

2G05

香味基因 *BADH2* 的序列 和功能研究

Sequence and function of aroma gene badh2

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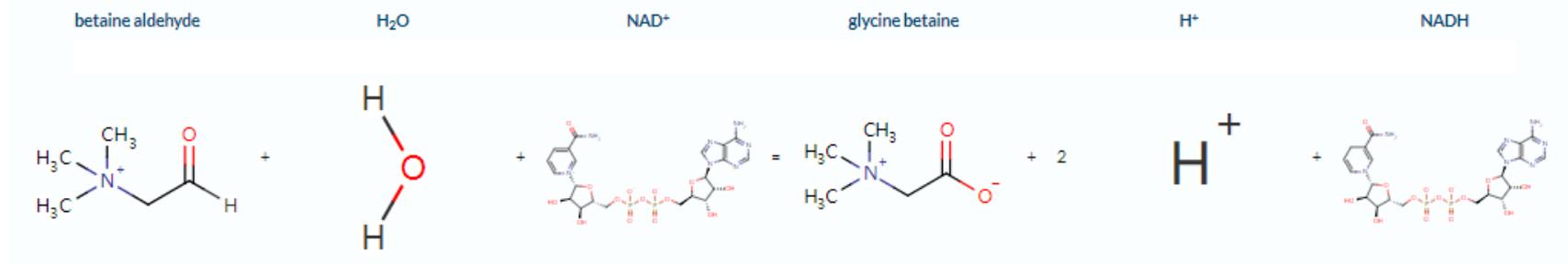
基因背景概述

大豆是我国主要作物之一，内含40%的蛋白质和20%脂肪，是人类主要蛋白质来源之一，广泛应用于食品行业和工业生产中

香气是大豆的品质性状之一，因此香味表型的相关基因的研究对于选育优质的大豆香型品种十分重要。其中2-乙酰-1-吡咯啉(2-acetyl-1-pyrroline, 2AP)是一种类似爆米花香的挥发性化合物,其含量高低直接影响消费者的偏好和接受度。在对诸多作物的研究当中均发现 BDAH2 是控制这个香味形成的关键基因



基因背景概述



此外，BADH是渗透调节物质，能够帮助植物对抗逆境胁迫，从而提高植物适应性。

目前，*OsBADH2* 基因已经被验证是水稻香气的主要决定基因，通过同源基因的查找来确定大豆中 *GmBADH2*基因的参考序列。

在uniprot中得到水稻的BADH序列



Entry ▲	Entry Name ▲	Protein Names ▲	Gene Names ▲	Organism ▲	Length ▲
<input type="checkbox"/> Q84LK3	 BADH2_ORYSJ	Betaine aldehyde dehydrogenase 2[...]	BADH2[...]	Oryza sativa subsp. japonica (Rice)	503 AA
<input type="checkbox"/> O24174	 BADH1_ORYSJ	Betaine aldehyde dehydrogenase 1[...]	BADH1[...]	Oryza sativa subsp. japonica (Rice)	505 AA

得到了该基因的蛋白序列和氨基酸序列

在NCBI中得到大豆的BADH序列

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
betaine aldehyde dehydrogenase 1 [Glycine max]	Glycine max	786	786	100%	0.0	74.16%	503	NP_001234990.1
betaine aldehyde dehydrogenase [Glycine max]	Glycine max	774	774	100%	0.0	75.35%	503	NP_001238427.1
aldehyde dehydrogenase family 2 member C4 isoform X1 [Glycine max]	Glycine max	355	355	97%	3e-117	41.78%	501	XP_003528912.1
aldehyde dehydrogenase family 2 member C8 [Glycine max]	Glycine max	354	354	96%	4e-117	42.21%	501	NP_001354140.1
aldehyde dehydrogenase family 2 member C4 [Glycine max]	Glycine max	351	351	96%	1e-115	41.92%	505	XP_003530501.1
aldehyde dehydrogenase [Glycine max]	Glycine max	352	352	97%	2e-115	41.70%	538	NP_001235519.2
aldehyde dehydrogenase family 2 member C1 [Glycine max]	Glycine max	348	348	96%	2e-114	40.98%	502	NP_001354120.1
aldehyde dehydrogenase family 2 member C4 [Glycine max]	Glycine max	345	345	96%	2e-113	44.06%	501	XP_003534192.1
aldehyde dehydrogenase family 2 member C4 [Glycine max]	Glycine max	343	343	97%	8e-113	43.50%	501	XP_003528913.1
aldehyde dehydrogenase family 2 member C7 [Glycine max]	Glycine max	343	343	96%	1e-112	40.16%	499	NP_001354139.1
aldehyde dehydrogenase family 2 member C5 [Glycine max]	Glycine max	342	342	96%	5e-112	41.60%	501	NP_001354132.1

用水稻的蛋白质序列在蛋白质参考数据库中搜索在大豆中的同源基因

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
betaine aldehyde dehydrogenase 1 [Glycine max]	Glycine max	786	786	99%	0.0	74.16%	503	NP_001234990.1
betaine aldehyde dehydrogenase [Glycine max]	Glycine max	774	774	99%	0.0	75.35%	503	NP_001238427.1
aminoaldehyde dehydrogenase [Glycine max]	Glycine max	772	772	99%	0.0	73.41%	504	AGC7996.1
betaine aldehyde dehydrogenase [Glycine max]	Glycine max	771	771	99%	0.0	75.15%	503	ADN03184.1
Betaine aldehyde dehydrogenase 1_chloroplast [Glycine max]	Glycine max	767	767	99%	0.0	74.31%	510	KAH1248730.1
hypothetical protein GYH30_011446 [Glycine max]	Glycine max	765	765	99%	0.0	75.15%	501	KAH1132611.1
hypothetical protein JHK85_016055 [Glycine max]	Glycine max	734	734	93%	0.0	73.78%	482	KAG5032073.1
hypothetical protein JHK85_011998 [Glycine max]	Glycine max	726	726	99%	0.0	70.38%	658	KAG5039522.1
hypothetical protein GLYMA_06G186300v4 [Glycine max]	Glycine max	609	609	72%	0.0	77.75%	364	KAG4389934.1
hypothetical protein GLYMA_05G033500v4 [Glycine max]	Glycine max	595	595	72%	0.0	78.57%	364	KAG4390731.1
betaine aldehyde dehydrogenase [Glycine max]	Glycine max	450	450	61%	1e-156	73.63%	311	ADN03180.1
Aldehyde dehydrogenase family 2 member C4 [Glycine max]	Glycine max	357	357	96%	3e-117	42.21%	501	KAH1234252.1
hypothetical protein JHK82_017923 [Glycine max]	Glycine max	355	355	97%	6e-117	41.05%	501	KAG5142228.1
aldehyde dehydrogenase family 2 member C4 isoform X1 [Glycine max]	Glycine max	355	355	97%	8e-117	41.78%	501	XP_003528912.1

用水稻的核酸序列在核酸的非冗余数据库中搜索在大豆中的同源蛋白质序列

➤ 得到的结果一致

在NCBI中得到大豆的BADH序列

默认参数 (种子长度28) , 以OsBADH2的核酸序列在Nt中检索大豆的核酸序列→ **没有得到相关序列**

	Word size	24	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>			PREDICTED: Glycine max uncharacterized LOC121172684.(LOC121172684).ncRNA	Glycine max	126	126	12%	3e-27	79.26%	1147	XR_005889308.1

更改种子长度为24, 再次搜索得到一条序列 (如上图) → 两条序列覆盖率为**12%**

	Word size	20	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>			Glycine max betaine aldehyde dehydrogenase (ALDH2A1), mRNA	Glycine max	606	606	85%	9e-172	75.19%	1875	NM_001251498.2
<input checked="" type="checkbox"/>			Glycine max cDNA, clone: GMFL01-09-C21	Glycine max	606	606	85%	9e-172	75.19%	1760	AK244648.1
<input checked="" type="checkbox"/>			Glycine max cDNA, clone: GMFL01-01-J08	Glycine max	606	606	85%	9e-172	75.19%	1771	AK243907.1
<input checked="" type="checkbox"/>			Glycine max cDNA, clone: GMFL01-07-G20	Glycine max	606	606	85%	9e-172	75.19%	1746	AK285321.1
<input checked="" type="checkbox"/>			Glycine max mRNA for peroxisomal betaine-aldehyde dehydrogenase, complete cds	Glycine max	606	606	85%	9e-172	75.19%	1512	AB333793.1
<input checked="" type="checkbox"/>			Glycine max cDNA, clone: GMFL01-19-116	Glycine max	606	606	80%	7e-158	75.24%	1764	AK245037.1

更改种子长度为20, 再次搜索得到大豆同源序列 (如上图) → 将NP_001238427.1作为大豆的参考序列

大豆BADH与水稻BADH基因序列分析

OsBADH2基本信息

基因号：Os08g0424500

染色体定位：8号染色体上

基因全长：6264bp，15个外显子

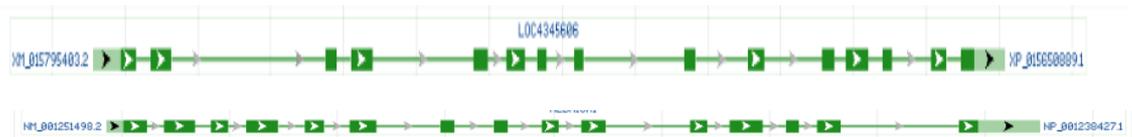
CDS序列长度：1512bp

编码氨基酸：503aa

功能：编码甜菜碱脱氢酶, 甘氨酸、丝氨酸和苏氨酸代谢、氧化还原酶活性

OsBADH2基因结构图

GmBADH2基因结构图



GmBADH2基本信息

基因号：Gly.ma05G033500

染色体定位：5号染色体上

基因全长：4323bp，15个外显子

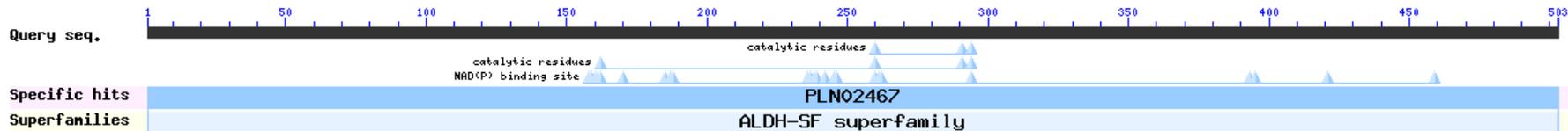
CDS序列长度：1512bp

编码氨基酸：503aa

功能：编码甜菜碱脱氢酶, 甘氨酸、丝氨酸和苏氨酸代谢、氧化还原酶活性

结构域基本信息

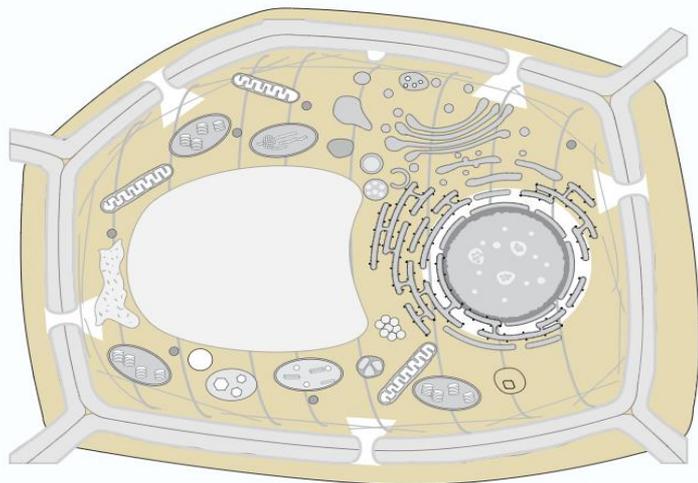
<https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi>



- 大豆BADH氨基酸序列功能结构域分析结果如图所示,其多肽链属于ALDH-SF超家族、醛脱氢酶家族,该家族具有氧化还原酶活性,可以催化醛基氧化为羧基。BADH的3个功能结构域分别为: catalytic residues [active site], catalytic residues [active site]和 **NAD(P)binding site** [chemical binding site]。

亚细胞定位预测

<http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/>



水稻

Your input sequence (503aa) is:

```
> Glycine max
MSIPIPHRQLFIDGDWKPVLKNRIPINPSTQHHIGDIPAATKEDVDLAVAAAKAALSR
NKGADWASASGSVRARYLRAIAAKITEKKPELAKLEAIDCGKPLDEAAWDIDDVAGCFEF
YADLAEKLDAQQKAHVSLPMDTFKSYVLKEPIGVVALITPWNYPLLMATWKVAPALAAGC
AAILKPSELASVTCLELAEICKEVGLPPGVNLITGLGPEAGAPLAAHPDVKIAFTGSS
ATGSKIMTAAQLIKPVSELEGGKSPIIVFEDVDLDKAAEWTFGCFWNGQICSATSRL
IVHESIATEFLNRIVKVVKNIKISDPLEEGCRLGPVSEGQYEKILKFISNAKSEGATIL
TGGSRPEHLKKGFFVEPTVITDVTSMQIWREEVFGPVLVVKTFSTEEEAIDLANDTVYG
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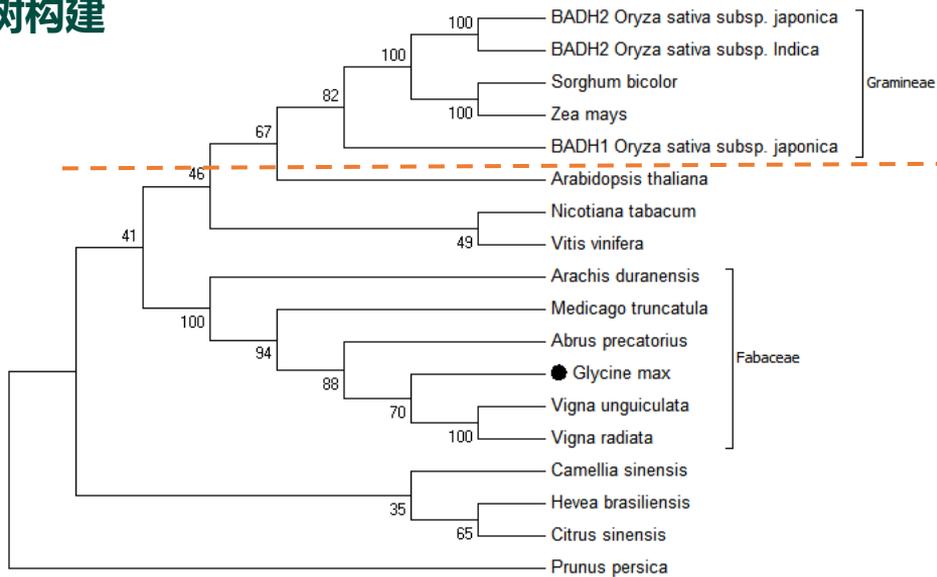
----- Plant-mPloc Computation Result -----

Query protein	Predicted location(s)
Glycine max	Chloroplast. Mitochondrion. Peroxisome.

大豆

亚细胞定位预测：在水稻中，从Uniprot中得知OsDADH2定位于细胞质和过氧化酶体中。再次基础之上，利用Cell-PLoc 2.0对大豆BADH蛋白基因预测，GmBADH蛋白主要定位于叶绿体、线粒体和过氧化酶体中。

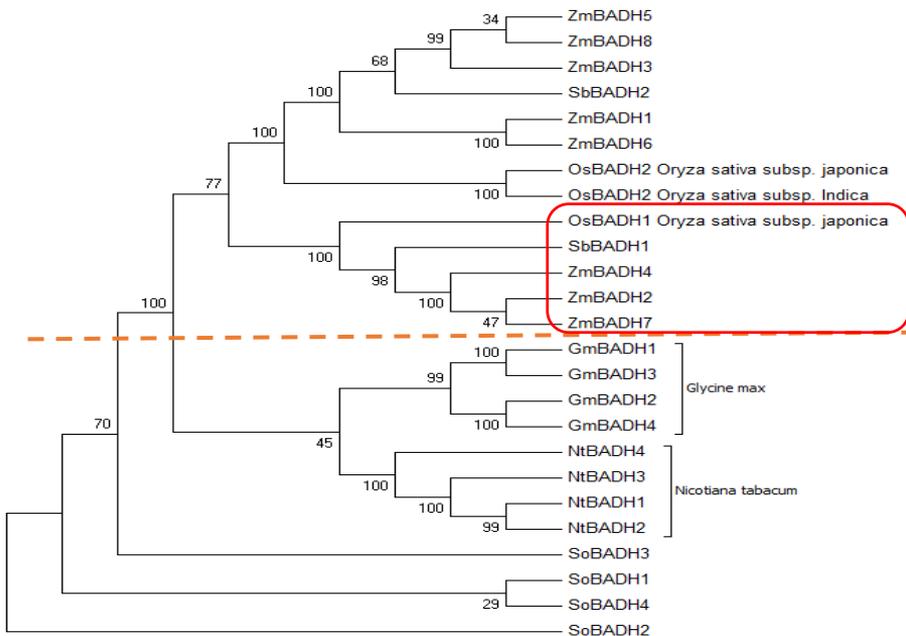
进化树构建



在NCBI数据库中，对GmBADH的氨基酸序列进行Blastp比对，显示其与豆科植物的氨基酸序列相似性均较高，分别与豇豆、绿豆和红豆BADH的相似性为94.83%、94.63%与94.23%。

- 用邻接法构建进化树显示，按种属关系可分为两支分别为单子叶植物、双子叶植物；
- BADH与定位于叶绿体上的豇豆和绿豆的亲缘关系最近,因此推测GmBADH蛋白质也可能定位于叶绿体；
- 大豆BADH与双子叶植物物种同源性较高，聚为一类；单子叶植物与大豆BADH蛋白质序列亲缘关系较远，聚为一类→随时间变化双子叶植物BADH遗传变异度小于单子叶植物

进化树构建



该系统进化树包括6个物种，每个物种均有多个基因。

结果表明:

- 单子叶植物和双子叶植物从类群上完全区分开来;
- 大豆、烟草等双子叶植物BADH序列都很好得聚类在一起，表明在双子叶植物中，该基因是“**先有物种，后有基因**”
- 在单子叶植物中，不同物种的BADH基因混杂在一起，表明在进化过程中出现了较大程度的变异，并且出现“**先有基因，后有物种**”的现象。其中水稻的BADH2基因没有因品种的分化而出现较大的差异，但是OsBADH1和OsBADH2序列发生了较大程度的变异，OsBADH1与高粱BADH1的序列亲缘关系最近。

利用ClustalX2进行蛋白的同源性分析

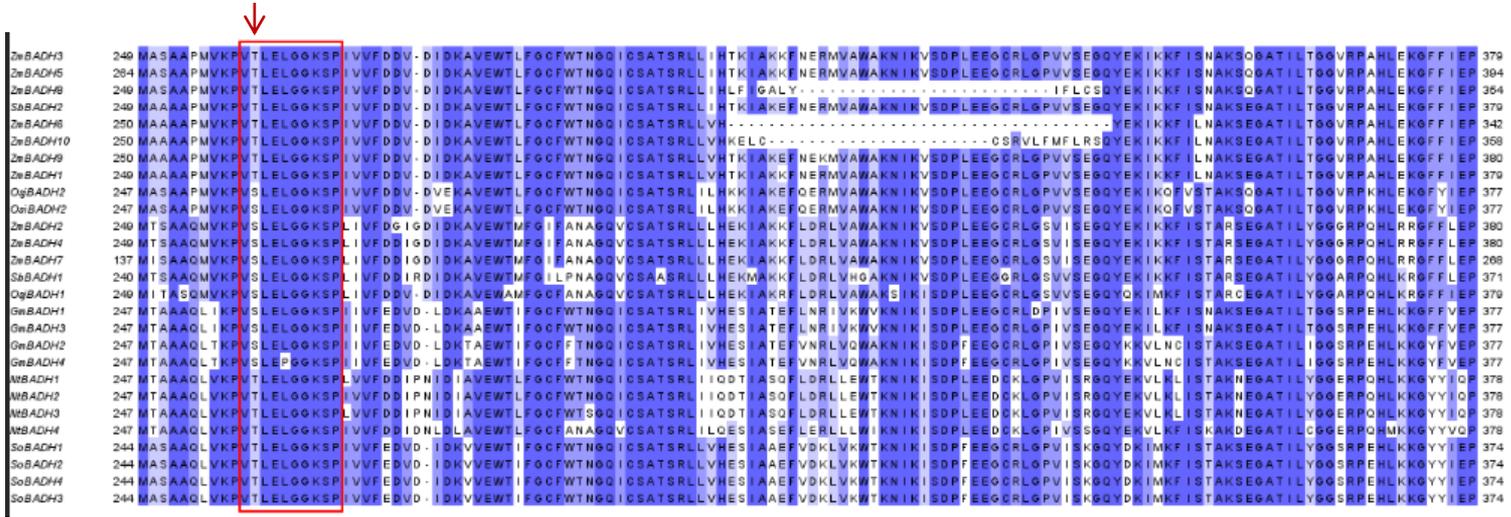
ZmBADH3	117	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPSGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	248
ZmBADH5	132	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPSGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	263
ZmBADH8	117	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPSGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	248
ZmBADH2	117	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPSGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	248
ZmBADH6	118	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	249
ZmBADH10	118	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	249
ZmBADH9	118	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	249
ZmBADH1	117	AQCFEYFADQAEALDKRQNSPVSLPMETFKCHLRREP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPDAGAPLSAHPDVKVAF	TGSFETGKKI	248
OgBADH2	115	AQCFEYFADLAEALDKRQNAVSLPMENFKCYLRKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELADV	ICEVGLPSGVLN	IVTGLGSEAGAPLSSHPDVKVAF	TGSYETGKKI	246
OaBADH2	115	AQCFEYFADLAEALDKRQNAVSLPMENFKCYLRKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELADV	ICEVGLPSGVLN	IVTGLGSEAGAPLSSHPDVKVAF	TGSYETGKKI	246
ZmBADH2	117	AACFEYYADLAEALDGKQSP	ISLPMENFKSYVLKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPEAGAPLSSSHVDKVAFTGSFETGKR	248
ZmBADH4	117	AACFEYYADLAEALDGKQSP	ISLPMENFKSYVLKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPEAGAPLSSSHVDKVAFTGSFETGKR	248
ZmBADH7	5	AACFEYYADLAEALDGKQSP	ISLPMENFKSYVLKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPEAGAPLSSSHVDKVAFTGSFETGKR	130
SbBADH1	108	AACFEYYADLAEALDGKQSP	ISLPMENFKSYVLKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPEAGAPLSSSHVDKVAFTGSFETGKR	239
OgBADH1	117	AACFEYYADLAEALDGKQSP	ISLPMENFKSYVLKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPEAGAPLSSSHVDKVAFTGSFETGKR	248
GmBADH1	115	AQCFEYFADLAEALDKRQNAVSLPMENFKCYLRKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPEAGAPLSSSHVDKVAFTGSFETGKR	246	
GmBADH3	115	AQCFEYFADLAEALDKRQNAVSLPMENFKCYLRKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGPEAGAPLSSSHVDKVAFTGSFETGKR	246	
GmBADH2	115	IGCFNYYAELAEALDGKQSP	ISLPMENFKSYVLKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGNEAGAPLSSSHVDKVAFTGSFETGKR	246
GmBADH4	115	IGCFNYYAELAEALDGKQSP	ISLPMENFKSYVLKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGNEAGAPLSSSHVDKVAFTGSFETGKR	246
NBADH1	115	VACFEYYADLAEALDSKSKTEMKLHLDSEFKTHVYKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGHEAGAPLSSSHVDKVAFTGSFETGKR	246	
NBADH2	115	VACFEYYADLAEALDSKSKTEMKLHLDSEFKTHVYKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGHEAGAPLSSSHVDKVAFTGSFETGKR	246	
NBADH3	115	AASFEYYADLAEALDSKSKTEMKLHLDSEFKTHVYKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGHEAGAPLSSSHVDKVAFTGSFETGKR	246	
NBADH4	115	AASFEYYADLAEALDSKSKTEMKLHLDSEFKTHVYKEP	IGVVGLITFNWYPLLMTW	IIAPALAAAGCAAVLKPSELASVTCLELAD	ICEVGLPPGVLN	IVTGLGHEAGAPLSSSHVDKVAFTGSFETGKR	246	
SoBADH1	112	ASCFEYFAGQAEALDGKQKAPV	TLPMERFKSHVLRQPLGVVGLIS	FNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLEFGEV	NEVGLPPGVLN	IVTGLGPDAGAPLVSHPDVKIAFTGSATGSKV	243
SoBADH2	112	ASCFEYFAGQAEALDGKQKAPV	TLPMERFKSHVLRQPLGVVGLIS	FNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLEFGEV	NEVGLPPGVLN	IVTGLGPDAGAPLVSHPDVKIAFTGSATGSKV	243
SoBADH	112	ASCFEYFAGQAEALDGKQKAPV	TLPMERFKSHVLRQPLGVVGLIS	FNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLEFGEV	NEVGLPPGVLN	IVTGLGPDAGAPLVSHPDVKIAFTGSATGSKV	243
SoBADH3	112	ASRFEYFAGQAEALDGKQKAPV	TLPMERFKSHVLRQPLGVVGLIS	FNWYPLLMTW	IIAPALAAAGCTAVLKPSELASVTCLEFGEV	NEVGLPPGVLN	IVTGLGPDAGAPLVSHPDVKIAFTGSATGSKV	243

Betaine aldehyde binding

NAD

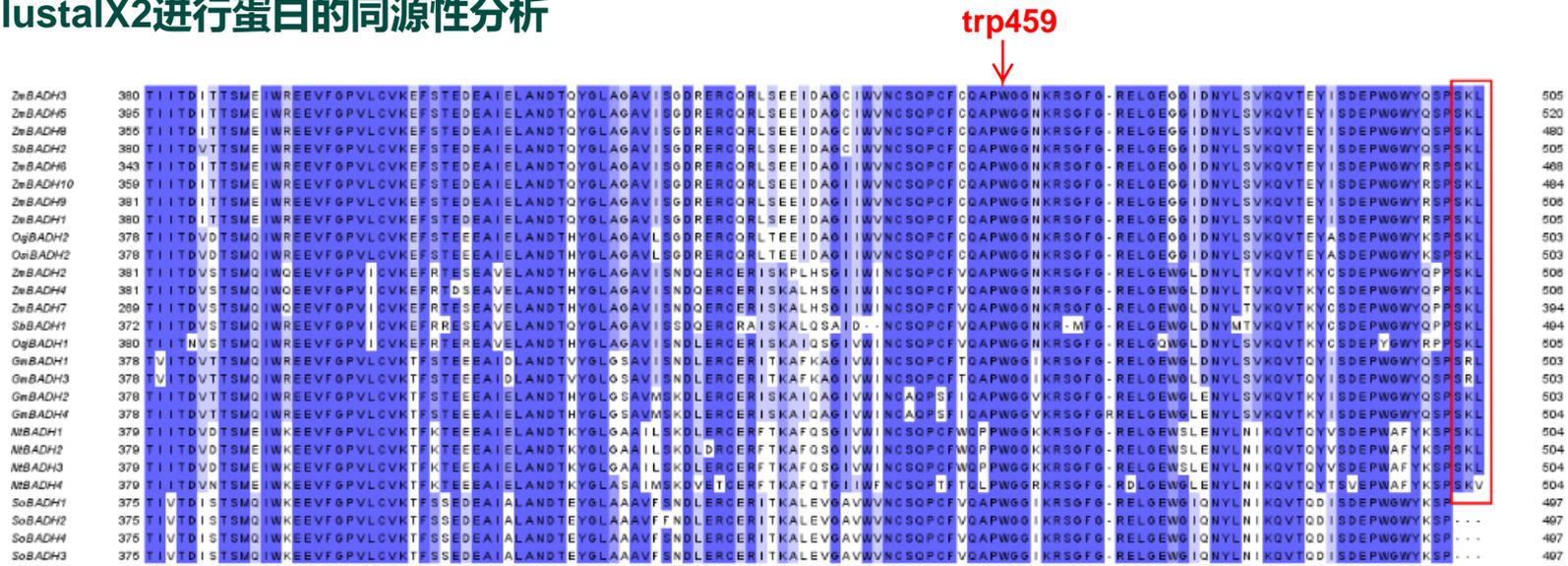
从uniprot中水稻序列的结构域分析得知：161-170为甜菜碱结合位点;从物种同源序列分析得到该结构域高度保守；238-243为NAD结合域，同源序列分析得到该结构域第3、4位点不保守，说明该结构域1、2、5、6位点与酶的功能高度相关。

利用ClustalX2进行蛋白的同源性分析



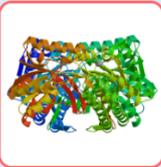
- 大多数植物的醛脱氢酶在相似的位置上都具有高度保守的**十肽(VTLELGKSP)基序**；在功能上，该基序与半胱氨酸(Cysteine, Cys)残基结合，从而决定甜菜碱醛脱氢酶的催化活性^[1]。
- 从图上来看，大豆(Glycine max) GmBADH、玉米 (Zea mays) ZmBADH、水稻OsBADH和高粱 SbBADH15一些蛋白序列出现了第2位的**苏氨酸(Threonine, Thr)残基被丝氨酸(Serine, Ser)残基所替代**的现象

利用ClustalX2进行蛋白的同源性分析



与水稻、玉米、高粱、菠菜和大豆中的BADH氨基酸序列进行同源比对发现，菠菜的氨基酸序列末端缺失了“微体定向信号”，该结构域主要介导甜菜碱脱氢酶定向在过氧化酶体中，因此大豆中BADH序列比对的结构表明，大豆BADH序列含有该结构域，再次印证了亚细胞定位的结果。

利用Swiss-Model预测大豆和水稻BADH的空间结构



Model 01
Structure Assessment

Model-Template Alignment

QMEANDisCo Local	QMEAN Z-Scores
Template 4v37.1.A	Description BETAIN ALDEHYDE DEHYDROGENASE, CHLOROPLASTIC Crystal structure of betaine aldehyde dehydrogenase from spinach showing a thiohemiacetal with 3-aminopropionaldehyde

Model-Template Alignment

Model-Template Alignment



Model 01
Structure Assessment

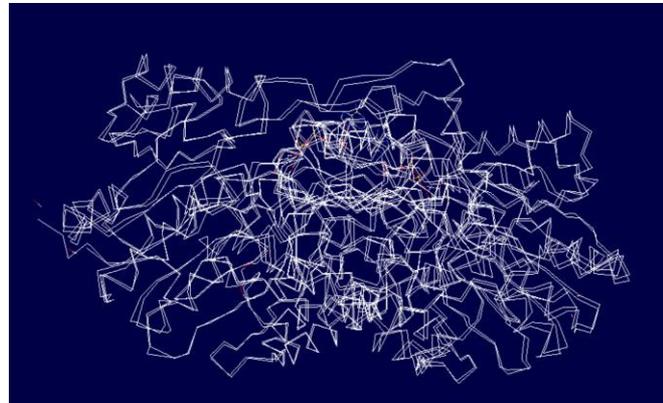
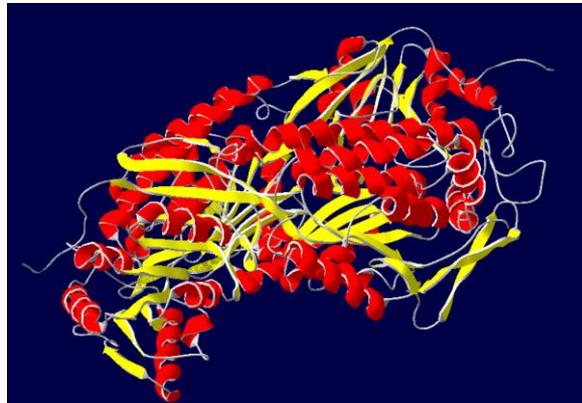
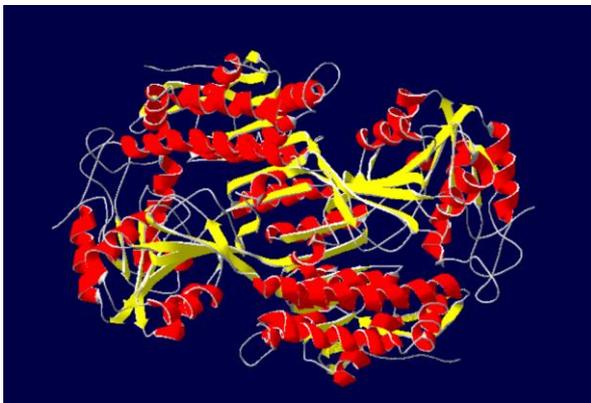
Model-Template Alignment

QMEANDisCo Local	QMEAN Z-Scores
Template 4i8p.1.A	Description Aminoaldehyde dehydrogenase 1 Crystal structure of aminoaldehyde dehydrogenase 1a from Zea mays (ZmAMADH1a)

Model-Template Alignment

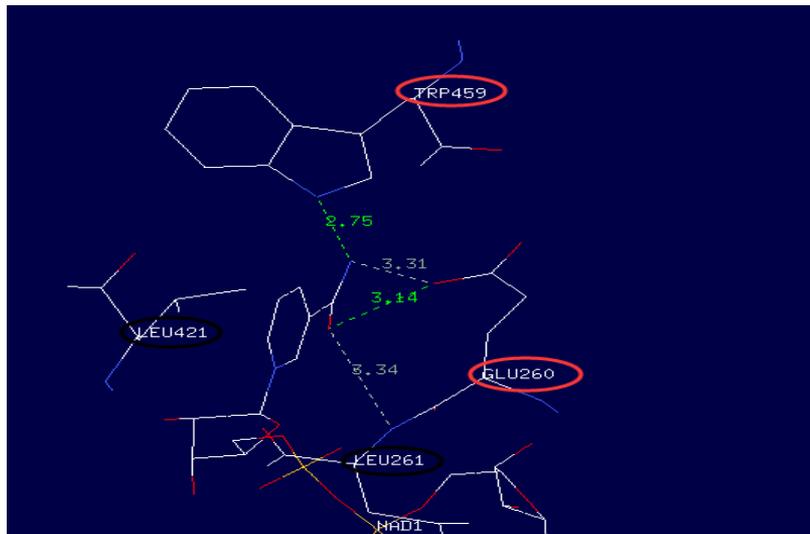
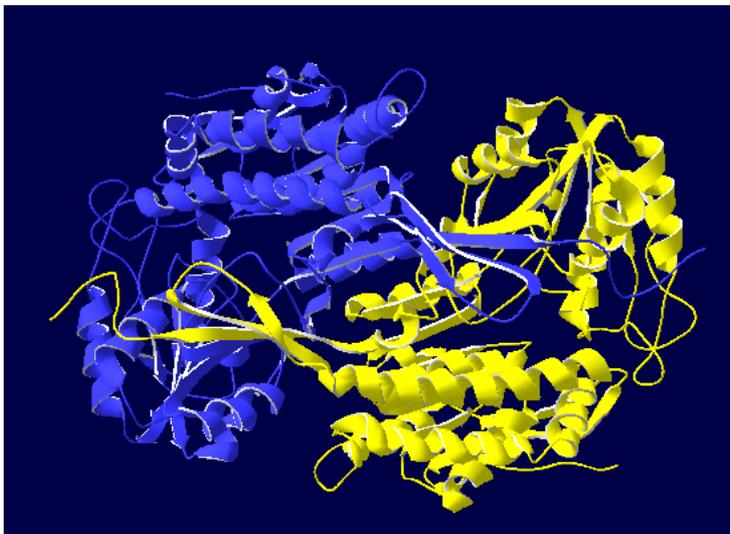
- 大豆和水稻分别参考不同的空间结构
- 相似性程度分别为76.66% 89.44%

利用spdbv观察大豆和水稻BADH的二级结构并比对



- 将大豆和水稻的蛋白空间结构模型以PDB格式下载，在spdbv中观察二级结构（图一、图二）
- 将二者通过“fit”中的结构叠合工具“magic fit”，得到这两个蛋白质分子的结构总体十分相似（图三）→可以以水稻研究内容作为参考进而探索大豆

利用spdbv观察大豆BADH的三级结构



从图上可看出，该蛋白由两条链组成，且在空间上对称；

- 通过检索工具得到可能与NAD形成化学键的四个氨基酸：色氨酸trp459、亮氨酸leu421、亮氨酸leu261、glu谷氨酸260。进一步通过计算氢键和氢键的距离，锁定glu谷氨酸260和色氨酸trp459是与NDA结合的氨基酸。同源序列比对结果，也表明了这两个位点高度保守。

感谢您的聆听

— 2G05 —