

# 玉米TIFY家族 *ZmJAZ4*基因的分 析

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# 报告内容

- 研究背景
- 序列分析
- 翻译后修饰分析
- 后续实验

# 研究背景

## TIFY家族简介

TIFY结构域曾被称为ZIM (Zinc-finger expressed in inflorescence meristem) 结构域，是植物特有的家族，但在最早的绿藻中并没有此类基因，而只存在于陆生植物中，所以推测TIFY家族是在产生陆生植物时产生的。苔藓被公认为陆生植物的鼻祖，在其中鉴定出两个TIFY成员，这两个成员被分成两个亚群，在拟南芥中已鉴定出18个成员，同样也分为两组，说明在植物进化过程中TIFY家族也不断的发展，并赋予植物更多样化的生理功能和发育机制。

## TIFY家族成员JAZ蛋白概述

JAZ (JASMONATE ZIM-DOMAIN) 蛋白是JA (茉莉酸) 信号通路中的一个重要因子，在拟南芥中JAZ蛋白有12个成员，它们含有三个保守区，分别为NT、TIFY和Jas结构域，其中TIFY结构域包含于ZIM结构域。但是人们仍沿用JAZ、PPD、ZIM和ZML为其命名，因为其命名同其蛋白功能相关。ZIM和ZLM (ZIM-LIKE) 是推测的转录因子，含有GATA-锌指结构；JAZs是阻遏蛋白不含DNA结合结构域，并必须依赖蛋白间的互作行使功能 ([Vanholme et al. 2007b](#))。PPD类蛋白不能结合DNA，但可以依赖TIFY结构域形成同源或异源二聚体，目前此类蛋白功能未知。

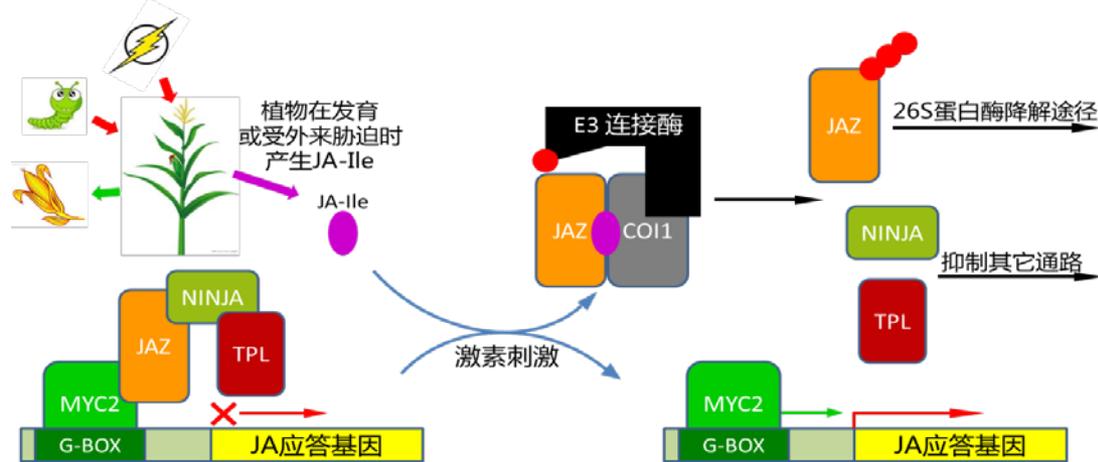
人们发现JAZ蛋白可以与其它转录因子结合，从而调控植物生理过程与应激反应，其本身并不是转录因子，而是转录调节因子。

本研究对玉米转录因子TIFY家族进行预测，找出玉米特异的TIFY转录因子，结合芯片数据进行分析，确定并克隆得到了玉米胚乳特异表达的JAZs基因。对其进行了功能鉴定：通过蛋白亚细胞定位、激素与胁迫处理等实验阐述他们的响应激素与胁迫的能力，为诠释蛋白功能提供了线索。



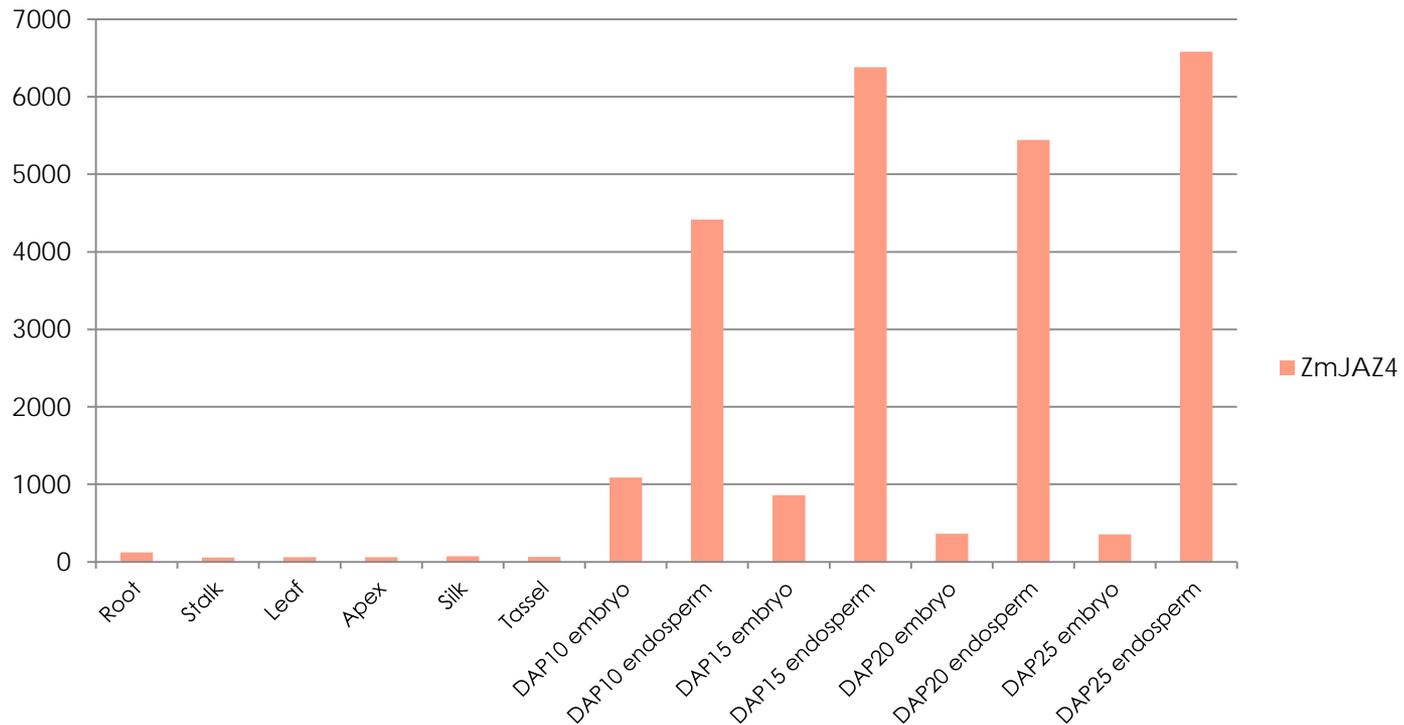
JAZs由3个结构域组成，分别为N-末端的NT结构域，ZIM结构域和C-末端的Jas结构域，其中ZIM结构域中有保守的TIFY基序。三个结构域都可以与其它因子形成二聚体。其中Jas结构域具有核定位信号使JAZs具有核定位特点。

## JAZ蛋白与茉莉酸信号通路



当植物体处于正常的生理环境时，体内JAs水平很低，JAZs结合MYC2并召集共阻遏蛋白NINJA、TPL抑制JA应答基因的转录；当植物体受到外界刺激或生物和非生物胁迫时，体内产生具有生物活性的JA-Ile激素，并促使COI1与JAZs的结合，形成的SCFCOI1/JAZs复合体被E3泛素连接酶识别，JAZs进入26S蛋白酶解途径，MYC2被释放从而起动机JA应答基因的转录。

通过玉米全基因组序列分析，预测了玉米中所有编码转录因子的基因。对所得全部玉米转录因子进行筛选，从中排除掉与水稻、拟南芥蛋白水平同源性高于55%的结果，从而获得了玉米特异的转录因子。其中ZmJAZ4在营养器官中无表达，但在籽粒的胚与胚乳中有表达，并且是随着生育期的进行在胚乳中的表达量持续升高，表达量最高至6000余倍。



利用 Affymetrix Maize Genome Array 表达谱芯片检测玉米B73自交系植株的根、茎、叶、茎尖、花丝、雄花以及授粉后10天、15天、20天、25天的胚和胚乳组织中各基因表达情况

# 序列分析

○ 序列来源:

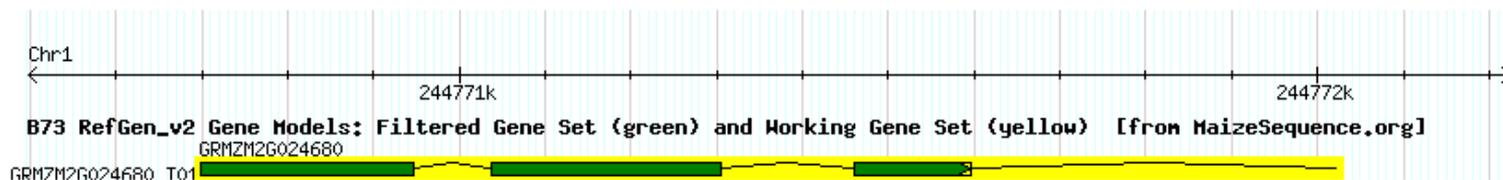


## ▼ Description

**Gene ID:** GRMZM2G024680

**Canonical Transcript (?):** GRMZM2G024680\_T01

**Canonical Position:** Chr1:244,770,698-244,772,023 (Forward strand)



**Line:** B73

**Reference Sequence:** This gene model has 1 transcript(s) on B73 RefGen\_v2.

# 蛋白序列

- >|cl|GRMZM2G024680\_P01 seq=translation;  
coord=1:244770698..244772023:1;  
parent\_transcript=GRMZM2G024680\_T01;  
parent\_gene=GRMZM2G024680

MAASGNNRFSLTCARLRKFMMEQNRKVRMDDL  
V  
GSSSFQRPLQLTPVPVATGPAAAAAWETGTSRTTLP  
LFPVRTAGTAEITRPEEGKATLTIFYQGQVSTFHHFP  
ADRAKVLMMQMASSVTGNTPEKGVAVATAVPKKAQ  
TSDDQPSPAGAGMPPIARKLTLQNFLRKRKNRIAGT  
DDADRNEDASPWKKRDSAAGAGGTPAGDVPDDAS  
WLALRL

# Blast

## Graphical overview

Color code for identity 0  
-100% =

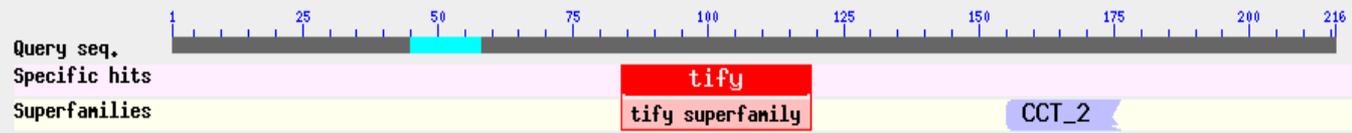


Accession	Entry name	0Query hit216	0Match hit (sqrt scale)1346	Name (Organism)
<input type="checkbox"/> Query	2013062340CT3SO27V			
<input type="checkbox"/> C5WN74	C5WN74_SORBI			Putative uncharacterized protein... (Sorghum bicolor)
<input type="checkbox"/> K4AFH1	K4AFH1_SETIT			Uncharacterized protein (Setaria italica)
<input type="checkbox"/> K4AF91	K4AF91_SETIT			Uncharacterized protein (Setaria italica)
<input type="checkbox"/> K3XS39	K3XS39_SETIT			Uncharacterized protein (Setaria italica)
<input type="checkbox"/> K4AG81	K4AG81_SETIT			Uncharacterized protein (Setaria italica)
<input type="checkbox"/> I1I3L9	I1I3L9_BRADI			Uncharacterized protein (Brachypodium distachyon)
<input type="checkbox"/> I1QU49	I1QU49_ORYGL			Uncharacterized protein (Oryza glaberrima)
<input type="checkbox"/> I1H906	I1H906_BRADI			Uncharacterized protein (Brachypodium distachyon)
<input type="checkbox"/> C5WVS7	C5WVS7_SORBI			Putative uncharacterized protein... (Sorghum bicolor)
<input type="checkbox"/>	A03010_ORYGL			Putative uncharacterized protein... (Oryza glaberrima)

# 多序列比对

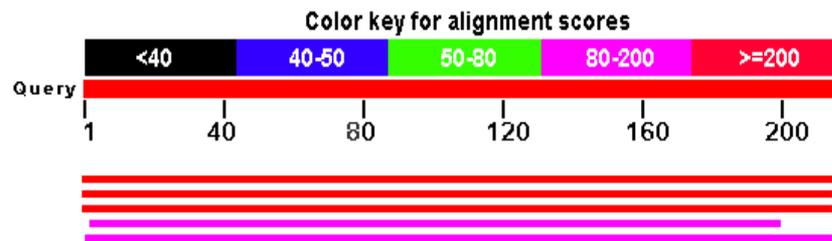
1	MASE-HA-----QLS-AKAAAGSRFAVTYGLLRQYMKEQGGSGATRCLAS-----	43	B6T038	B6T038_MAIZE
1	MA-----ASARPVGVGGERATSFAMACSLLSRYVRQNGAAAAELGLGIRGEGEAPRA	52	A2YNP2	A2YNP2_ORYSI
	. . *:: . * :: : .			
46	LQLTPVPVATG--PAG--SWDTGA--TTLSLFPAG-TG-----TEVIRPEETKA	87	C5WN74	C5WN74_SORBI
41	LQLTPVPVATG--PAAAAAWETGTSRTTLPLFPVRTAG-----TAETRPEEGKA	87	M2G024	M2G024_STRMG
45	A-V-AMGLMPEADAAAA---ATEERTVLELFPQQAGTLKDEQ-----QRKRKEPADGRA	94	C5WVS7	C5WVS7_SORBI
35	-----EAVAEEDARTMQLFPPRAAADG-----VATPSAGTA	70	Q7XEZ1	Q7XEZ1_ORYSJ
51	A-VTAMSLMTG---GADAEAEPEVRKTMELFPQQAGTLKDTQ-----ERKEI---TEKA	98	Q8GRS2	Q8GRS2_ORYSJ
44	A-V-GVGLMPETDAAAAAEAETEERTTVLELFPQQAGTLKDEQ-----QRKRKERADGRA	96	B6T038	B6T038_MAIZE
53	A-PGTMSLLPG-----EAERKKETMELFPQSAGFGQDDAITADSAADAREQEPEKR	102	A2YNP2	A2YNP2_ORYSI
	. : ***			
<b>TIFY domain</b>				
88	LTIFYGQVATFHNFPADRAKDLLQMAGSVTGKAPEKGFLOMADSVTGKAPEKGVMMTA	147	C5WN74	C5WN74_SORBI
88	LTIFYGQVSTFHHFPADRAKVLMMQMASSVTGNTPEKGVAV-----ATA	132	M2G024	M2G024_STRMG
95	LTIFYGKMMVVDFFPAEKAEELMQLAGSGGNAAPA-----A-----	132	C5WVS7	C5WVS7_SORBI
71	LTIFYGRMVVDDVPVEKAAELMRLAGSACSPP-----	105	Q7XEZ1	Q7XEZ1_ORYSJ
99	LTIFYGSSVVVDFFPAEKAGELMQLAGSRDSTAAAA-----VSDA-----	140	Q8GRS2	Q8GRS2_ORYSJ
97	LTIFYGKMMVVDFFPAEKAEELMQLAGSGNA-----A-----	130	B6T038	B6T038_MAIZE
103	LTIFYGKVLVFNDFPADKAKGLMQLASKGSTVAPQNAVAPAAVTDNTKAPMAVPAP	162	A2YNP2	A2YNP2_ORYSI
	***** * : .....*.*:* *:::*			
<b>Jas motif</b>				
148	V--PGKAE-TSDEPADAGAMPPIARKLTLQRFLEKRKNRIAGTDDDGDHNEALPWKKR	204	C5WN74	C5WN74_SORBI
133	V--PKKAQTSDDQPSAGAGMPPIARKL-LQNFLRKRKNRIAGTDD-ADRNEALPWKKR	188	M2G024	M2G024_STRMG
133	-----ENALGQPSLTDMPIARKVSLKRFLEKRKNRIATA-ADPYPAASE-----	175	C5WVS7	C5WVS7_SORBI
106	-----QPAHAAALPEMPIARKASLQRFLEKRKNRIITTTSEPYKKAASV-----	149	Q7XEZ1	Q7XEZ1_ORYSJ
141	-----GAAAGQPCLPDMPIARKVSLQRFLEKRKNRIIVV-AEPLPESEKKE-----	184	Q8GRS2	Q8GRS2_ORYSJ
131	-----QDALGQPSLTDMPIARKVSLKRFLEKRKNRIATA-ADPYPAASATA-----	174	B6T038	B6T038_MAIZE
163	VSSLPTAQADAQKPARANASDMPIARKASLHRFLEKRKNRIATA-KTPYQASPS-----	214	A2YNP2	A2YNP2_ORYSI
	*:*** *:*** **			
205	DS-AGTGNNPAEDAPDDASWLRL-----	226	C5WN74	C5WN74_SORBI
189	DSAAGAGGTPAGDVPDDASWLALRL-----	213	M2G024	M2G024_STRMG
176	----SSTKQPPAVKEEGAPWLVSSALSLS-----	201	C5WVS7	C5WVS7_SORBI
150	-----DAEKSEAVAVKDEPATWVCT-----	171	Q7XEZ1	Q7XEZ1_ORYSJ

Putative conserved domains have been detected, click on the image below for detailed results.

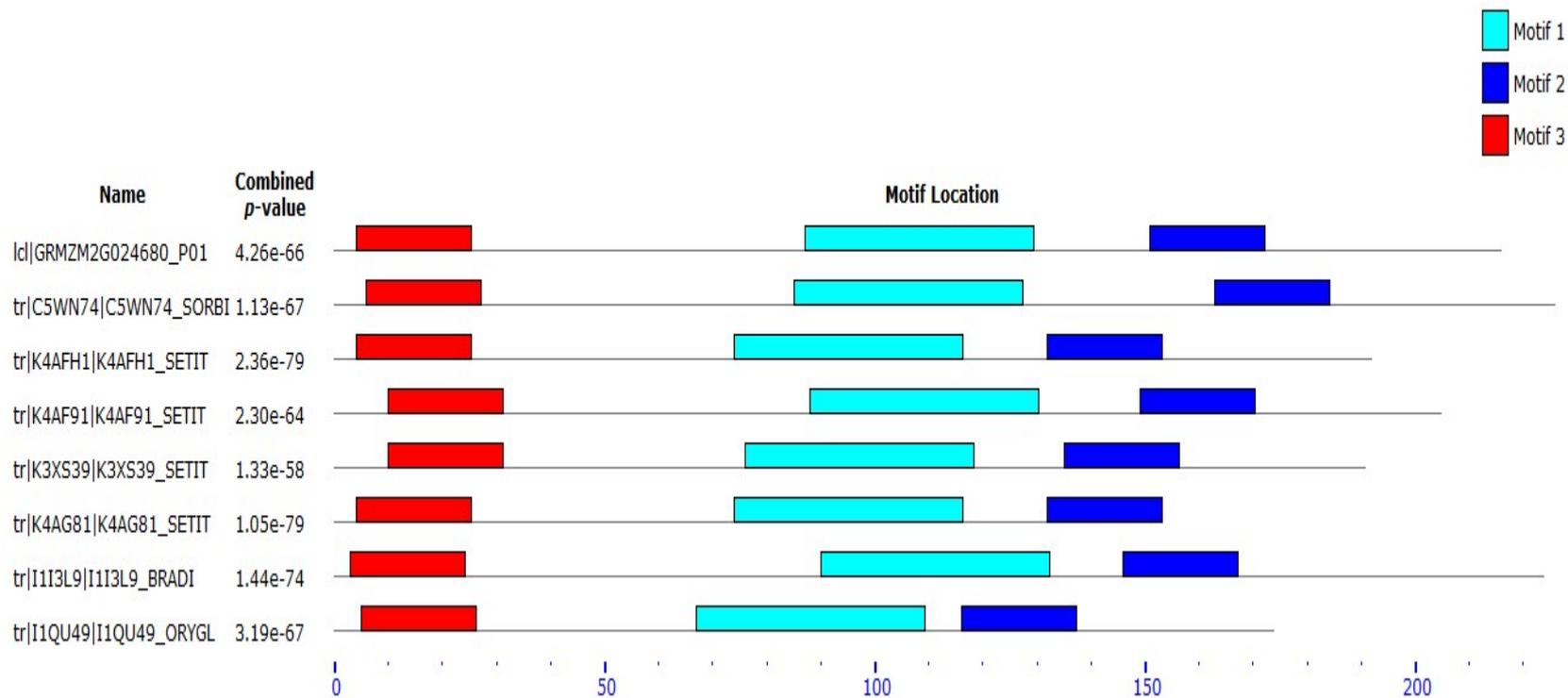


### Distribution of 100 Blast Hits on the Query Sequence

Mouse-over to show define and scores, click to show alignments



# MEME分析保守结构域



## MOTIF 1

### Summary

E-value 1.6e-101  
Width 42  
Sites 8  
[show more](#)

### Sequence Logo



Standard

Download LOGO

Orientation:  SSC:  Format:  Width:  cm Height:  cm

Regular expression

## MOTIF 2

[Previous](#) [Next](#) [Top](#)

### Summary

E-value 4.6e-059  
Width 21  
Sites 8  
[show more](#)

### Sequence Logo



Standard

Download LOGO

Orientation:  SSC:  Format:  Width:  cm Height:  cm

Regular expression

## MOTIF 3

[Previous](#) [Top](#)

### Summary

E-value 5.8e-055  
Width 21  
Sites 8  
[show more](#)

### Sequence Logo



Standard

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Orientation:  SSC:  Format:  Width:  cm Height:  cm

Regular expression

# 亚细胞定位分析

- Wolf PSORT

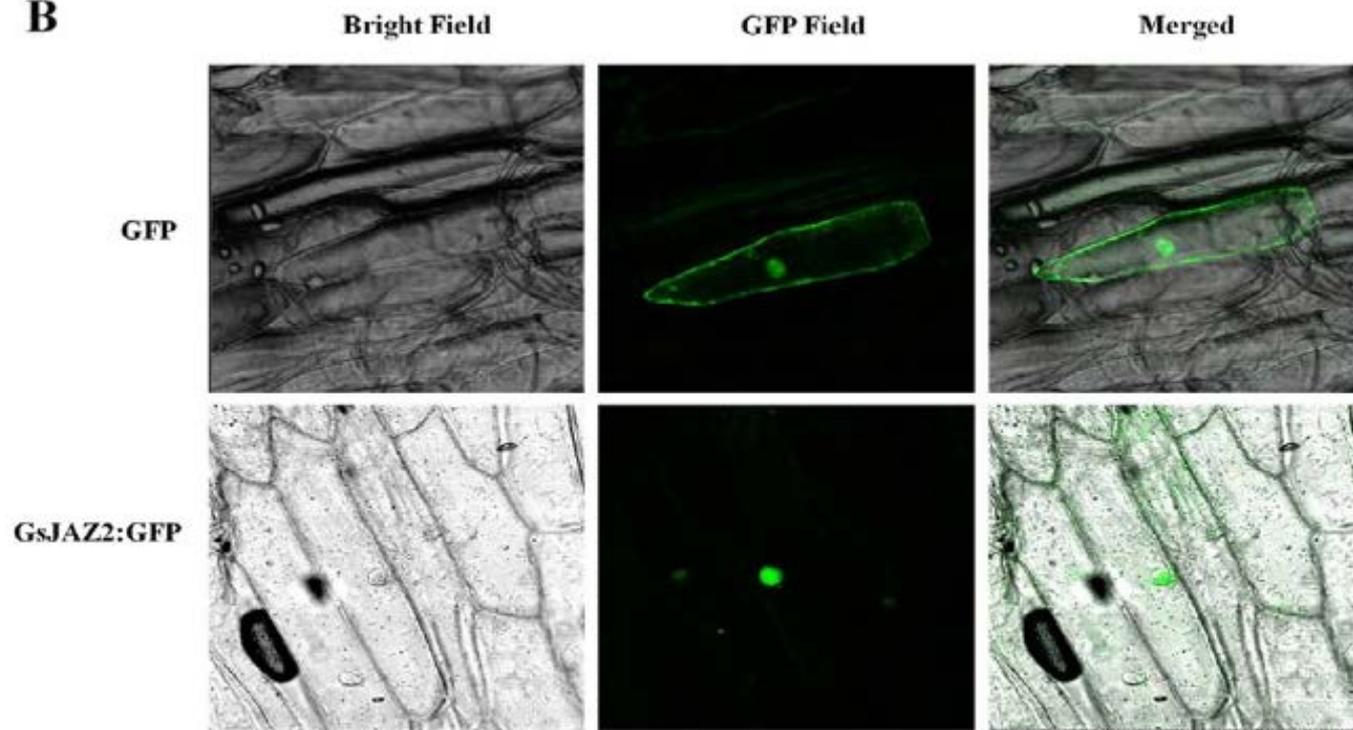
---

*k used for kNN is: 14*

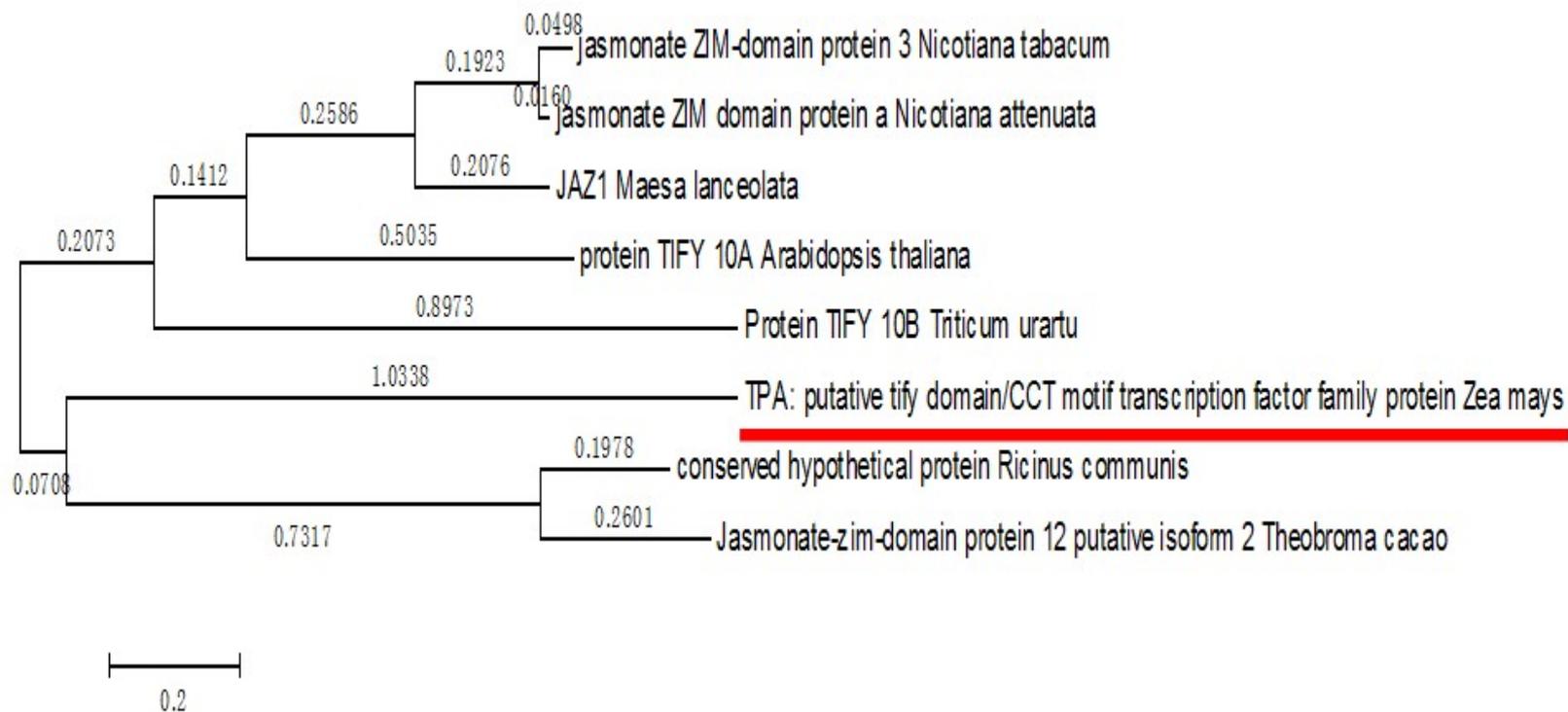
1c1|GRMZM2G024680\_P01 [details](#) chlo: 7.0, nucl: 5.0, mito: 2.0

# 亚细胞定位

**B**



# 系统发育分析



# Protein-protein interaction



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**DIP**  
39129N

### BROWSE LINKS



Protein: Protein TIFY 10A

**Binary** [Complex](#)

[Functional](#)

DIP			Cross Reference			Protein Name/Description
Interaction	Interactor(s)	Links	PIR	SWISSPROT	GENBANK	
<a href="#">DIP:186146E</a>	<a href="#">DIP:58585N</a>	●	---	<a href="#">Q9FIP9</a>	---	Transcription factor ATR2
<a href="#">DIP:80588E</a>	<a href="#">DIP:31324N</a>	●	---	<a href="#">O04197</a>	---	Coronatine-insensitive protein 1
<a href="#">DIP:185673E</a>	<a href="#">DIP:40581N</a>	●	---	<a href="#">Q9SV55</a>	---	uncharacterized protein AT4g28910
<a href="#">DIP:186145E</a>	<a href="#">DIP:53279N</a>	●	---	<a href="#">Q9C5K8</a>	---	Protein TIFY 3B
<a href="#">DIP:122448E</a>	<a href="#">DIP:40533N</a>	●	---	<a href="#">Q39204</a>	---	Transcription factor AtMYC2

# 酵母双杂交实验

- ZmJAZ4与TIFY 3B、AtMYC2等是否互作。

# 翻译后修饰分析

- 磷酸化:DISPHOS,NetPhos 2.0 Server
- 泛素化:BDM-PUB,UbPred

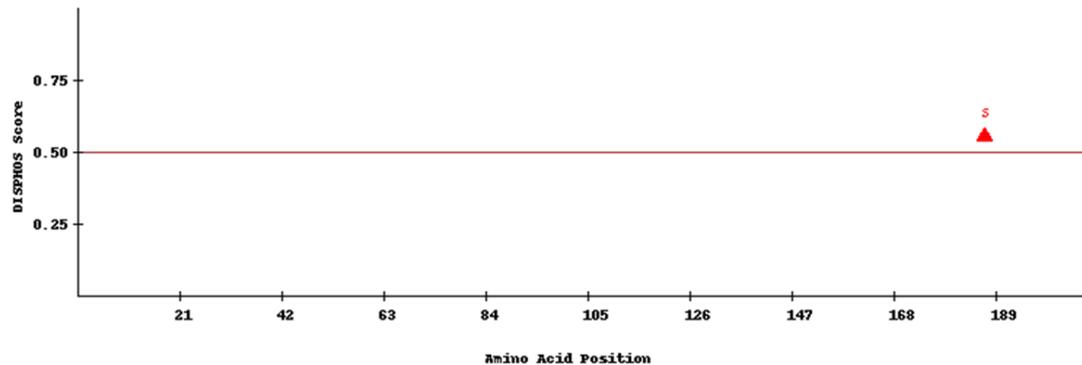
# DISPHOS 1.3

*Disorder-Enhanced Phosphorylation  
Sites Predictor*

## DISPHOS Results

MAASGNNRFSLTCARLRKFMMEQNRKVRMDDLVGSSSFQRPLQLTPVPVATGPAAAAAWE TGTSR<sup>T</sup>TLPLFPVRTAGTAE  
ITRPEEGKATLTI<sup>F</sup>YQGQV<sup>S</sup>TFHHFPADRAKVLQM<sup>A</sup>SSVT<sup>G</sup>NTPEKGVAVATAVPKKAQ<sup>T</sup>SDDQPS<sup>S</sup>PAGAGMPP<sup>I</sup>ARKL  
TLQNFLRKRKNRIAGTDDADR<sup>N</sup>EDAS<sup>P</sup>WK<sup>R</sup>RD<sup>S</sup>AAGAGGT<sup>P</sup>PAGDVPDDAS<sup>W</sup>LALRL

<Unknown> results



### Statistics

Number of phosphorylated serines: 1 out of 14 (7.143%)  
Number of phosphorylated threonines: 0 out of 20 (0.000%)  
Number of phosphorylated tyrosines: 0 out of 1 (0.000%)

## DISPHOS Results

Position	Residue	Score	Sequence	Yes/No
4	S	0.086	*MAA <b>S</b> GNNR	
10	S	0.100	NNRF <b>S</b> LTCA	
12	T	0.053	RF <b>S</b> LT <b>C</b> ARL	
35	S	0.164	DLVG <b>S</b> SS <b>F</b> Q	
36	S	0.063	LVG <b>S</b> SS <b>F</b> Q <b>R</b>	
37	S	0.147	VG <b>S</b> SS <b>F</b> Q <b>R</b> P	
45	T	0.106	PLQL <b>T</b> PPVPV	
51	T	0.164	VPVA <b>T</b> GPAA	
61	T	0.048	AAW <b>E</b> TGTSR	
63	T	0.038	W <b>E</b> TG <b>T</b> S <b>R</b> T <b>T</b>	
64	S	0.076	ETGT <b>S</b> R <b>T</b> T <b>L</b>	
66	T	0.018	G <b>T</b> S <b>R</b> T <b>L</b> PL	
67	T	0.071	T <b>S</b> R <b>T</b> L <b>P</b> L <b>F</b>	
75	T	0.019	FPV <b>R</b> TAGTA	
78	T	0.044	RTAG <b>T</b> A <b>E</b> IT	

92	T	0.016	KATL <b>T</b> IFYQ
95	Y	0.050	LT <b>I</b> F <b>Y</b> Q <b>Q</b> QV
100	S	0.022	Q <b>Q</b> Q <b>V</b> S <b>T</b> F <b>H</b> H
101	T	0.022	G <b>Q</b> V <b>S</b> T <b>F</b> H <b>H</b> F
118	S	0.055	M <b>Q</b> M <b>A</b> S <b>S</b> V <b>T</b> G
119	S	0.075	Q <b>M</b> A <b>S</b> S <b>V</b> T <b>G</b> N
121	T	0.011	A <b>S</b> S <b>V</b> T <b>G</b> N <b>T</b> P
124	T	0.182	V <b>T</b> G <b>N</b> T <b>P</b> E <b>K</b> G
133	T	0.020	V <b>A</b> V <b>A</b> T <b>A</b> V <b>P</b> K
141	T	0.198	K <b>K</b> A <b>Q</b> T <b>S</b> D <b>D</b> Q
142	S	0.284	K <b>A</b> Q <b>T</b> S <b>D</b> D <b>Q</b> P
147	S	0.367	D <b>D</b> Q <b>P</b> S <b>P</b> A <b>G</b> A
161	T	0.052	A <b>R</b> K <b>L</b> T <b>L</b> Q <b>N</b> F
176	T	0.159	R <b>I</b> A <b>G</b> T <b>D</b> D <b>A</b> D
186	S	0.492	N <b>E</b> D <b>A</b> S <b>P</b> W <b>K</b> K
193	S	0.562	K <b>K</b> R <b>D</b> S <b>A</b> A <b>G</b> A
200	T	0.171	G <b>A</b> G <b>G</b> T <b>P</b> A <b>G</b> D
210	S	0.195	P <b>D</b> D <b>A</b> S <b>W</b> L <b>A</b> L

YES



## NetPhos 2.0 Server - prediction results

Technical University of Denmark

216 Sequence

```
MAASGNNRFSLTCARLRKFMMEQNRKVRMDDLVGSSSFQRPLQLTPVPVATGPAAAAAWETGTSRTTLPLFPVRTAGTAE      80
ITRPEEGKATLTI FYQGQVSTFHHFPADRAKVLQMMASSVTGNTPEKGVAVATAVPKKAQTSDDQSPAGAGMPPIARKL      160
TLQNFLRKRKNRIAGTDDADRNEASPWKKRDSAAGAGGTPAGDVPDDASWLALRL                               240
.....S.....S.....T.....T...T.....T..      80
.T.....S...T.....T.....TS....S.....      160
.....S.....S.....T.....                               240
```

Phosphorylation sites predicted: Ser: 7 Thr: 9 Tyr: 0

Serine predictions

Pos	Context	Score	Pred
	v		
4	-MAASGNRR	0.004	.
10	NNRFSLTCA	0.988	*S*
35	DLVGSSSFQ	0.600	*S*
36	LVGSSSFQR	0.042	.
37	VGSSSFQRP	0.095	.
64	ETGTSRTTL	0.048	.
100	QGQVSTFHH	0.415	.
118	MQMASSVTG	0.003	.
119	QMASSVTGN	0.895	*S*
142	KAQTSDDQP	0.914	*S*
147	DDQPSPAGA	0.943	*S*
186	NEDASPWKK	0.980	*S*
193	KKRDSAAGA	0.925	*S*
210	PDDASWLAL	0.175	.
	^		

Threonine predictions

Pos	Context	Score	Pred
	v		
12	RFSLTCARL	0.078	.
45	PLQLTPVPV	0.938	*T*
51	VPVATGPAA	0.179	.
61	AAWETGTSR	0.061	.
63	WETGTSRTT	0.961	*T*
66	GTSRTTLPL	0.054	.
67	TSRTTLPLF	0.549	*T*
75	FPVRTAGTA	0.064	.
78	RTAGTAEIT	0.576	*T*
82	TAEITRPEE	0.504	*T*
90	EGKATLTIF	0.072	.
92	KATLTIFYQ	0.059	.
101	GQVSTFHHF	0.052	.
121	ASSVTGNTP	0.008	.
124	VTGNTPEKG	0.859	*T*
133	VAVATAVPK	0.502	*T*
141	KKAQTSDDQ	0.891	*T*
161	ARKLTLQNF	0.204	.
176	RIAGTDDAD	0.302	.
200	GAGGTPAGD	0.833	*T*
	^		

Tyrosine predictions

Pos	Context	Score	Pred
	v		
95	LTIFYQGQV	0.005	.
	^		

# 预测磷酸化位点

- 193位丝氨酸

# 泛素化位点分析

## ※ BDM-PUB: Prediction of Ubiquitination sites with Bayesian Discriminant Method

[Go back to BDM-PUB prediction page](#)

Predicted Ubiquitination sites:

Peptide	Position	Score	Threshold
LTCARLRKFMMEQNR	18	0.72	0.3
VTGNTPEKGVAVATA	127	0.65	0.3
AVATAVPKKAQTSDD	137	2.18	0.3
VATAVPKKAQTSDDQ	138	2.30	0.3
GMPPIARKLTLQNFL	159	2.66	0.3
QNFLRKRKNRIAGTD	170	1.36	0.3
NEDASPWKKRDSAAG	189	1.93	0.3

Download the TAB-delimited data file from [here](#).

[Go back to BDM-PUB prediction page](#)



## UbPred: predictor of protein ubiquitination sites



>

MAASGNRRFSLTCARLRKFMMEQNRKVRMDDLVGSSSFQRPLQLTPVPVATGPAAAAAWETGTSRTTLPLFPVRTAGTAEITRPEEGKATLTIFYQGQVSTFHHPADRAKVLMLQASSVTGNTPEKGVAVATAVPKKAQTSDDQSPAGAGMPPIARKLTLQNFLRKRKNRIAGTDDADRNEASPWKKRDSAAGAGGTPAGDVPDDASWLALRL

18 0.17 No

26 0.40 No

88 0.78 Yes Medium confidence

111 0.47 No

127 0.75 Yes Medium confidence

137 0.76 Yes Medium confidence

138 0.81 Yes Medium confidence

159 0.12 No

168 0.21 No

170 0.50 No

189 0.81 Yes Medium confidence

190 0.77 Yes Medium confidence

Legend:

Label Score range Sensitivity Specificity

Low confidence  $0.62 < s < 0.69$  0.464 0.903

Medium confidence  $0.69 < s < 0.84$  0.346 0.950

High confidence  $0.84 < s < 1.00$  0.197 0.989

# 泛素化位点

- 137,138,189位赖氨酸

# 后续实验

- 亚细胞定位
- 酵母双杂交
- 转入拟南芥中进行表型分析
- 定点突变

# 致谢

- 感谢罗老师的指导；
- 感谢小组成员的共同努力。

谢谢！