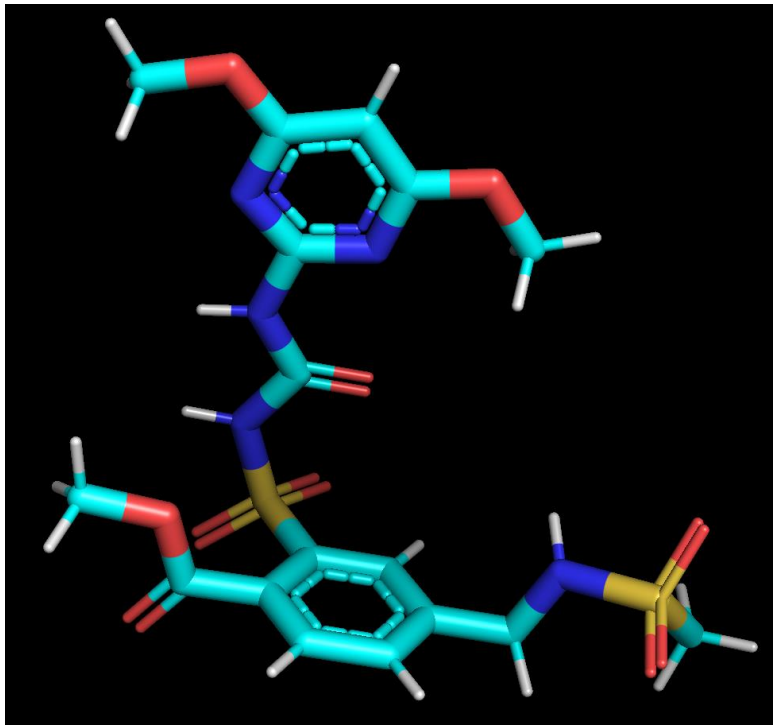
序列比对找突变位点，发现376位氨基酸位点突变，GAT变为GAG，氨基酸从D变为E

6. 结构预测

以拟南芥为模板，通过Swissmodel构建敏感型和抗药型黑麦草中ALS 3D结构，从Pubmed下载甲基二磺隆 3D结构，通过Autodock vina进行分子对接，利用DS可视化，分析比较敏感型和抗药型黑麦草中ALS与甲基二磺隆的相互作用



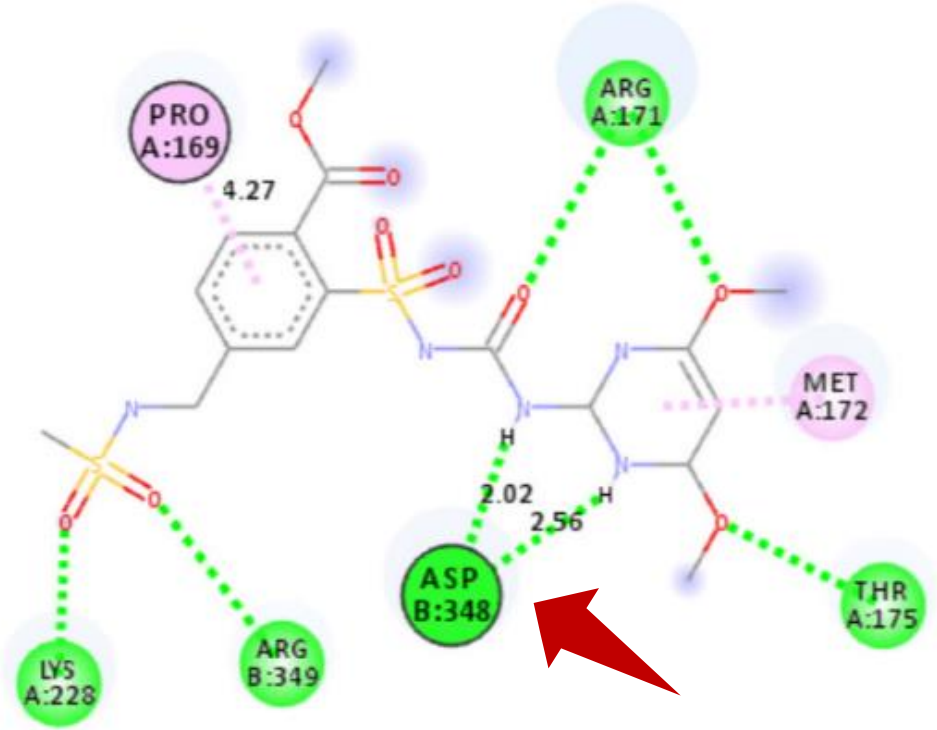
ALS 3D结构



甲基二磺隆 3D结构

敏感型黑麦草中ALS残基天冬氨酸与配体之间通过氢键相互作用，而抗药型黑麦草中ALS残基谷氨酸（ 由GAT突变为GAG导致 ）与配体之间结合不紧密，以排斥力为主

S



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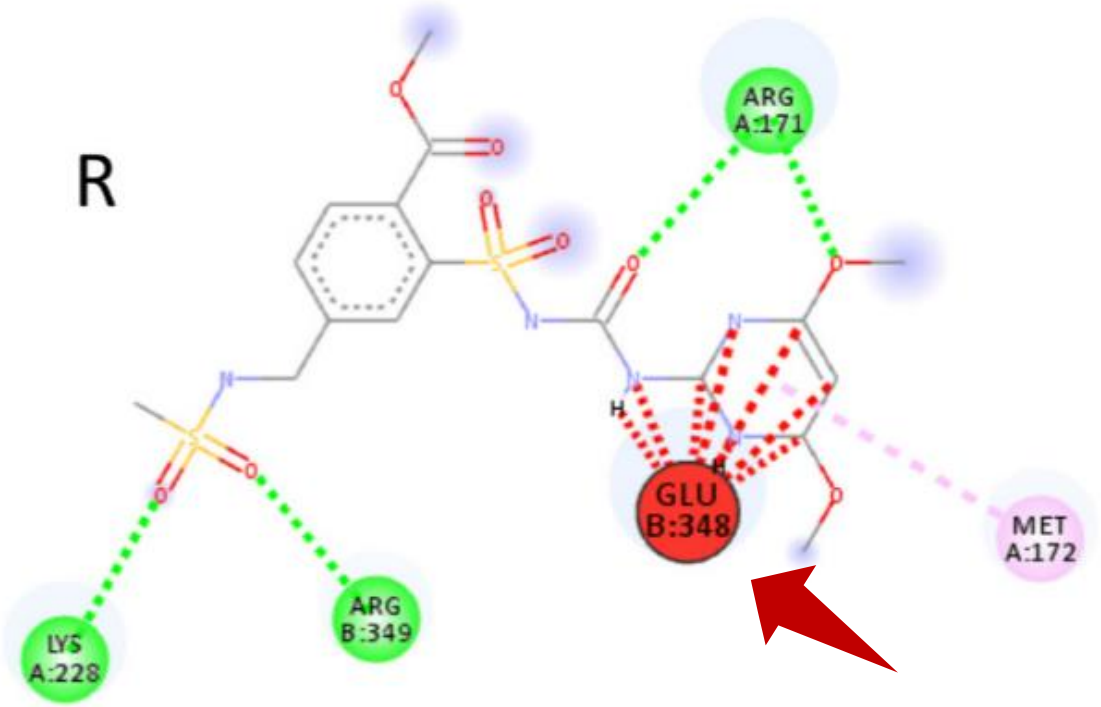

- **科学理论贡献：**黑麦草中乙酰乳酸合酶发生Asp376Glu突变，导致黑麦草对甲基二磺隆抗性增强，补充了靶标抗性的分子互作机制，为其他杂草抗性研究提供参考。

- **农业生产指导：**研究表明当前单一ALS类除草剂防控杂草逐渐失效，需及时调整防控方案，建议结合非ALS，同时综合耕作措施降低除草剂选择压力，延缓杂草抗性发展。此外，可考虑通过基因编辑等手段增强作物对除草记的抗性，提高除草剂使用效率。

组员合影（左至右依次为黄菲侠、陈星宇、薛熙豪、熊岳）：

THANKS!