

甘蓝型油菜甘油三磷酸脱氢酶(*BnaGPDH*)基因的克隆与表达

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摘要: 甘油三磷酸脱氢酶(GPDH)是三酰甘油(TAG)合成的关键酶。为了阐明甘蓝型油菜(*Brassica napus*) *GPDH* (*BnaGPDH*)的表达特性, 本研究采用反转录PCR (RT-PCR)方法, 获得*BnaGPDH*基因的3条全长编码序列(CDS), 分别命名为*BnaGPDH1*、*BnaGPDH2*、*BnaGPDH3*, 长度分别为1 395、1 395、1 389 bp。生物信息学分析表明, *BnaGPDHs*具有GPDH基因家族的特征, *BnaGPDHs*与其他植物GPDHs有高度同源性。时空表达分析表明, *BnaGPDHs*是组成型表达基因, 在花中的表达量最高, 叶中的表达量最低。 *BnaGPDHs*在幼嫩种子发育的第5周表达量普遍提高, 在果荚发育到第5周表达量明显降低。逆境分析表明, *BnaGPDHs*响应多种逆境胁迫, 可能与植物抗逆机制有关。

关键词: *GPDH*; 甘蓝型油菜; 基因克隆; 表达分析

甘油三磷酸脱氢酶(glycerol-3-phosphate dehydrogenase, GPDH)是磷酸二羟丙酮(dihydroxyacetone phosphate, DHAP)还原生成3-磷酸甘油(glycerol-3-phosphate, G3P)的催化酶。在植物油脂合成过程中, 以G3P和酰基辅酶A (acyl-coenzyme A, acyl-CoA)为底物在甘油-3-磷酸酰基转移酶(*sn*-glycerol-3-phosphate acyltransferase, GPAT)、溶血磷脂酰基转移酶(lysophospholipid acyltransferase, LPAT)、磷脂酸磷酸酶(phosphatidate phosphatase, PAP)、二酰甘油酰基转移酶(diacylglycerol acyltransferase, DGAT)等一系列酶作用下生成三酰甘油(triacylglycerol, TAG)是主要的合成途径(Kennedy 1961)。为研究上述催化酶对TAG合成的影响, 研究者的焦点集中在与TAG合成过程中间产物直接相关的酶GPAT (Gidda等2009; Shockey等2016; Yang等2012; Chen等2011)、LPAT (Zou等1997; Kim和Huang 2004; Ghosh等2009; Maisonneuve等2010; 威维聪2008; 陈四龙等2012)、PAP (Park等2015)、DGAT (Lai等2012; Li等2010; Banilas等2011; McFie等2010)上, 而对应重要底物G3P合成的催化酶GPDH的研究相对较少。

Gibon等(2002)以及Vigeolas和Geigenberger (2004)分别报道了G3P在拟南芥(*Arabidopsis thaliana*)和油菜(*Brassica napus*)中的表达量不能满足TAG合成过程中的需求。随后, Vigeolas和Geigenberger (2004)给油菜供给适当浓度的甘油后发现, 油菜G3P和TAG的合成量都增加了, 同时在油菜中异位超表达酵母*GPDH*也能提高油菜G3P的表达量, 促进种子中TAG的合成量增加40%。此外, Yao等(2014)通过超表达三角褐指藻(*Phaeodactylum tricornutum*)

细胞中*GPDH*基因发现细胞的中性脂含量增加了1.9倍。由此我们可以推测, TAG的合成与在一定浓度范围内的底物G3P的浓度存在正相关关系。因此, GPDH与TAG合成可能有非常重要的关系。

近年来, 对于*GPDH*基因对非生物逆境方面的响应已有一些研究。有研究显示, 适当浓度的渗透压能够诱导莱茵衣藻(*Chlamydomonas reinhardtii*)中*GPDH*基因的表达, GPDH催化合成的甘油作为一个相容性溶质应对细胞外的渗透压(Herrera-Valencia等2012; Hadi等2008; Mishra等2008)。He等(2009)对杜氏盐藻(*Dunaliella viridis*)耐盐胁迫的研究认为, 位于叶绿体中的GPDH编码基因可能参与盐胁迫逆境的响应。秀丽隐杆线虫(*Caenorhabditis elegans*) (Anderson等2016; Possik等2015; Burton等2017; Kaneshiro和Strome 2017)对抗高渗透压逆境时也能促进*GPDH*的表达。Barat等(2012)研究表明, 在5°C的低温环境下, 理氏裂腹鱼(*Schizothorax richardsonii*)细胞中*GPDH*的表达量增加了19倍, 由此我们猜测, 在植物中*GPDH*是否也会受低温冷胁迫逆境的调控? 有趣的是, Lai等(2015)研究发现, 35°C高温逆境也能诱导坛紫菜(*Pyropia haitanensis*)中*GPDH*的表达, 同时坛紫菜中G3P浓度增加, 而糖苷含量减少。

在甘蓝型油菜中, GPDH酶定位于质体、线粒

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体和细胞溶质,且GPDH在核基因组中分别存在多个拷贝。Nandi等(2004)通过构建拟南芥质体GPDH缺失突变体后发现, TAG的合成没有发生明显的变化,我们推测TAG的合成可能与细胞溶质中GPDH有关,而位于细胞溶质中的GPDH与TAG合成的关系还并不清楚。本文根据甘蓝型油菜定位于细胞溶质中GPDH的基因序列设计引物,从甘蓝型油菜‘湘油15’中克隆了3个GPDH拷贝,并进行了生物信息学分析及时空表达和逆境表达分析,为进一步分析GPDH功能提供参考。

材料与方法

1 试验材料

试验材料为国家油料改良中心湖南分中心基地开花期的甘蓝型油菜(*Brassica napus* L.) ‘湘油15’,用于提取总RNA。大肠杆菌[*Escherichia Coli* (Migula) Castellani et Chalmers]菌种DH5 α 由本实验室保存,克隆载体采用Promega公司PGEM-TEasy Vector。植物总RNA提取试剂盒TransZol UP、First-Strand cDNA Synthesis SuperMix、TaqTM-T DNA Polymerase、T4 DNA Ligase、DNA Marker、Tip Green qPCR SuperMix试剂盒由北京全式金公司提供;DNA胶回收试剂盒、质粒DNA小量提取试剂盒购自上海生工生物工程有限公司;引物由擎科生物技术有限公司合成;基因测序由上海生工生物科技有限公司完成。

2 总RNA提取和cDNA合成

以‘湘油15’油菜根、茎、叶、花、人工授粉后第1~5周的种子和果荚皮及非生物逆境胁迫下的叶为试验材料,采用TransZol UP试剂盒提取油菜总RNA。检测RNA浓度和质量后,采用反转录

试剂盒合成第一链cDNA,用于目标基因扩增和表达分析。

3 BnaGPDHs基因cDNA克隆

利用甘蓝型油菜BRAD数据库(<http://brassicadb.org/brad/>),对甘蓝型油菜和拟南芥位于细胞溶质GPDH的编码基因进行共线性分析,发现GPDH存在3个不同的拷贝,位于A05、C04、A03染色体上,分别为GSBRNA2T00132785001、GSBRNA2T000-24258001、GSBRNA2T00058395001,依次简化名称为GPDH1、GPDH2、GPDH3。根据目标基因和内参基因actin序列信息,用Primer Premier 5设计了7对不同的引物(表1),用于GPDH基因扩增和实时荧光定量反转录PCR (quantitative real-time reverse transcription PCR, qRT-PCR)。引物由擎科生物技术有限公司合成。

以合成的第一链cDNA为模板和相应的引物扩增GPDH基因。20 μ L PCR反应体系包括: cDNA模板1 μ L、10 mmol·L⁻¹ dNTP 0.5 μ L、5 U· μ L⁻¹ HiFi高保真DNA聚合酶0.5 μ L、10 μ mol·L⁻¹上下游引物(表1)各1 μ L、10 \times Buffer 2 μ L、双蒸水(double-distilled water, ddH₂O) 14 μ L。PCR条件为: 94 $^{\circ}$ C 3 min预变性; 94 $^{\circ}$ C 45 s, 59 $^{\circ}$ C 45 s, 72 $^{\circ}$ C 90 s, 35个循环; 72 $^{\circ}$ C延伸10 min。随后将PCR反应产物在1%凝胶中电泳检测,回收目标条带,与pGEM-TEasy Vector连接。分别吸取3 μ L连接产物转化到50 μ L DH5 α 大肠杆菌中,利用蓝白斑筛选阳性克隆,菌落PCR检测后送生工测序,每个基因测3个转化子。

4 GPDH基因生物信息学分析

对BnaGPDHs和GPDHs基因间序列同源性进行分析,作测序结果的初步鉴定;利用Primer Premier

表1 PCR引物与参数

Table 1 PCR primers and parameters

引物名称	上游引物(5'→3')	下游引物(5'→3')	退火温度/ $^{\circ}$ C	循环数	用途
GPDH1	GCTGTGGAAATAAAGAAAATG	ATTATATTGGAGCCATGACAT	59	35	qRT-PCR
GPDH2	GCTGTGGAAATAAAGAAAATG	TGAGTAATAGCTGACGAATCA	59	35	qRT-PCR
GPDH3	CGTTGCCTTTGATTTGTGTTG	TAGAAGTTCGGATTATGAGCC	59	35	qRT-PCR
BnaGPDH1	CTCATCACAAGAGAACAATCA	ATTATATTGGAGCCATGACAT	58	45	qPCR
BnaGPDH2	TAAGGGATGAAACACTGAATG	TAATAGCTGACGAATCAAGGC	62	45	qPCR
BnaGPDH3	AGTTAGACATTGCTCGTTGCC	TGAACAGACCCGTTTGAAGAGT	62	45	qPCR
actin	GGTTGGGATGGACCAGAAGG	TCAGGAGCAATACGGAGC	58	45	qPCR

5将核苷酸序列翻译成氨基酸序列,运用ExPASy ProtParam tool (<http://web.expasy.org/protparam/>)分析BnaGPDHs理化信息;利用在线软件GOR IV Secondary Structure Prediction Method (二级结构预测法, https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html)进行蛋白二级结构预测分析;在NCBI (<http://www.ncbi.nlm.nih.gov/cdd>)分析GPDH的保守功能域;搜索NCBI数据库查找同源物种GPDH氨基酸序列,用MEGA 6构建系统发育树。

5 时空表达分析

按照TransStart Tip Green qPCR SuperMix试剂盒中的方法,利用Bro-Rad公司实时荧光定量PCR仪分析BnaGPDH基因时空表达水平,内参基因为甘蓝型油菜‘湘油15’ *actin*基因,20 μL 实时荧光定量PCR (qPCR)反应体系包括:2 \times TransStart Tip Green qPCR SuperMix 10 μL 、cDNA模板1 μL 、10 $\mu\text{mol}\cdot\text{L}^{-1}$ 上下游引物(表1)各0.4 μL 、ddH₂O 8.2 μL 。反应程序为:94 $^{\circ}\text{C}$ 30 s预变性,45个循环(变性94 $^{\circ}\text{C}$ 5 s,退火15 s,72 $^{\circ}\text{C}$ 10 s),从60 $^{\circ}\text{C}$ 升温到90 $^{\circ}\text{C}$ 的熔解过程;引物、退火温度、循环次数信息如表1所示。运用CT法($2^{-\Delta\Delta C_t}$)分析GPDH基因相对于内参基因的表达变化趋势,并用GraphPad Prism 5作图分析。每个样品进行3个独立的生物学重复和3次技术重复,取3次重复试验的平均值。

6 非生物逆境胁迫表达分析

选取长势一致、发育良好、4~5片真叶的‘湘油15’植株分别进行盐(300 $\text{mmol}\cdot\text{L}^{-1}$ NaCl)、干旱[20%聚乙二醇4000 (polyethylene glycol-4000, PEG-4000)]、激素[50 $\mu\text{mol}\cdot\text{L}^{-1}$ 脱落酸(abscisic acid, ABA);

50 $\mu\text{mol}\cdot\text{L}^{-1}$ 6-苄基氨基嘌呤(6-benzylaminopurine, 6BA)]、水渍(清水淹没油菜根部)、低温(5 $^{\circ}\text{C}$)、高温(35 $^{\circ}\text{C}$)胁迫处理,参考Kim等(2010)、Chen等(2012)和Gong等(2005)的处理方法。在胁迫处理0、3、6、12和24 h后剪取200 mg油菜叶片,液氮速冻后-80 $^{\circ}\text{C}$ 保存,用于提取RNA,合成第一链cDNA,以上述cDNA为模板进行实时荧光定量PCR分析。PCR条件、数据处理方法同第5节所述。

实验结果

1 BnaGPDHs基因全长cDNA克隆及生物信息学分析

克隆获得的BnaGPDHs序列与BRAD数据库中GPDHs基因序列几乎一致,基因序列聚类结果如图1所示,表明克隆的拷贝在进化上具有高度的同源性。利用ExPASy ProtParam tool分析BnaGPDHs基因理化性质,如表2所示。利用在线软件GOR IV Secondary Structure Prediction Method分析GPDH蛋白二级结构,结果表明GPDH蛋白二级结构中只存在 α -螺旋、延伸链和无规则卷曲这三种结构,所占百分比如表3所示。利用NCBI分析BnaGPDHs保守功能域,结果显示三个蛋白都含有GpsA、NADB_Rossmann superfamily和NAD_Gly3P_dh_C superfamily这三个保守功能域(图2)。

为分析BnaGPDHs与其他高等植物GPDHs在进化上的关系,通过NCBI数据库BlastP,选取拟南芥(BAH20028.1)、天蓝遏蓝菜(*Noccaea caerulea*; JAU14322.1)等12种高等植物GPDH氨基酸序列,运用MEGA软件构建系统进化树(图3)。同源性聚类分析表明,BnaGPDHs与其他植物GPDHs进化上具有高度的同源性。其中,与拟南芥、天蓝遏蓝

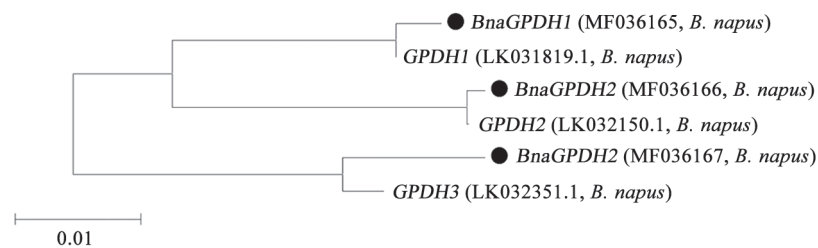


图1 GPDHs基因间相似性聚类图

Fig.1 Phylogenetic tree of identity among GPDHs

GPDH1、GPDH2、GPDH3来自BRAD数据库,分别定位于A05、C04、A03染色体;●标记的BnaGPDH1、BnaGPDH2、BnaGPDH3表示从甘蓝型油菜‘湘油15’中克隆的GPDH基因序列;MF036165、MF036166、MF036167、LK031819.1、LK032150.1和LK032351.1分别为基因GenBank登记号;图中比例尺代表相对遗传距离。

表2 甘蓝型油菜GPDHs基因编码蛋白理化性质分析结果

Table 2 Analysis of physicochemical property of proteins encoded by GPDHs in *B. napus*

理化性质	分析结果		
	BnaGPDH1	BnaGPDH2	BnaGPDH3
氨基酸数目/个	464	464	462
分子量/kDa	51.631 7	51.829 7	51.584 4
等电点(pI)	7.14	6.81	7.12
负电荷残基(Asp+Glu)/个	53	54	54
正电荷残基(Arg+Lys)/个	53	53	54
脂肪系数	100.50	100.06	100.28

脂肪系数: 由脂肪族侧链占据蛋白的相对体积(丙氨酸、缬氨酸、异亮氨酸、亮氨酸)。它被认为是提高球状蛋白热稳定性的一个积极因素。

表3 甘蓝型油菜GPDHs基因编码蛋白二级结构分析结果

Table 3 Analysis of secondary structure of proteins encoded by GPDHs in *B. napus*

二级结构	比例/%		
	BnaGPDH1	BnaGPDH2	BnaGPDH3
α -螺旋	41.81	41.38	42.64
延伸链	14.87	15.95	14.72
无规则卷曲	43.32	42.67	42.64

菜的GPDH同源性约为95%; 三种植物同为双子叶十字花科, 亲缘关系最近, 进化上属于同一个分支, 表明这三种植物有共同的进化起源和类似的催化功能。而BnaGPDHs与木豆(*Cajanus cajan*; XP_020207239.1)、菜豆(*Phaseolus vulgaris*; XP_007162159.1)、蒺藜苜蓿(*Medicago truncatula*; XP_003625105.2) GPDHs同源性较远, 与其他植物如川桑(*Morus notabilis*; XP_010090209.1)、芝麻(*Sesamum indicum*; XP_011075353.1)、大叶藻(*Zostera marina*;

KMZ-61791.1)、蓖麻(*Ricinus communis*; EEF-49622.1)、麻风树(*Jatropha curcas*; XP_012086218.1)、土瓶草(*Cephalotus follicularis*; GAV83468.1)、桃(*Prunus persica*; XP_007202031.1)的GPDHs亲缘关系最远, 属于不同的进化分支。

2 甘蓝型油菜BnaGPDHs的时空表达模式

用实时荧光定量PCR检测BnaGPDHs基因在根、茎、叶、花和1~5周的种子和角果中的表达情况, 结果如图4所示。BnaGPDHs基因在各个组织中均有表达, 其中3个基因在花中的表达量都是最高的, 远远高于同时期根、茎、叶中的表达量。其次, BnaGPDH1和BnaGPDH3在根、茎中有一定的表达量, BnaGPDH2在根中也有少量的表达, 3个基因在叶中的表达量均是最低的。

BnaGPDH1和BnaGPDH2在胚中表达量的变化趋势不规律, 均在第5周表达量达到了最大值。BnaGPDH3在胚中第3周的表达量明显高于其他时期。

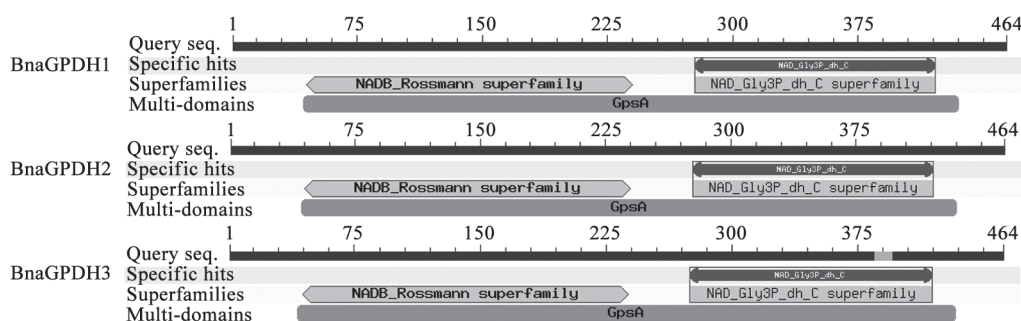


图2 甘蓝型油菜GPDHs蛋白保守结构域

Fig.2 Conserved domain prediction of GPDHs in *B. napus*

Query seq.: 查询序列; Specific hits: 特异位点; Superfamilies: 超家族; Multi-domains: 域; NADB_Rossmann superfamily: NADB_Rossmann超家族; NAD_Gly3P_dh_C superfamily: NAD_Gly3P_dh_C超家族; GpsA: GpsA域。

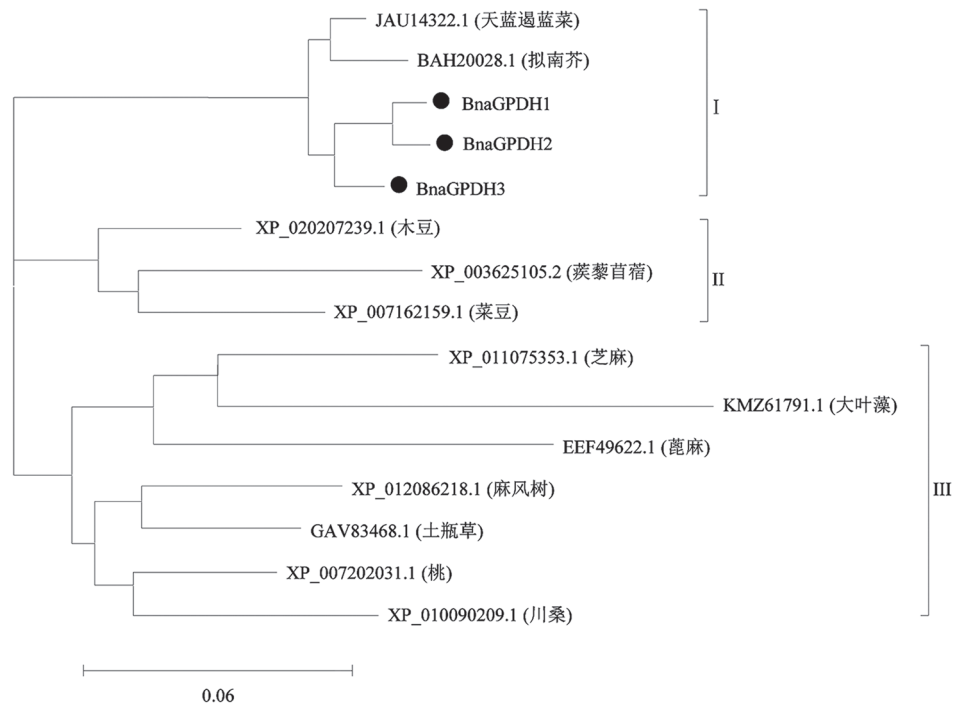


图3 不同植物GPDHs蛋白的进化树分析

Fig.3 Phylogenetic analysis of GPDHs in different plant species

●标记的BnaGPDH1、BnaGPDH2、BnaGPDH3表示从甘蓝型油菜‘湘油15’中克隆的GPDH氨基酸序列; 图中比例尺代表相对遗传距离; I、II、III表示不同植物GPDHs按进化上的亲缘关系大致分为3大类。

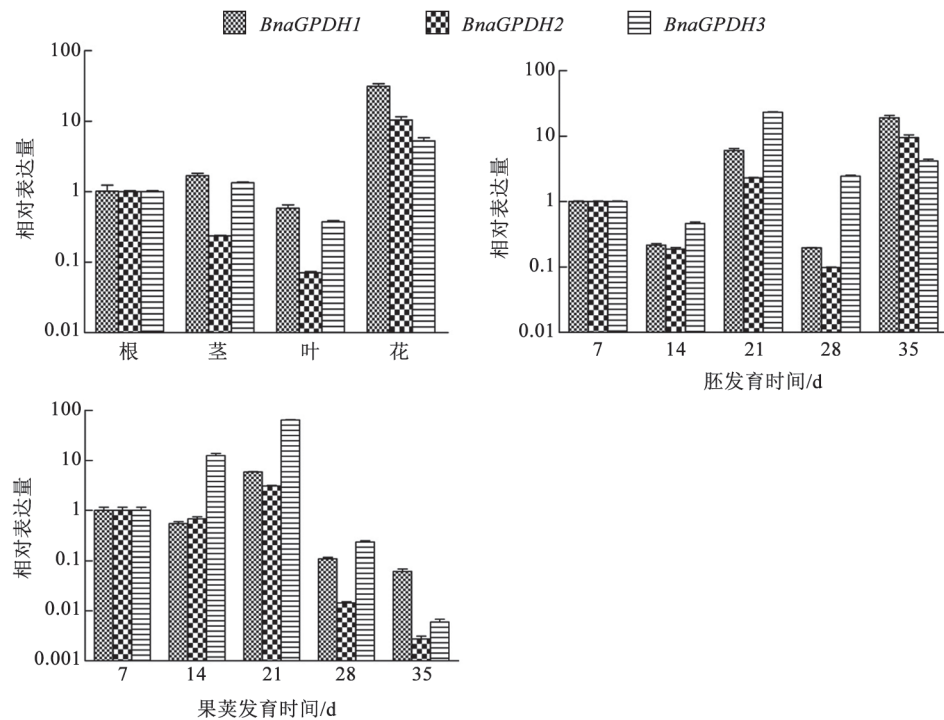


图4 qPCR分析甘蓝型油菜BnaGPDHs基因的时空表达

Fig.4 Spatio-temporal expression of BnaGPDHs in B. napus by qPCR

*BnaGPDH1*和*BnaGPDH2*在果荚中表达量的变化是先降低后升高再降低,第3周表达量最高,到第4、5周的表达量已经非常低了。*BnaGPDH3*在果荚中表达量则是先升高再降低,同*BnaGPDH1*和*BnaGPDH2*一样,在第5周表达量最低。

3 甘蓝型油菜*BnaGPDHs*在逆境中的表达模式

实时荧光定量PCR分析逆境胁迫处理后3个基因表达模式,结果如图5所示。在NaCl、6BA、PEG-4000、ABA、水渍、低温(5°C)、高温(35°C)处理下,3个基因的表达量均有明显变化,说明它们对这七种逆境胁迫都有响应。

在NaCl、PEG-4000、ABA、水渍逆境处理过程中,*BnaGPDH1*呈现出先降低后升高再降低的表达模式。在低温处理下,*BnaGPDH1*表现非常敏感,表达量先快速上升后再下降到对照组水平。而在高温处理下,*BnaGPDH1*表达量一直低于对照组水平。

在NaCl和ABA逆境处理下,*BnaGPDH2*表达量始终低于对照组。而在水渍、PEG-4000的处理下则表现出先降低后升高再降低的表达模式,且表达量最大值高于对照组水平。在高温和低温的处理下,*BnaGPDH2*和*BnaGPDH1*的表达模式是相似的。

在NaCl和6BA处理下,*BnaGPDH3*表达量随时间增加逐渐下降,低于对照组水平。在PEG-4000和ABA处理下,呈现出先下降后上升的表达模式。在水渍逆境处理下,表达量先上升后下降,总体来说仍然高于对照组水平。

讨 论

本研究从甘蓝型油菜‘湘油15’中克隆得到了*BnaGPDHs*的3个拷贝,*BnaGPDHs*编码的蛋白定位于细胞溶质中。经生物信息学分析显示,*BnaGPDH1*、*BnaGPDH2*、*BnaGPDH3*分别编码464、464、462 aa的蛋白,3个蛋白的脂肪系数都偏高,这可能与其参与TAG合成有关。它们都有NADB_Rossmann超家族、GpsA和NAD_Gly3P_dh_C超家族保守功能域,以Rossmann折叠的方式与NADH结合,具有GPDH结构特征,这与Shen等(2010)研究分析的结果是一致的,可见*BnaGPDHs*依赖辅助因子NADH的协同作用。

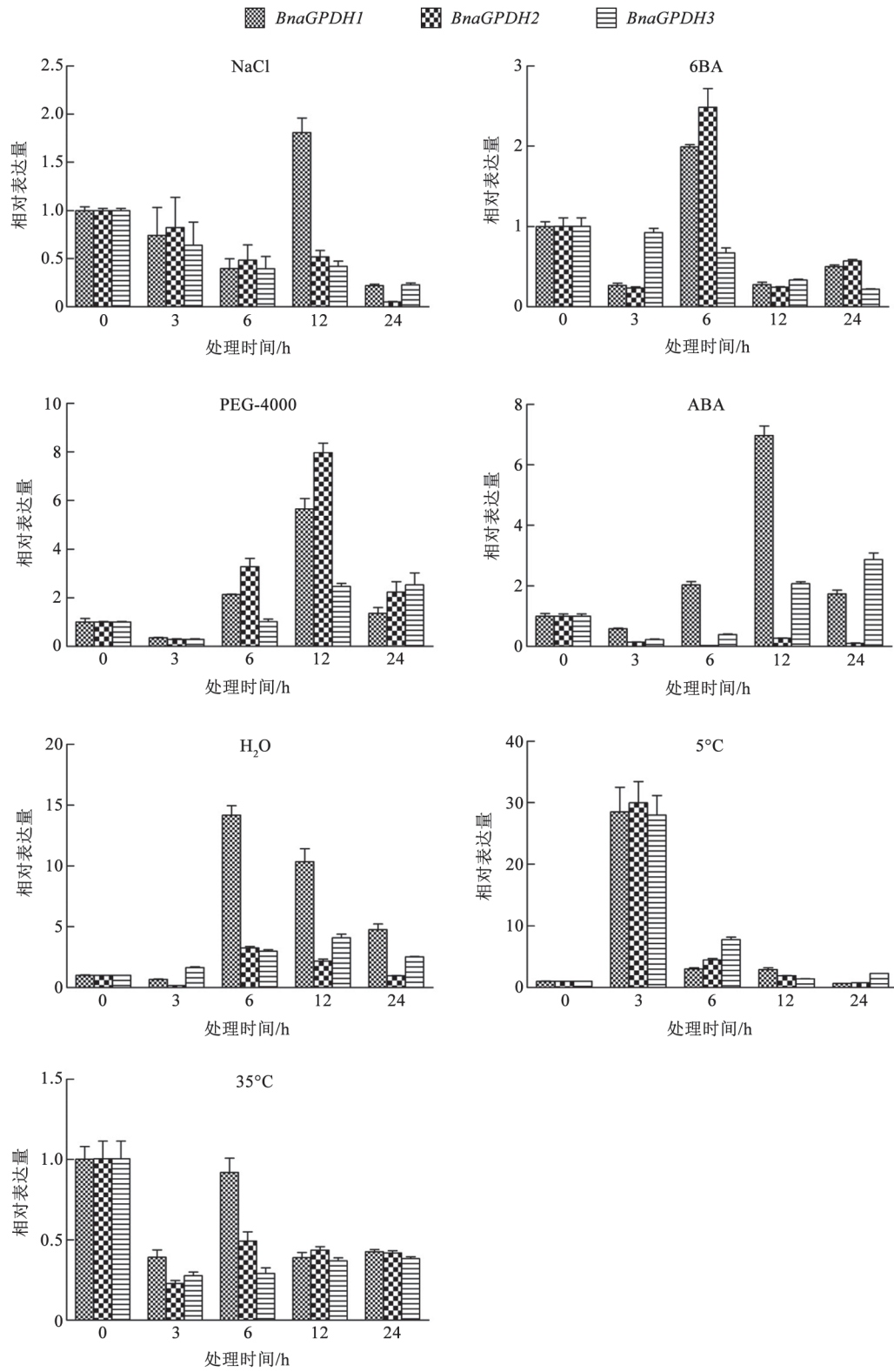
将*BnaGPDHs*氨基酸序列与其他植物GPDHs聚类分析,显示*BnaGPDHs*与同为双子叶十字花科的天蓝遏蓝菜、拟南芥亲缘关系最近,同源性高

达95%,属于进化树的同一分支,同其他高等植物也具有较高的同源性,说明GPDH蛋白在进化上具有高度的保守性。

时空表达分析结果显示,*BnaGPDHs*在油菜植株的各个组织中均有表达,说明*BnaGPDHs*属于组成型表达基因。在根、茎、叶、花中,*BnaGPDHs*有类似的表达谱,它们在花中的表达量明显高于其他组织中,叶中的表达量最低,有一定的组织特异性;*BnaGPDH1*、*BnaGPDH2*均在油脂积累的高峰期第5周达到最大值,说明这两个基因可能与TAG合成密切相关;*BnaGPDHs*在角果发育第3周的时候表达量最高,其中*BnaGPDH3*的表达量明显高于另外两个基因,由此推测*BnaGPDH3*可能与角果的发育有关。

*BnaGPDHs*在甘蓝型油菜植株中响应非生物逆境胁迫的结果表明,在与渗透压相关的逆境NaCl处理下,*BnaGPDH1*的表达量先降低后升高再降低,这可能是由于前期的逆境对植株有一定的伤害,抑制了表达,中后期植株细胞内通过调节甘油的合成量来适应植株外渗透压的变化(刘冠楠2011);而*BnaGPDH2*和*BnaGPDH3*表达量一直低于对照组,受到严重抑制,说明植株受渗透胁迫作用时,主要通过调节*BnaGPDH1*的表达来缓解不利环境对自身的伤害。在PEG-4000和水渍处理下,*BnaGPDHs*表达量在中后期高于对照组。有意思的是,在生长素类似物6BA和ABA的处理下,6BA抑制*BnaGPDH1*和*BnaGPDH3*的表达,ABA却促进*BnaGPDH1*和*BnaGPDH3*的表达,表现出相反的结果;*BnaGPDH2*的表达受生长素类似物的抑制。5°C低温环境下,*BnaGPDHs*的表达非常敏感,表达量都有不同程度的增加,这和Barat等(2012)对理氏裂腹鱼适应低温环境的研究有类似的结论,可能是通过促进*BnaGPDHs*的表达,从而增加甘油的合成来避免植株受到低温伤害的。35°C高温中,*BnaGPDHs*的表达受到抑制,与低温环境中的表现相反。

本研究明确了甘蓝型油菜中位于细胞溶质中GPDH的编码基因逆境响应的表达模式,但它们在植株抗逆机制中发挥的作用还存在许多疑问。如:(1)植株细胞中G3P浓度是否发生变化?(2)GPDH蛋白的活性变化情况。(3)GPDH表达量的变化受到哪些转录因子调控?(4)TAG的含量是否发生变化?更多的问题需要在以后的研究中进一步解答。

图5 不同非生物胁迫下甘蓝型油菜*BnaGPDHs*基因的表达模式Fig.5 Expression pattern of *BnaGPDHs* in *B. napus* under various abiotic stresses

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Molecular cloning and expression profile of *BnaGPDH* gene in *Brassica napus*

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Abstract: Glycerol-3-phosphate dehydrogenase (GPDH) is a key enzyme in triacylglycerol (TAG) biosynthesis. In order to elucidate the expression feature of *BnaGPDHs* in *Brassica napus*, three *GPDH* full-length coding sequences (1 395, 1 395 and 1 389 bp) were obtained by reverse transcription PCR, and designated as *BnaGPDH1*, *BnaGPDH2* and *BnaGPDH3*, respectively. Bioinformatic analysis shows that they have the characteristics of the *GPDH* family, and amino acid sequences of *BnaGPDHs* are highly homologous to *GPDHs* in other plants. Spatio-temporal expression results show that *BnaGPDHs* are genes of constitutive expression, with the highest expression in flower and the lowest expression in leaf. *BnaGPDH* expression levels were generally increased in developing seed at 35 days after flowering, at the same time they were significantly decreased in developing capsule. *BnaGPDHs* responded to abiotic stresses, indicating they might be concerned with the mechanism of plant stress resistance.

Key words: *GPDH*; *Brassica napus*; gene cloning; expression analysis

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