利用酵母双杂交系统筛选水稻组蛋白去乙酰化酶 HDA705的互作蛋白

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摘要:为了解水稻(Oryza sativa)组蛋白去乙酰化酶 HDA705 的生物学功能,构建了 HDA705 酵母双杂交诱饵表达载体与双杂 交文库,并筛选了与 HDA705 相互作用的蛋白。结果表明, HDA705 的诱饵载体无自激活活性且对酵母无毒性作用,文库的 滴度也适合常规的酵母双杂交文库筛选。通过对酵母双杂交文库的筛选,共获得了 164 个阳性克隆,经 DNA 测序分析,这些 克隆编码 47 个可能与 HDA705 相互作用的蛋白,其中包括 3 个在逆境响应或激素信号转导过程中起到重要作用的(辅)转录因 子、6 个参与光合作用的叶绿体蛋白、1 个含有 R3H 结构域的蛋白以及 22 种酶类等。这为进一步研究 HDA705 的生物学功 能提供了重要的线索。

关键词:水稻;组蛋白去乙酰化酶;酵母双杂交;互作蛋白 doi: 10.11926/j.issn.1005-3395.2015.01.007

Screening of Proteins Interacting with Histone Deacetylase HDA705 in Rice (*Oryza sativa*) by Yeast Two Hybrid System

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Abstract: In order to understand the biological function of histone deacetylase HDA705 in rice, the yeast twohybrid bait vector of HDA705 and the cDNA library were constructed and the interaction partners of HDA705 were screened. The results showed that HDA705 bait vector showed no self-activating and no toxicity to yeast cells. Furthermore, the titer of cDNA library was suitable for normal yeast two-hybrid screening assay. There were 164 positive clones by yeast two-hybrid screening. DNA sequencing analysis indicated that these positive clones encoded 47 proteins which may interact with HDA705. These proteins included 3 transcription factors (or transcription co-factors) which play key roles in stress responses and hormone signaling pathways, 6 chloroplast located proteins which were involved in photosynthesis, 1 protein containing the R3H domain structure, and 22 enzymes in plant, etc. These may provide important cues to further study the biological function of HDA705 in rice. **Key words:** Rice; Histone deacetylase; Yeast two-hybrid; Interaction protein

核心组蛋白的乙酰化与去乙酰化作用在真核 生物的基因转录调控中起到重要的作用^[1]。组蛋白 去乙酰化酶(Histone deacetylase, HDAC)是通过移除 染色质组蛋白上的乙酰基,使染色质的结构变得紧密从而抑制基因的转录^[2-8]。高等植物中,HDACs家族成员被划分为 RPD3/HDA1 超家族、SIR2 家

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族和 HD2 家族 3 大类^[9-12]。HDACs 成员广泛参与 植物的生长发育过程及对逆境胁迫的响应途径^[13]。

水稻(Oryza sativa)中已鉴定出 18个 HDACs 成员,其中, HDA705属于 RPD3/HDA1 超家族 成员,具有典型的 HDAC 功能结构域^[14]。目前, 对 HDA705 的功能研究仅局限于生物信息学分 析、不同发育阶段和不同逆境条件下的表达谱分 析、以及启动子特异性表达分析等方面,罕见有 关其参与水稻的生长发育及对逆境胁迫的响应 等生物学功能方面的研究报道[14-16]。有研究表 明, 拟南芥(Arabidopsis thaliana)中的组蛋白去乙 酰化酶 HDA15、HDA6和 HDA19分别与 PIF3 (Phytochrome-interacting factor 3) AS1 (Asymmetric leaf 1)及 HSL1 (HSI2-like 1)等蛋白互作,调控叶绿 素的生物合成、叶片发育及种子成熟等相关基因的 表达[17-19], 表明 HDACs 通常通过与其它蛋白质的 相互作用共同调控植物基因表达和生长发育过程。 因此,筛选和鉴定 HDA705 的互作蛋白对于解析其 在水稻中的生物学功能具有重要的意义。

酵母双杂交(Yeast two hybrid)是一种高效的互 作蛋白筛选方法,通过转录激活报告基因表达,能 迅速、灵敏地在酵母体内检测蛋白的互作。酵母双 杂交技术己在细胞周期与分化、信号传导和基因 表达调控等蛋白质互作研究方面得到了广泛的应 用^[20-21]。

本研究中,我们构建了水稻 HDA705 的酵母 双杂交诱饵表达载体和双杂交文库,并通过接合型 酵母双杂交文库筛选,获得了与 HDA705 互作的蛋 白,为 HDA705 生物学功能及作用机制的研究提 供了重要的线索。

1 材料和方法

1.1 材料

大肠杆菌(Escherichia coli)菌株 DH10B、酵母 (Saccharomyces cerevisiae)菌株 AH109和Y187、 pGADT7 DNA-AD Cloning Vector、pGBKT7 DNA-BD Cloning Vector、酵母双杂交试剂盒及酵母培养 基各组分购自 Clontech 公司;水稻(Oryza sativa)品 种'中花 11'('Zhonghua11', ZH11)为中国科学院 华南植物园生物技术育种课题组保存;质粒提取试 剂盒和 DNA 胶回收试剂盒购自天根生化科技(北 京)有限公司;RNA 提取试剂 Trizol 购自 Invitrogen 公司;mRNA 纯化试剂 Oligotex-dT30<Super>mRNA Purification Kit (From Total RNA)、限制性核酸内切 酶、T4 DNA 连接酶、PCR 所用 Ex Taq 酶, RNA 反转录试剂盒购自宝生物工程(大连)有限公司;其 他为国产分析纯试剂。

1.2 诱饵载体构建

以水稻 ZH11 的两周幼苗为材料,采用 Trizol 法提取总 RNA 后逆转录为 cDNA。以此 cDNA 为 模板,采用 TaKaRa 公司的高保真酶(Ex Taq)扩增 HDA705 基因的全长序列,引物分别为OsHDA705F: 5'-GAAGATCTATGGCGGCGTCCGGCGAGGG-3' 和OsHDA705R: 5'-GAAGATCTCTAGGAATCATC-ATTCGATT-3'。回收扩增片段后连接到 T 载体,并 转化到大肠杆菌 DH10B,提取质粒进行测序验证。 以测序正确的质粒为模板,采用 In-fusion 技术进 行 PCR 扩增,引物分别为 OsHDA705L: 5'-CATG-GAGGCCGAATTCATGGCGGCGTCCGGCGAGG-GGGCG-3' 和 OsHDA705R: 5'-GCAGGTCGACG-GATCCCTAGGAATCATCATTCGATTCATC-3'。 回 收PCR产物,并用BamHI和EcoRI进行双酶切, 获得的片段与 pGBKT7-DNA-BD 载体连接,构建 为 pGBKT7-HDA705 诱饵载体,转化大肠杆菌后提 取质粒进行测序。pGBKT7-HDA705诱饵载体转 化酵母菌后,即可表达由 ADH I 启动子驱动表达的 GAL4 BD-HDA705 诱饵蛋白。

1.3 诱饵蛋白毒性及自激活检测

为检测诱饵蛋白 GAL4 BD-HDA705 对酵母菌 的毒性和自身激活能力,分别将 pGBKT7-HDA705 诱饵载体和 pGBKT7-DNA-BD 空载体质粒转化到 酵母菌株 AH109 中,并将菌液涂抹在 SD/-Trp/X-α-Gal 平板上培养。4 d 后分别挑选 10 个 2 mm 大小 的单克隆进行摇菌培养,至菌液浓度 OD₆₀₀ 为 0.5 左右时,分别取 0.5 μL 菌液在 SD/-Trp/X-α-Gal 固 体培养基上点样培养,于 30℃恒温箱中培养4 d 后, 观察酵母菌的生长情况。

1.4 cDNA文库的构建及丰度检测

采用 Trizol 法提取水稻 ZH11 两周幼苗的总 RNA,用 mRNA 纯化试剂盒分离纯化水稻 mRNA。 以分离的 mRNA 为模板,用 Oligo (dT) 18-Anchor Primer [5'-(GA)10ACTAGTCTCGAG(T)₁₈V-3' (V: A/C/G)]进行逆转录反应,合成单链 cDNA,双链 化后进行平末端处理,并连接上 EcoRI Adaptor [5'-OH-AATTCGGCACGAGG-3' (3'-GCCGTGCT-CCp-5')], 进行 EcoR I/Xho I 双 酶 切 后 连 接 到 pGADT7载体,得到酵母双杂交 cDNA 文库。将 cDNA 文库质粒转化大肠杆菌进行培养,利用血 球计数板计算滴度达到 1.0×10⁶ cfu mL⁻¹ 后涂平 板扩增放大 cDNA 文库。大量提取 cDNA 文库质 粒后,取15 µg 质粒转化到酵母感受态 Y187 中, 将 25 mL 转化液均匀涂抹到 160 个直径为 9 cm 的 SD/-Leu 平板上,于 30℃培养 4 d。采用 500 mL Freezing medium 洗脱菌体,并取 100 µL 菌液,稀释 5倍后采用血球计数板计算滴度。将洗脱的菌液离 心浓缩成文库所需滴度,分装后放入-80℃冰箱保 存。构建成功的 cDNA 文库可以在 ADH I 启动子 驱动下表达 GAL4 AD-cDNA 融合蛋白。

1.5 cDNA文库的鉴定

从 SD/-Leu 平板中随机挑取 30 个单菌落,采用 pGADT7 通用引物进行菌落 PCR 反应, PCR 产物用 1% 琼脂糖凝胶电泳进行检测,根据扩增片段的大小和个数,统计 cDNA 文库的重组率和插入片段大小的分布情况。

1.6 酵母双杂交文库筛选与HDA705相互作用的蛋白

挑取表达诱饵载体的 AH109 单菌落(大小约 2~3 mm)至 50 mL SD/-Trp 液体培养基中培养,至菌 液浓度 OD₆₀₀ 约为 0.8 时在 1000×g 下离心 5 min,

用 4~5 mL SD/-Trp 液体培养基进行重悬浮 (>1×10⁸ cfu mL⁻¹)。取 1 mL cDNA 文库菌液与 4~ 5 mL 诱饵载体菌液混合,并添加 45 mL 2×YPDA 液体培养基进行低速摇菌培养(30~50 r min⁻¹)。20 h 后,取菌液在显微镜下观察,出现三叶草或者米老 鼠状酵母接合体,说明酵母接合杂交成功。将菌液 在1000×g下离心10min,弃上清液后用50mL0.5× YPDA 液体培养基重悬浮菌体,再次离心(1000×g, 10 min),弃上清液后重悬浮于 10 mL 0.5×YPDA 液 体培养基中。取 10 µL 菌液稀释 1/10000 涂至 SD/ -Trp、SD/-Leu 和 SD/-Leu/-Trp 平板上(100 µL 板⁻¹), 用各板生长菌落数计算接合效率和杂交克隆数。 将剩余未稀释的菌液涂于 55 个 SD/-Ade/-His/-Leu/ -Trp/X-α-Gal 平板上。挑取生长良好、显蓝色的克 隆进行菌落 PCR 检测,并提取质粒,用 pGADT7 通用引物进行测序,在GenBank数据库中进行 BLAST 序列比对分析,以序列相似度最高的水稻 基因为目的基因。

2 结果和分析

2.1 HDA705诱饵蛋白表达载体的构建及序列测定

将 pGBKT7-HDA705 连接产物转化大肠杆菌 后,采用载体两端的引物进行菌落 PCR 分析,结果 得到大小为 1300~1500 bp 的 PCR 产物(图 1: A), 由于 HDA705 cDNA 全长 1377 bp,表明得到的克 隆均为阳性克隆。将阳性克隆进行摇菌培养,提取 质粒后进行酶切检测。HDA705 cDNA 的 1099 bp



图 1 pGBKT7-HDA705 诱饵表达载体的菌落 PCR 检测(A)和酶切验证(B)。M: 2000 bp DNA marker; 1~6: 阳性克隆; 7: 对照; 8: Pst I 酶切; 9: EcoR I 酶切。

Fig. 1 Colony PCR (A) and enzymatic digestion detection (B) of pGBKT7-*HDA705* bait vector. M: 2000 bp DNA marker; 1–6: Positive colonies; 7: Control; 8: *Pst* I digestion; 9: *Eco*R I digestion.

处有1个 EcoR I 酶切位点, 991 bp 处有1个 Pst I 酶切位点,而在 pGBKT7-DNA-BD 载体的 1300 bp 处也有1个 EcoR I, 1326 bp 处有1一个 Pst I,因 此可以采用 EcoR I 或 Pst I 对构建的载体质粒进行 单酶切检测。酶切后电泳结果表明, EcoR I 酶切得 到的片段约为 1100 bp, 而 Pst I 酶切得到约为 400 bp 的片段,均符合预期的片段大小(图 1: B)。将酶切 检测正确的质粒送交测序,序列比对结果显示序列 正确(图 2),表明诱饵蛋白表达载体构建成功。

2.2 诱饵蛋白载体的毒性和自激活检测

将 pGBKT7-HDA705 重 组 质 粒 和 pGBKT7-DNA-BD 空载体质粒分别转化到 AH109 菌株中, 摇菌培养后,取菌液点在 SD/-Trp/X-α-Gal 平板上 培养3d。结果表明,同转化空载体的阴性对照一 致,转化 pGBKT7-HDA705 诱饵载体的菌落在 SD/ -Trp/X-α-Gal 培养基上能正常生长,且不显蓝色(图

3), 表明 pGBKT7-HDA705 诱饵蛋白在 AH109 酵 母细胞内无毒性,且无自激活活性,可以用于酵母 双杂交的文库筛选实验。

2.3 文库载体的丰度检测

取1mL酵母 cDNA 文库菌液稀释5倍,血球 计数板上每格约 200 个菌体。因此,酵母 cDNA 文 库滴度为 2.5×10⁸ cfu mL⁻¹,达到酵母双杂交所需的 文库滴度要求(2.0×10⁷ cfu mL⁻¹)。

2.4 酵母cDNA文库片段大小和重组率的测定

将构建的 cDNA 文库质粒转化酵母后,随机 挑选了 30 个菌落进行 PCR 检测。结果表明,挑选 的 30 个菌落中除 10、19 和 24 号菌落没有扩增出 任何条带外(图 4: 白色箭头),其余 27 个菌落均扩 增出外源片段(图 4),表明文库的重组率约为 90%。 同时,重组外源片段大小为 250~2000 bp (图 4)。

HDA705	1	ATGGCGGCGTCCGGCGAGGGGGCGTCGCTGGCGTCGGCGGGGGGGG
pGBKT7-HDA705	1	${\tt CCATGGAGGCCGAATTCATGGCGGCGTCCGGCGAGGGGGGGG$
HDA705	58	CGGCGGCGGCGGGTGAGCTACTTCTACGAGCCGTCGATCGGGGACTACTACTACGGGCAGGGGCACCCGATGAA
pGBKT7-HDA705	75	${\tt CGGCGGCGGCGGGGGGGGGCACTTCTACGAGCCGTCGATCGGGGACTACTACTACGGGCAGGGGCACCCGATGAA}$
HDA705	132	${\tt GCCCCATCGCATCCGCATGGCGCACTCGCTGGTGGTCCACTACGGCCTCCACCGCCTCCTCGAGCTCTCCCGCCCCCCCC$
pGBKT7-HDA705	149	${\tt GCCCCATCGCATCGCATGGCGCACTCGCTGGTGGTCCACTACGGCCTCCACCGCCTCCTCGAGCTCTCCCGCCCCCCCC$
HDA705	206	${\tt cctacccggcctccgacgccgacatccgccgcttccactccgacgactacgtcgccttcctcgcctccgccacccccccc$
pGBKT7-HDA705	223	${\tt cctacccggcctccgacgccgacatccgccgcttccactccgacgactacgtcgccttcctccgccacc}$
HDA705	280	GGGAACCCCGCCTGCTCGACGCCCGCCGCCGTCAAGCGCTTCAACGTCGGCGAGGACTGCCCGGTCTTCGACGG
pGBKT7-HDA705	297	GGGAACCCCGCCGCGCGCGCGCGGGGGGGGGG
HDA705	354	${\tt cctcttccccttctgccaggcctccgcggggggcagcatcggcgccgccgtcaagctcaaccgcggcgacgccg}$
pGBKT7-HDA705	371	$\tt CCTCTTCCCCTTCTGCCAGGCCTCCGCGGGGGGGGGGGG$
HDA705	428	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$
pGBKT7-HDA705	445	ACATCACCGTCAACTGGGCGGGGGGGGCCTCCACCACGCCAAGAAGGGCGAGGCCTCCGGCTTCTGCTACGTCAAC
HDA705	502	${\tt GACATCGTCCTCGCCATCCTCGAGCTCCTCAAGTTCCACAGGCGTGTGCTATATGTAGACATTGATGTCCACCA}$
pGBKT7-HDA705	519	${\tt GACATCGTCCTCGCCATCCTCGAGCTCCTCAAGTTCCACAGGCGTGTGCTATATGTAGACATTGATGTCCACCA}$
HDA705	576	${\tt TGGAGATGGCGTGGAGGAAGCTTTCTTCACTACGAATCGAGTCATGACTTGTTCCTTTCACAAGTATGGGGACT$
pGBKT7-HDA705	593	${\tt TGGAGATGGCGTGGAGGAAGCTTTCTTCACTACGAATCGAGTCATGACTTGTTCCTTTCACAAGTATGGGGACT$
HDA705	650	${\tt TTTTCCCTGGTACTGGGCATATCACTGATGTTGGAGCAGGCGAAGGAAAACATTATGCTTTAAATGTGCCCCTG$
pGBKT7-HDA705	667	${\tt TTTTCCCTGGTACTGGGCATATCACTGATGTTGGAGCAGGCGAAGGAAAACATTATGCTTTAAATGTGCCCCTG$
HDA705	724	${\tt AGTGATGGCATTGATGATGATACCTTTCGTGATCTGTTCCAGTGCATCATCAAAAAGGTAATGGAGGTTTATCA}$
pGBKT7-HDA705	741	${\tt AGTGATGGCATTGATGATGATACCTTTCGTGATCTGTTCCAGTGCATCATCAAAAAGGTAATGGAGGTTTATCA}$
HDA705	798	${\tt GCCAGATGTAGTTGTTCTCCAGTGTGGAGCCGACTCTTTGGCTGGAGACAGGTTAGGTTGCTTCAACCTGTC TG$
pGBKT7-HDA705	815	GCCAGATGTAGTTGTTCTCCAGTGTGGAGCCGACTCTTTGGCTGGAGACAGGTTAGGTTGCTTCAACCTGTCTG
HDA705	872	${\tt TGAAAGGTCATGCAGACTGCCTCCGTTACCTTAGGTCATTCAATATTCCTATGATGGTTTTGGGAGGTGGG~GGT$
pGBKT7-HDA705	889	${\tt TGAAAGGTCATGCAGACTGCCTCCGTTACCTTAGGTCATTCAATATTCCTATGATGGTTTTGGGAGGTGGGGGTGGGGGTGGGGGTGGGGGG$
HDA705	946	TACACCATCAGAAATGTTGCTCGCTGCTGGTGCTATGAGACTGCAGTTGCTGTTGGAGTTGAACCTGATAACAA
pGBKT7-HDA705	963	TACACCATCAGAAATGTTGCTCGCTGCTGGTGCTATGAGACTGCAGTTGCTGTTGGAGTTGAACCTGATAACAA
HDA705	1020	GTTACCTTATAATGACTACTATGAGTACTTTGGTCCTGATTATAATCTCCATATTCAACCCAGAAGTGTGGAGA
pGBKT7-HDA705	1037	GTTACCTTATAATGACTACTATGAGTACTTTGGTCCTGATTATAATCTCCATATTCAACCCAGAAGTGTGGAGA
HDA705	1094	ACCTGAATTCGACTAAAGACCTGGAGAACATAAAGAGCATGATATTGGATCATCTCTCAAAAATTGAGCATGTT
pGBKT7-HDA705	1111	ACCTGAATTCGACTAAAGACCTGGAGAACATAAAGAGCATGATATTGGATCATCTCTCAAAAATTGAGCATGTT
HDA705	1168	$\tt CCGAGCACTCAGTTCCATGACAGACCATCAGATCCTGAAGCTCCAGAGCAGGAAGAGGAGGACATGGACAAGAG$
pGBKT7-HDA705	1185	CCGAGCACTCAGTTCCATGACAGACCATCAGATCCTGAAGCTCCAGAGCAGGAAGAGGAGGACATGGACAAGAG
HDA705	1242	ACCACCTCAGCGCAGTAGGTTATGGAGTGGAGGAGCTTATGAATCTGATACAGAGGATCCTGACAACATGAAAA
pGBKT7-HDA705	1259	ACCACCTCAGCGCAGTAGGTTATGGAGTGGAGGAGCTTATGAATCTGATACAGAGGATCCTGACAACATGAAAA
HDA705	1316	CTGAGACCAATGACTTATCTGCCAGCTCTGTCATGAAGGATGAATCGAATGATGATTCCTAG
pGBKT7-HDA705	1333	CTGAGACCAATGACTTATCTGCCAGCTCTGTCATGAAGGATGAATCGAATGATGATTCCTA GGGATCC

图 2 pGBKT7-HDA705 测序结果与原序列比对

Fig. 2 Sequence alignment of pGBKT7-HDA705 and HDA705

2.5 酵母双杂交筛选与HDA705相互作用的蛋白

将接合杂交后的酵母菌液 10 μL 稀释至 1/10000 后涂板(每板 100 μL)。在 SD/-Leu/-Trp 培 养基长出 31 个单克隆菌落,在 SD/-Leu 培养基上 长出约 170 个菌落,在 SD/-Trp 培养基上长出约 560 个单克隆菌落(图 5),因此,筛选得到文库克隆



图 3 pGBKT7-HDA705 诱饵蛋白在 AH109 菌株内的自激活活性和毒性检测

Fig. 3 Autoactivation and toxicity detection of pGBKT7-HDA705 bait protein in AH109



图 4 cDNA 文库的 PCR 检测。1~30: 克隆编号; M: 2000 bp DNA marker。 Fig. 4 PCR detection of cDNA library. 1–30: Clone No.; M: 2000 bp DNA marker.



图 5 酵母接合杂交后的培养。A: SD/-Leu/-Trp; B: SD/-Trp; C: SD/-Leu。 Fig. 5 Culture of yeast after mating. A: SD/-Leu/-Trp; B: SD/-Trp; C:SD/-Leu.

总数约为3.56×10⁷个,杂交率约为18%,满足酵母 双杂交文库筛选的杂交效率(2%)。将其余未稀释 的杂交转化菌经过两轮SD/-Ade/-His/-Leu/-Trp/ X-α-Gal培养基筛选(图6),共获得164个阳性克隆, 对这些克隆进行DNA测序分析,最终得到了47个 非重复的候选基因(克隆)序列,表明筛选得到了47 个可能与HDA705互作的蛋白。在这些蛋白中,包 括3个转录因子或者辅转录因子,RSS3、RHSF10 和含有WD40结构域的GAMYB-binding蛋白,6 个叶绿体蛋白,1个含有R3H结构域的蛋白,22 个具有催化作用的蛋白,10个含有一些特殊结构 域的蛋白以及5个未知蛋白(表1)。



图 6 酵母接合杂交后经两轮 SD/-Ade/-His/-Leu/-Trp/X-α-Gal 培养 基筛选

Fig. 6 Twice screen of mating yeast on SD/-Ade/-His/-Leu/-Trp/X- α -Gal plate

第23卷

表1酵母双杂交筛选到的水稻中编码与 HDA705 互作蛋白的基因

Table 1 Genes encoding HDA705 interactors screened by yeast two hybrid

Lond Control accession No. Protein accession No. Protein accession No. Protein accession No. October 20 A7533600 NM. 001068312 Similar to CAMVE-binding protein NP. 001061777.1 1 0x05g60116600 NM. 001068373 Single-stranded nucleic acid binding R3H domain containing NP. 001065178.2 25 0x06g6056000 NM. 00106989 Similar to Asstrate stranscription factor \$917, beat shock NP. 001065186.1 1 0x06g6056000 NM. 00107555 Similar to 308 ribosomal protein \$13, chloroplast precursor NP. 001051020.2 4 0x05g6056000 NM. 001068585 Piatosystem I reaction center suburit 11 NP. 001051950.1 9 0x05g6056000 NM. 001068585 Piatosystem I reaction center suburit 11 NP. 001055906.1 10 0x05g6056000 NM. 001068535 Hance previous NP. 001055906.1 20 0x05g6056000 NM. 001068535 Hance previous NP. 00105501.1 10 0x05g6056000 NM. 001068535 Hance previous NP. 001045203.1 1 0x05g6056000 NM. 001069358 Similar to Acta Acta Acta Acta Acta Acta Acta Acta	基因	基因序列登录号	功能描述	蛋白质序列登录号	克隆数 Number
AB. (3:80) AD (3) 5800 Role and setting of the end of	Gene	Gene accession No.	Function description	Protein accession No.	of clone
Outgr@ndc200 NM_00106312 Similar to GRAM 1=bandaring protein NP_00105533 25 0x66g011000 NM_001060273 Single-tranded nucleis acid binding R3H domain containing NP_001055338.2 25 0x66g015000 NM_001060293 Single-tranded nucleis acid binding R3H domain containing NP_001055338.2 25 0x66g0170000 NM_001069395 Sininlar to 305 ribosomal protein S13, chloroplast precursor NP_001051300.1 9 0x66g055000 NM_00106365 Pintososystem 1 reaction center subunit V1 NP_001056301.1 1 0x66g056000 NM_001066407 Rieske iron salfur protein family protein NP_001056301.1 1 0x66g050700 NM_001066407 Rieske iron salfur protein family protein NP_001058231.1 1 0x66g017700 NM_001069358 Similar to Photosystem 1 reaction center subunit V1 NP_001048323.1 1 0x66g017700 NM_001069358 Similar to Photosystem 1 reaction center subunit V1 NP_001048323.1 1 0x66g017700 NM_001069358 Similar to Relatingplatement centors NP_001048323.1 1 0x66g007700 NM_001069358 Similar to Relatingplat	AB753860	AB/55860	Rice sait sensitive 3, regulates root cell elongation	BAM02800.1	2
Outsgon Find NM_00106.075 Simplestrance nucleic acid minuting Sist annum community NP_00103538.2 25 0.009/0455000 NM_00106989 Similar to heat stoss transcription factor Spl7, heat shock NP_00103364.1 1 0.003/00000 NM_001057555 Similar to 305 ribosomal protein S13, chloroplast precursor NP_00105100.2 4 0.003/00000 NM_001063839 Floatistic - specific 305 ribosomal protein I NP_00105630.1 10 0.003/0016600 NM_001066497 Riskek iron salfar protein family protein NP_00105630.1 10 0.003/0016600 NM_001066497 Riskek iron salfar protein family protein NP_00106530.1 10 0.003/0016600 NM_001066497 Riskek iron salfar protein family protein NP_00106251.1 35 0.003/0016600 NM_001066497 Riske iron salfar protein family protein NP_00104323.1 1 0.003/001600 NM_00106121 Cytosolic 6-phosphogluconate dehydrogenase NP_001045361.2 1 0.003/0017600 NM_001007178 Similar to NLobros bipshophate carboxylas etroase NP_001045431.1 1 0.0119/0070700 NM_00107218 Similar to	Os08g0408200	NM_001068312	Similar to GAM YB-binding protein	NP_001061///.1	1
0.0090455000 NP_00106399 Similar to heat stress transcription factor Spl7, heat shock NP_00105364.1 1 0.033g0704000 NM_001057555 Similar to 305 ribosomal protein S13, chloroptast precursor NP_001051020.2 4 0.033g0055500 NM_001063485 Similar to a 305 ribosomal protein S13, chloroptast precursor NP_001050630.1 10 0.005g005000 NM_001063485 Finder Stellin Control Stellin Ste	<i>Os</i> 05g0411600	NM_001062073	single-stranded nucleic acid binding K3H domain containing protein	NP_001055538.2	25
0483g0704000 NM_001057555 Similar to 306 rbbsomal protein S13, chloroplast precursor NP_00105102.2 4 0473g0856550 NM_001058485 Similar to plastid-specific 308 rbbsomal protein 1 NP_0010550.1 10 0473g0856500 NM_00106305 Plastocyami, chloroplast precursor NP_00105503.1 10 0473g055200 NM_00106305 Plastocyami, chloroplast precursor NP_00105503.1 10 0473g055200 NM_001069046 Similar to Photosystem I reaction center subunit II NP_00104520.1 1 0403g0507000 NM_001069176 Similar to 3-ketoacy-IcoA thiolase (fragmenn) NP_00104523.1 1 0404g0578600 NM_001061176 Evric reductase-like transmembrane component family protein NP_00105641.2 1 041g0577000 NM_00107017 Similar to ND1-dependent cylincras/dehydratuse family protein NP_001065852.1 1 041g0577000 NM_00105212 Similar to ND1-dependent cylincras/dehydratuse family protein NP_001065851.1 1 041g0507000 NM_00105647 Flavonol synthaseffavance 3-hydroxylase AL58118.1 1 041g0607000 NM_00105550 Leiner-rich r	Os09g0456800	NM_001069899	Similar to heat stress transcription factor Spl7, heat shock factor RHSF10	NP_001063364.1	1
0.6326055600 NM_0010518485 Similar to plastid-specific 30S ribosonal protein 1 NP_001051950,1 1 0.6326056000 NM_00106239 Photosystem 1 raccion center subuni VI NP_001056304,1 10 0.66960101000 NM_001066907 Rieske iron suffur protein family protein NP_00105904,1 20 0.6082656700 NM_001069046 Similar to Photosystem 1 reaction center subunit II NP_00104280,1 1 0.60826817000 NM_0010550S Similar to 3-ketosy-1CA chiloske (fingment) NP_00104823,1 1 0.608268177000 NM_0010550S Similar to 3-ketosy-1CA chiloske (fingment) NP_00104823,1 1 0.608268177000 NM_00105218 Cytosolic 6-phosphogluconate dehydrogenase NP_00104857,1 1 0.6180707000 NM_00107508 Similar to Ribulses bisphosphate carboxylase activase (FC 6.3.4.) (fingment) NP_00104587,1 1 0.6180707000 NM_001052142 Similar to ADDI -dependent hydroxypruvate reductase NP_00104352,1 1 0.6180707000 NM_001052145 Similar to ADDI -dependent hydroxypruvate reductase NP_00104352,1 1 0.61807060000000 NM_001052145	Os03g0704000	NM_001057555	Similar to 30S ribosomal protein S13, chloroplast precursor (CS13) – (CS13),	NP_001051020.2	4
Ou58g056000 NM_001062339 Photosystem I reaction center subunit VI NP_00106503.1 1 0.007g0556200 NM_001066497 Rieske ion sulfur protein family protein NP_001095962.1 20 0.007g0556200 NM_001064937 Rieske ion sulfur protein family protein NP_00104280.1 1 0.007g057600 NM_001069358 Similar to Photosystem I reaction center subunit II NP_00104280.1 1 0.005g011500 NM_001061558 Similar to Stetoseyl-CoA thiolase (fragment) NP_00106541.2 1 0.005g011500 NM_00106176 Ferric reductase-like transmerhrane component family protein NP_00106541.2 1 0.011g007000 NM_00107307 Similar to Ribulose bisphosphate carboxylase activase NP_001065451.2 1 0.011g007000 NM_001051214 Similar to NADI14ependent hydroxypruvate reductase family protein NP_001045589.1 1 0.011g007000 NM_001051214 Similar to Paulo synthase/flavanone 3-hydroxylase AAL58118.1 1 0.011g007000 NM_001051214 Similar to Paulo synthase/flavanone 3-hydroxylase AP_00104552.2 1 0.011g0005000 NM_001051214 S	Os03g0856500	NM_001058485	Similar to plastid- specific 30S ribosomal protein 1	NP_001051950.1	9
0:06g/010600 NM_001063065 Plastocyanin, chloroplast precursor NP_00105550.1 10 0:06/g/0555200 NM_001060404 Similar to Photosystem I reaction center subunit II NP_00102511.1 35 0:06/g/050700 NM_001060356 Similar to Photosystem I reaction center subunit II NP_00104250.1 1 0:06/g/01700 NM_001063121 Cytosolic 6-phosphogluconate dehydrogenase NP_001056556.1 1 0:06/g/01700 NM_001060176 Ferric reductase-like transmembrane component family protein NP_001056552.1 1 0:01/g/057000 NM_00107019 NAD-dependent epimerase/dehydratase family protein NP_001065455.2 1 0:11/g/07/000 NM_001075087 Similar to Ribulose bisphosphate earboxylase activase NP_001065455.2 1 0:01/g/07/000 NM_00105212 Similar to NAD1-dependent hydroxylase AAL58118.1 1 0:02/g/01/200 NM_00105212 Similar to NAD1-dependent hydroxylase NP_001043559.1 1 0:02/g/02/200 NM_00105237 Similar to AD1-dependent hydroxylase NP_00104359.1 1 0:02/g/02/200 NM_001015235 Similar to NAD1-dependent hydrox	Os05g0560000	NM_001062839	Photosystem I reaction center subunit VI	NP_001056304.1	1
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0x88g6569900 NM_001069046 Similar to Photosystem I reaction center subunit II NP_00104251.1 35 0x81g62947700 NM_001043335 Haem peroxidase, plant/fungal/bacterial family protein NP_001043823.1 1 0x80g6817700 NM_00105058 Similar to 3-ketoacyl-CoA thiolase (fragment) NP_00106586.1 1 0x80g676600 NM_001060121 Cytosolic 6-phosphogluconate dehydrogenase NP_001065487.1 1 0x81g6756900 NM_001072019 NAD-dependent epimerase/dehydratase family protein NP_001065487.1 1 0x81g6756900 NM_001072019 NAD-dependent epimerase/dehydratase family protein NP_001065487.1 1 0x81g6766900 NM_001072019 NAD-dependent hydroxyphase activase NP_001045548.1 1 0x81g6717000 NM_00105214 Similar to ADH-dependent hydroxypyruvate reductase NP_001045508.1 1 0x81g6701500 NM_001054501 Elavonol synthase/Ela domain containing protein NP_001043929.2 1 0x81g6701500 NM_001054501 Elavonol synthase/Ela domain containing protein NP_001044641.1 4 0x82g6736700 NM_001052979 Lipsolytic enzy	Os07g0556200	NM_001066497	Rieske iron sulfur protein family protein	NP_001059962.1	20
0x01gd294700 NM_001043335 Haem peroxidase, plant/fungal/bacterial family protein NP_001042800.1 1 0x02g0817700 NM_00105508 Similar to 3-ketoacyL-CoA thiolase (fragment) NP_001056586.1 1 0x04g07700 NM_001001612 Cytosic is c-hopsphogluconate dehydrogenase NP_001056386.1 1 0x04g0757000 NM_0010710 Ferric reductase-like transmembrane component family protein NP_00105451.2 1 0x11g0707000 NM_00107507 Similar to Ribulose bisphosphate carboxylase activase NP_001068555.2 1 0x11g0707000 NM_00105214 Similar to NADH-dependent hydroxylase AAL58118.1 1 0x02g0101500 NM_00105214 Similar to ADH-dependent hydroxylase AAL58118.1 2 0x01g0600000 NM_00105014 Poten kinase-like domain containing protein NP_001043292.2 1 0x01g07073200 NM_0010537 Similar to β-glucanase NP_00104052.1 1 0x00gd525000 NM_001054510 Lipolytic enzyme, G-D-S-L family protein NP_001046441.4 4 0x00gd525000 NM_00105433 Sparatic protein aseryzasin 1 precursor NP_00106371.1<	Os08g0560900	NM_001069046	Similar to Photosystem I reaction center subunit II	NP_001062511.1	35
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Ox02g0101500 NM_001052124 Similar to NADH-dependent hydroxypyruvate reductase (EC 1.1.1.29) (fragment) NP_001045589.1 1 Ox01g0600800 NM_001050464 Protein kinase-like domain containing protein NP_001043929.2 1 Ox01g0600800 NM_001185939 Leucine-rich repeat, plant specific containing protein NP_001172668.1 2 Ox01g0713200 NM_001050877 Similar to β-glucanase NP_001044052.1 1 Ox02g0250400 NM_00105450 Peptidase A1, pepsin family protein NP_001046444.1 4 Ox02g030500 NM_001064321 Lipolytic enzyme, G-D-S-L family protein NP_001050175.1 1 Ox05g0567100 NM_001063283 Aspartic proteinase oryzasin 1 precursor NP_00105348.1 1 Ox05g0533900 NM_001070306 Similar to CEL5=CELLULASE 5 NP_00106478.1 1 Ox05g067300 NM_001061339 Transferase family protein NP_001048041.1 1 Ox05g067300 NM_00107131 Similar to Transferase family protein NP_00106478.1 1 Ox05g067300 NM_00107136 Similar to Transferase family protein NP_001049201.1 1 <td>OSJNBb0060105.4</td> <td>AC092697</td> <td>Flavonol synthase/flavanone 3-hydroxylase</td> <td>AAL58118.1</td> <td>1</td>	OSJNBb0060105.4	AC092697	Flavonol synthase/flavanone 3-hydroxylase	AAL58118.1	1
Os01g0690800NM_001050464Protein kinase-like domain containing proteinNP_001043929.21Os02g0232500NM_001185939Leucine-rich repeat, plant specific containing proteinNP_001172868.12Ox601g0713200NM_001050587Similar to β-glucanaseNP_001044052.11Os02g0250400NM_001052979Lipase, GDSL domain containing proteinNP_001046444.14Os02g0730700NM_001054550Peptidase A1, pepsin family proteinNP_001048015.11Os06g0531900NM_001064321Lipolytic enzyme, G-D-S-L family proteinNP_001050175.11Os05g0567100NM_001062883Aspartic proteinase oryzasin 1 precursorNP_001050175.11Os05g0533900NM_001070306Similar to CEL5=CELLULASE 5NP_00105484.11Os03g0186100NM_00101213Similar to Starch synthase IINP_00105478.11Os03g0186100NM_00107136Similar to ADR11 protein (fragment)NP_00105478.11Os06g0532800NM_00107131Similar to ADR11 protein (fragment)NP_001058178.11Os06g0652800NM_001071866Similar to Tfm5 proteinNP_001058178.11Os06g06552800NM_001053707Protein of unknown function DUF789 family proteinNP_00105487.11Os02g05656700NM_001059435Protein of unknown function DUF597 family proteinNP_00105290.12Os02g0565700NM_001059435Protein of unknown function DUF597 family proteinNP_00105457.11Os02g0567700NM_001059660XYPPX repeat containing prot	Os02g0101500	NM_001052124	Similar to NADH-dependent hydroxypyruvate reductase (EC 1.1.1.29) (fragment)	NP_001045589.1	1
Os02g0232500 NM_001185939 Leucine-rich repeat, plant specific containing protein NP_001172868.1 2 Os01g0713200 NM_01050587 Similar to β-glucanase NP_001044052.1 1 Os02g0250400 NM_001052979 Lipase, GDSL domain containing protein NP_001046444.1 4 Os02g0730700 NM_001054550 Peptidase A1, pepsin family protein NP_001048015.1 1 Os06g0531900 NM_001064321 Lipolytic enzyme, G-D-S-L family protein NP_0010507786.1 1 Os05g0567100 NM_001062883 Aspartic proteinase oryzasin 1 precursor NP_00105371.1 1 Os05g0567100 NM_001062883 Aspartic proteinase oryzasin 1 precursor NP_001063771.1 1 Os05g0179300 NM_001061339 Transferase family protein NP_00106478.1 1 Os05g0186100 NM_00105776 Similar to Starch synthase II NP_00104201.1 1 Os06g0643500 NM_001071866 Similar to Tfm5 protein NP_0010658178.1 1 Os10g0552800 NM_001071866 Similar to Tfm5 protein NP_001042847.1 1 Os10g05574700 <	Os01g0690800	NM_001050464	Protein kinase-like domain containing protein	NP_001043929.2	1
Os01g0713200 NM_001050587 Similar to β-glucanase NP_001044052.1 1 Os02g0250400 NM_001052979 Lipase, GDSL domain containing protein NP_001046444.1 4 Os02g0730700 NM_00105550 Peptidase A1, pepsin family protein NP_001048015.1 1 Os06g0531900 NM_001064321 Lipolytic enzyme, G-D-S-L family protein NP_001050775.1 1 Os05g0567100 NM_00106833 Aspartic proteinase oryzasin 1 precursor NP_001056348.1 1 Os05g057300 NM_001061339 Transferase family protein NP_001054804.1 1 Os05g0179300 NM_001061339 Transferase family protein NP_00105478.1 1 Os05g0179300 NM_001061339 Transferase family protein NP_00105478.1 1 Os05g0179300 NM_001061339 Transferase family protein NP_00105478.1 1 Os05g0179300 NM_001061713 Similar to Transferase family protein NP_00105478.1 1 Os05g0643500 NM_001064713 Similar to Trins protein (fragment) NP_00105834.1 1 Os010g0552800 NM_001019382.1	Os02g0232500	NM_001185939	Leucine-rich repeat, plant specific containing protein	NP_001172868.1	2
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Os02g0730700NM_001054550Peptidase A1, pepsin family proteinNP_001048015.11Os06g0531900NM_001064321Lipolytic enzyme, G-D-S-L family proteinNP_001057786.11Os03g0365800NM_001056710Lipolytic enzyme, G-D-S-L family proteinNP_001050175.11Os05g0567100NM_001062883Aspartic proteinase oryzasin 1 precursorNP_001056348.11Os05g0533900NM_001070306Similar to CEL5=CELLULASE 5NP_001063771.11Os05g0179300NM_001061339Transferase family proteinNP_001054804.11Os05g0437600NM_001071213Similar to Starch synthase IINP_001064678.11Os05g0643500NM_001064713Similar to Uroporphyrinogen III synthaseNP_001049201.11Os06g0643500NM_001064713Similar to Tfm5 proteinNP_00105334.11Os02g0566500NM_0010191866Similar to Tfm5 proteinNP_001042847.11Os02g0566500NM_001053707Protein of unknown function DUF789 family proteinNP_001042847.11Os02g0566500NM_001059435Protein of unknown function DUF597 family proteinNP_0010505290.12Os03g0431100NM_001050960XYPPX repeat containing proteinNP_00105476.12Os03g0431100NM_001060982Similar to B22EL8 proteinNP_00105447.11Os05g0346100NM_001061794Phox-like domain containing proteinNP_001055259.12Os05g0346100NM_001061784Frigida-like family proteinNP_00105003.11 <td>Os02g0250400</td> <td>NM_001052979</td> <td>Lipase, GDSL domain containing protein</td> <td>NP_001046444.1</td> <td>4</td>	Os02g0250400	NM_001052979	Lipase, GDSL domain containing protein	NP_001046444.1	4
Oso6g0531900NM_001064321Lipolytic enzyme, G-D-S-L family proteinNP_001057786.11Oso3g0365800NM_001056710Lipolytic enzyme, G-D-S-L family proteinNP_001050175.11Oso5g0567100NM_001062883Aspartic proteinase oryzasin 1 precursorNP_001056348.11Oso9g0533900NM_001070306Similar to CEL5=CELLULASE 5NP_001063771.11Oso5g0179300NM_001061339Transferase family proteinNP_001054804.11Os05g037600NM_001071213Similar to Starch synthase IINP_001064678.11Os06g0643500NM_001055736Similar to Uroporphyrinogen III synthaseNP_00105178.11Os06g0643500NM_001064713Similar to ADR11 protein (fragment)NP_00105334.11Os02g0566500NM_001071866Similar to Tfm5 proteinNP_001042847.11Os02g0566500NM_001053707Protein of unknown function DUF789 family proteinNP_001052900.12Os04g0445200NM_001072008Protein of unknown function DUF597 family proteinNP_00105476.12Os03g0431100NM_001059600XYPPX repeat containing proteinNP_001050425.17Os05g0346100NM_00106982Similar to B22EL8 proteinNP_00105447.11Os05g0346100NM_001065388Frigida-like family proteinNP_001055259.12Os05g0346100NM_001065538Frigida-like family proteinNP_00105903.11	Os02g0730700	NM_001054550	Peptidase A1, pepsin family protein	NP_001048015.1	1
Os03g0365800NM_001056710Lipolytic enzyme, G-D-S-L family proteinNP_001050175.11Os05g0567100NM_001062883Aspartic proteinase oryzasin 1 precursorNP_001056348.11Os05g0533900NM_001070306Similar to CEL5=CELLULASE 5NP_001063771.11Os05g0179300NM_001061339Transferase family proteinNP_001054804.11Os10g0437600NM_001071213Similar to Starch synthase IINP_001064678.11Os05g06643500NM_00105576Similar to Uroporphyrinogen III synthaseNP_001058178.11Os06g0643500NM_001064713Similar to ADR11 protein (fragment)NP_001058178.11Os02g0552800NM_001071866Similar to Tfm5 proteinNP_001065334.11Os02g0566500NM_001053707Protein of unknown function DUF789 family proteinNP_001047172.11Os04g0445200NM_001059455Protein of unknown function DUF861, cupin_3 domain containing proteinNP_001052900.12Os03g0431100NM_001056960XYPPX repeat containing proteinNP_00105476.12Os05g03411300NM_001060982Similar to B22EL8 proteinNP_00105447.11Os05g0346100NM_001061794Phox-like domain containing proteinNP_001055259.12Os05g0346100NM_001065538Frigida-like family proteinNP_00105903.11	Os06g0531900	NM_001064321	Lipolytic enzyme, G-D-S-L family protein	NP_001057786.1	1
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Os03g0186100 NM_001055736 Similar to Uroporphyrinogen III synthase NP_001049201.1 1 Os06g0643500 NM_001064713 Similar to ADR11 protein (fragment) NP_001058178.1 1 Os10g0552800 NM_001071866 Similar to Tfm5 protein NP_001065334.1 1 Os01g0306900 NM_001049382.1 Protein of unknown function DUF789 family protein NP_001042847.1 1 Os02g0566500 NM_001053707 Protein of unknown function DUF1644 family protein NP_001052900.1 2 Os04g0445200 NM_001059435 Protein of unknown function DUF861, cupin_3 domain containing protein NP_001065476.1 2 Os03g0431100 NM_001056960 XYPPX repeat containing protein NP_00105447.1 1 Os05g0111300 NM_001060982 Similar to B22EL8 protein NP_001054447.1 1 Os05g0346100 NM_001061794 Phox-like domain containing protein NP_001055259.1 2 Os07g0173200 NM_001065538 Frigida-like family protein NP_00105903.1 1	Os10g0437600	NM_001071213	Similar to Starch synthase II	NP_001064678.1	1
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Os01g0306900 NM_001049382.1 Protein of unknown function DUF789 family protein NP_001042847.1 1 Os02g0566500 NM_001053707 Protein of unknown function DUF1644 family protein NP_001047172.1 1 Os04g0445200 NM_001059435 Protein of unknown function DUF861, cupin_3 domain containing protein NP_001052900.1 2 Os10g0574700 NM_001072008 Protein of unknown function DUF597 family protein NP_001065476.1 2 Os03g0431100 NM_001056960 XYPPX repeat containing protein NP_001050425.1 7 Os05g0111300 NM_001060982 Similar to B22EL8 protein NP_001055259.1 2 Os07g0173200 NM_001065538 Frigida-like family protein NP_001059003.1 1	Os10g0552800	NM_001071866	Similar to Tfm5 protein	NP_001065334.1	1
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Os04g0445200 NM_001059435 Protein of unknown function DUF861, cupin_3 domain containing protein NP_001052900.1 2 Os10g0574700 NM_001072008 Protein of unknown function DUF597 family protein NP_001065476.1 2 Os03g0431100 NM_001056960 XYPPX repeat containing protein NP_001050425.1 7 Os05g0111300 NM_001060982 Similar to B22EL8 protein NP_0010554447.1 1 Os05g0346100 NM_001061794 Phox-like domain containing protein NP_001055259.1 2 Os07g0173200 NM_001065538 Frigida-like family protein NP_001059003.1 1	Os02g0566500	NM_001053707	Protein of unknown function DUF1644 family protein	NP_001047172.1	1
Os10g0574700 NM_001072008 Protein of unknown function DUF597 family protein NP_001065476.1 2 Os03g0431100 NM_001056960 XYPPX repeat containing protein NP_001050425.1 7 Os05g0111300 NM_001060982 Similar to B22EL8 protein NP_001054447.1 1 Os05g0346100 NM_001061794 Phox-like domain containing protein NP_001055259.1 2 Os07g0173200 NM_001065538 Frigida-like family protein NP_001059003.1 1	Os04g0445200	NM_001059435	Protein of unknown function DUF861, cupin_3 domain	NP_001052900.1	2
Os03g0431100 NM_001056960 XYPPX repeat containing protein NP_001050425.1 7 Os05g0111300 NM_001060982 Similar to B22EL8 protein NP_001054447.1 1 Os05g0346100 NM_001061794 Phox-like domain containing protein NP_001055259.1 2 Os07g0173200 NM_001065538 Frigida-like family protein NP_001059003.1 1	Os10g0574700	NM 001072008	Protein of unknown function DUF597 family protein	NP 001065476.1	2
Os05g0111300 NM_001060982 Similar to B22EL8 protein NP_001054447.1 1 Os05g0346100 NM_001061794 Phox-like domain containing protein NP_001055259.1 2 Os07g0173200 NM_001065538 Frigida-like family protein NP_001059003.1 1	Os03g0431100	– NM 001056960	XYPPX repeat containing protein	NP_001050425.1	7
Os05g0346100 NM_001061794 Phox-like domain containing protein NP_001055259.1 2 Os07g0173200 NM_001065538 Frigida-like family protein NP 001059003.1 1	Os05g0111300	– NM 001060982	Similar to B22EL8 protein	_ NP 001054447.1	1
Os07g0173200 NM_001065538 Frigida-like family protein NP_001059003.1 1	Os05g0346100	NM 001061794	Phox-like domain containing protein	NP 001055259.1	2
	Os07g0173200	 NM_001065538	Frigida-like family protein		1

续表(Continued)

基因	基因序列登录号	功能描述	蛋白质序列登录号	克隆数 Number
Gene	Gene accession No.	Function description	Protein accession No.	of clone
Os09g0115500	NM_001069119	CBS domain containing protein	NP_001062584.1	1
Os03g0200800	NM_001055827	ADP-ribosylation factor family protein	NP_001049292.1	1
Os06g0602200	NM_001187958	Hypothetical protein	NP_001174887.1	1
Os03g0213500	NM_001055898	Hypothetical protein	NP_001049363.1	1
Os04g0679900	NM_001060818	Conserved hypothetical protein	NP_001054283.1	1
Os07g0189700	NM_001065619	Conserved hypothetical protein	NP_001059084.1	2
Os06g0280900	NM_001187784	Conserved hypothetical protein	NP_001174713.1	1

3 讨论

本研究中,采用酵母接合型双杂交系统筛选与 组蛋白去乙酰化酶 HDA705 互作的蛋白,结果酵母 接合效率约为 18%,远远高于实验所要求的最低值 2%,也显著高于国内报道的酵母双杂交最高接合 效率(约 12%)^[22],说明通过一定的条件优化,酵母双 杂交的接合效率还有一定的提升空间。较高的接 合率将扩大候选互作蛋白的覆盖度,使低表达的互 作蛋白也能被有效的筛选出来。

本研究中,利用酵母接合型双杂交系统成功筛 选出 47 个可能与 HDA705 在胞内存在互作的蛋白 质。这47个候选蛋白中,有3个在逆境响应或激 素信号转导过程中起到重要作用的转录因子或者 辅转录因子 RSS3、RHSF10 和1个含有 WD40 结 构域的 GAMYB-binding 蛋白。已有研究表明,水 稻 RSS3 蛋白作为负调控者抑制逆境条件下 JA 响 应基因的表达^[23], HDA705可能通过与RSS3蛋 白互作,改变 JA 响应基因的组蛋白乙酰化水平从 而抑制其表达。RHSF10属于HsfB1家族蛋白, 该家族蛋白具有辅助转录激活或者转录抑制的功 能。在番茄(Lycopersicon peruvianum)中, RHSF10 的同源蛋白 LpHsfB1 可以通过 C 末端的 Histone-Like Motif 和一些 CREB 接合蛋白互作调节下游基 因的表达^[24]。在大豆(Glycine max)中, RHSF10的 同源蛋白 GmHsfB1 通过 C 端调节区域的抑制结 构域(RD)和 TFIIB 结合域与一些 GTFs 互作从而 发挥转录抑制作用^[25]。在拟南芥中, RHSF10的 同源蛋白 AtHsfB1/B2b 与 HsfA 家族的热激因子互 作从而调节热休克反应的关闭[26],并参与抑制一些 热诱导的热激因子和热激蛋白基因的表达^[27]。我 们的研究表明, HDA705 能与 RHSF10 进行互作, 揭示 HDA705 可能参与水稻热诱导基因的表达调 控过程。本实验筛选到的水稻 GAMYB-binding 蛋 白又称 SRWD4 蛋白,属于 SRWD (Salt responsive WD40 protein)亚家族成员。水稻中共有 5 个此类 蛋白(SRWD1~SRWD5),它们的表达都受到盐胁迫 的诱导,其中 SRWD4 蛋白在花药和胚乳中有较高 的表达^[28]。本研究中鉴定 HDA705 能与 SRWD4 蛋白互作,揭示 HDA705 可能通过与 SRWD4 互作 调控下游基因的表达,进而影响水稻对盐胁迫的响 应过程。

近期的研究结果表明, 拟南芥中与光合作用 相关的蛋白包括叶绿素结合蛋白 LHCB、光系统 II 亚基和 Rubisco 大小亚基等能够发生乙酰化修饰 的变化^[29-30]。本研究中, 筛选出与 HDA705 互作 的 6 个参与光合作用的叶绿体蛋白, 表明在水稻 中 HDA705 可能参与这些叶绿体蛋白的乙酰化修 饰及光合作用的调控途径。此外, 在筛选到的与 HDA705 互作的蛋白中, 有 22 个具有催化作用的 蛋白。这些结果暗示 HDA705 除了拥有组蛋白的 去乙酰化效应外, 还可能参与细胞内的非组蛋白的 去乙酰化作用。

综上所述,本研究利用酵母双杂交系统筛选出 47个可能与HDA705在胞内相互作用的蛋白质, 其中有3个在逆境响应或激素信号转导过程中起 到重要作用的(辅)转录因子、6个参与光合作用的 叶绿体蛋白、1个含有R3H结构域的蛋白、22个 细胞内的各种酶类、10个含有一些特殊结构域的 蛋白以及5个未知功能蛋白。这些互作蛋白的鉴 定为进一步研究和了解HDA705参与调控水稻生 长发育和逆境响应的分子机制提供了重要的信息。

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