高等植物醇脱氢酶及其基因家族研究进展

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摘要: 醇脱氢酶属于高等植物中普遍存在的一个锌结合脱氢/还原蛋白超家族, 根据作用底物不同, 将高等植物中的醇脱氢酶分为3个家族: 乙醇脱氢酶(alcohol dehydrogenase, ADH)、肉桂醇脱氢酶(cinnamyl alcohol dehydrogenase, CAD)、甲醛脱氢酶(formaldehyde dehydrogenase, FDH)。3个家族均不同程度地响应植物逆境胁迫, 不仅受低氧胁迫等逆境的诱导, 也受ABA等激素的调控。CAD催化木质素合成, 参与构建植物防御体系。ADH在植物香气物质合成中发挥作用, 受乙烯等激素调控, 选择性地进行短的直链醇和醛之间的相互转化, 催化香气物质前体的合成。本文综述了醇脱氢酶家族在高等植物中对逆境的响应、木质素和香气物质合成方面的研究概况, 以期为醇脱氢酶的深入研究提供参考。

关键词: 乙醇脱氢酶; 肉桂醇脱氢酶; 甲醛脱氢酶; 逆境胁迫; 香气

Advances in Alcohol Dehydrogenase Enzymes and Their Gene Families in Higher Plants

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Abstract: Alcohol dehydrogenases belong to a Zn-binding dehydrogenase/reductase protein superfamily commonly existing in higher plants, and which are classified into three families according to their substrate specialties: alcohol dehydrogenase (ADH), cinnamyl alcohol dehydrogenase (CAD), and formaldehyde dehydrogenase (FDH). All the three family members act differently in response to stresses, and they are not only response to the stresses such as oxygen deficiency, but also regulated by hormones such as ABA. CADs family members catalyze the synthesis of lignin and take part in the formation of defense. In addition, ADHs also act in the synthesis of aroma volatiles in which pathway they are regulated by hormones such as ethylene and inter-convert ethanol and acetaldehyde (and other short linear alcohol/aldehyde pairs) selectively to form the precursors of aroma volatiles. This paper reviews the research status of alcohol dehydrogenase superfamily in higher plants response to stresses, lignin and aroma volatiles synthesis, proposing to provide a reference for alcohol dehydrogenase further study.

Key words: alcohol dehydrogenase; cinnamyl alcohol dehydrogenase; formaldehyde dehydrogenase; stresses; aroma volatiles

醇脱氢酶是广泛存在于所有真核生物和某些原核生物中的一类酶, 该家族有短链(shortchain dehydrogenase/reductase, SDR)和中链(medium-length dehydrogenase/reductase, MDR)之分。多数醇脱氢酶隶属于MDR超家族(Riveros-Rosas等2003; Persson等2008; Jörnvall等2010), Persson等(2008)将MDR超家族分成8个家族, 但根据Chase(1999)在高等植物中的研究, 依据不同成员作用底物的特点, 将醇脱氢酶分成三类, 分别是: (1)乙醇脱氢酶(alcohol dehydrogenase, ADH, EC 1.1.1.1)亚家族, 主要依赖NAD, 催化短的直链醛和醇之间的相互转化; (2)肉桂醇脱氢酶(cinnamyl alcohol dehydrogenase, CAD, EC 1.1.1.195)亚家族, 依赖NADP, 主要在木质素生物合成中起作用; 和(3)甲醛脱氢酶(formaldehyde dehydrogenase, FDH, EC 1.2.1.1)亚家族, 依赖NAD, 催化还原甲醛(formaldehyde,

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FA)和*S*-羟甲基谷胱甘肽(*S*-hydroxymethyl glutathione, HMGSH), 维持体内一氧化氮(NO)和*S*-亚硝基硫醇(*S*-nitrosothiols, SNOs)平衡(Chase 1999)。最近的研究证明, FDH催化HMGSH的活性高于催化甲醛的活性, 所以很多研究者将FDH改称为*S*-亚硝基谷胱甘肽还原酶(*S*-nitrosoglutathione reductase, GSNOR, EC 1.1.1.284) (Kubienova等2013), 本文仍称呼为FDH亚家族。

自1960年在玉米中分离并鉴定出第一个醇脱氢酶后(Hageman和Flesher 1960), 相继在众多植物中均有发现, 且该类酶在植物生长发育、逆境胁迫(干旱、低温、淹水和机械损伤等)响应、木质素合成及香气物质合成中均起作用(Manriquez等2006; Moummou等2012)。因此, 近年来关于醇脱氢酶的研究日益受到国内外学者的关注, 本文主要从植物逆境响应、木质素合成和香气物质合成3个方面综述了醇脱氢酶及其基因家族的研究进展。

1 醇脱氢酶在逆境条件下的作用

植物的生命周期中经常会遇到一些胁迫, 如水涝、干旱、冷害、高盐和机械损伤等, 阻碍其生长发育, 但是经过数百万年的进化, 植物形成了一系列的防御机制来抵御各种生物和非生物胁迫, 醇脱氢酶家族就是进化出的众多抗逆机制之一(Drew 1997)。ADH亚家族成员在防御生物和非生物胁迫中起作用, 并且还是很多激素作用途径中的参与者(Zeevaart和Creelman 1988; Peña-Cortès等1991; Strommer和Garabagi 2009)。CAD亚家族成员又分为CAD I和CAD II。CAD I催化很多种苯醛衍生物, 即催化苯环类香气物质的合成, CAD II成员主要在防御机制和木质素生物合成中起作用(Goffner等1998; Umezawa 2010)。FDH的氨基酸序列非常保守, 拟南芥(*Arabidopsis thaliana*)、水稻(*Oryza sativa*)和豌豆(*Pisum sativum*)体内FDH的氨基酸序列相似性高达95% (Shafqat等1996)。该亚家族在植物的很多生理过程中均发挥重要作用: 调节信号传导(Rustérucci等2007)、调控生长发育(Lee等2008)、控制多种植物抗病模式(Feechan等2005)、调控细胞死亡(Chen等2009)、保护细胞免受硝酸化胁迫(Liu等2001; Sakamoto等2002; Lamotte等2005; Valderrama等2007)等, 此外, 还参与到各种生物和非生物胁迫的响应机制中。

1.1 醇脱氢酶在抗淹水胁迫中的作用

淹水胁迫下, 缺乏呼吸底物, 能量代谢降低, 并积累大量的有毒末端产物, 发生较强烈的过氧化损伤, 造成根部伤害甚至死亡。植物将代谢途径转变到乙醇发酵途径, 在丙酮酸脱氢酶复合体(pyruvate dehydrogenase complex, PDC)和ADH的作用下, 产生少量腺嘌呤核苷三磷酸(adenosine triphosphate, ATP), 使NAD(P)H再生成NAD(P), 来维持糖酵解, 产生乙醇这类无毒或更易扩散到胞外的物质(Drew 1997; Srommer 2011)。

对ADH酶活性的研究亦可证明ADH对植物抗涝的重要性。水涝胁迫下, 玉米(*Zea mays*)根部ADH对乙醛的亲和力增加, 对乙醇的亲和力下降, 其增减幅度均与品种耐涝性相反(刘晓忠等1991)。‘中农8号’和‘宝优1号’两个品种的黄瓜(*Cucumis sativus*)体内, ADH活性均受低氧的强烈诱导(马月花和郭世荣2005)。耐涝性较强的浙江乌桕(*Sapium sebiferum*)种源体内的ADH活性受水涝胁迫的诱导而显著增加, 且一直保持在较高水平, 而不耐涝的福建种源体内的ADH活性显著低于对照(蔡金峰等2013)。水稻的*rad*突变体中, ADH酶活性下降, 受低氧胁迫诱导而升高, 主要集中在根冠部位; 虽然野生型和突变体中*Adh* mRNA的表达量正常, 但是突变体中ADH蛋白含量却很低, 可能是mRNA未转录或转录产物不稳定, 致使突变体不耐长期水涝(Matsumura等1998)。对菘蓝(*Isatidis indigotica*) (唐晓清等2011)和薏苡(*Coix lacrymajobi*) (吴功庆2006)的研究也得到类似的结论。

大量研究表明, 淹水胁迫可诱导植物中*ADH* mRNA表达量的升高。玉米体内的*PDC*、*ZmAdh1*、*ZmAdh2*的转录本在低氧下均升高, 在随后的无氧状态下逐渐降低(Andrews等1993, 1994a, b); 但在未经低氧预处理, 而直接受无氧胁迫的植株根中, 这些转录本均迅速升高, 通常在6 h达到最高峰, 之后又迅速消失(Drew 1997)。大豆(*Glycine max*)根系遭遇无氧时, ADH是在根系中鉴定出的仅有的4种重要酶之一(Russell等1990), 并且*ADH1*的表达呈降低-升高-降低的趋势, 但在任何一个时期, 处理组的表达量均高于对照组(沙向红等2011)。在大豆(Komatsu等2011)、马铃薯(*Solanum tuberosum*) (Matton等1990)、水稻(Minhas和Grover 1999;

赵森等2008; 梁燕2013)、黄瓜(Qi等2012)和葡萄(*Vitis vinifera*) (Tesniere等2006)等作物中均有相似报道, 证明ADH受低氧诱导并在植物体内抗低氧过程中发挥作用。

对*ADH*进行沉默和超表达分析则更能说明其耐受低氧的机制, 拟南芥的*ADH*缺失突变体在低氧或无氧条件下发芽率明显降低(Conley等1999), 玉米的*ADH1*缺失突变体根系在无氧条件下更易死亡(Fan等1988; Saint-Ges等1991), 而*ADH*超表达的拟南芥则促进了根系的生长, 提高了根毛对低氧的耐受能力, 即增强了耐涝性(Shiao等2002)。湿地物种之所以能够长期耐受无氧状态, 就是因为其组织器官中包含有持续的、占主导地位的乙醇发酵途径(Bucher和Kuhlemeier 1993; Armstrong等1994), 这一特性已通过*PDC*和*ADH*缺失突变体的表型得到证实(Drew 1997; Baxter-Burrell等2002; Gibbs和Greenway 2003; Kursteiner等2003)。以上研究均表明, ADH受水涝胁迫诱导, 可一定程度上缓解胁迫伤害, 在植物的抗涝机制中起着非常重要的作用。

FA是低氧胁迫下产生的有毒物质之一, 如果细胞中有自由形式的还原型谷胱甘肽(glutatione, GSH), 它会立刻与FA结合形成HMGSH, 之后由FDH氧化形成*S*-甲酰基谷胱甘肽(*S*-formylglutathione), 从而解除FA的致毒作用。该途径中FDH既是限速酶, 又是清除FA的主要酶(Martinez等1996; Fliegmann和Sandermann 1997; Just等2011)。

1.2 ADH在抗干旱胁迫中的作用

干旱是由于土壤水分不足而对植物造成的水分胁迫。ADH很可能是植物在干旱胁迫下求得生存的策略之一。分析拟南芥*Adh*的启动子发现含有响应干旱胁迫的G-box-1序列, 突变该段序列可减弱*Adh*受干旱胁迫的诱导程度; 进一步研究发现, *Adh*启动子转基因植株在干旱胁迫下的GUS染色呈整株分布, 且颜色较深, 对照只在根部有较浅的GUS染色, 证明*Adh*启动子受干旱胁迫强烈诱导(Dolferus等1994)。干旱和盐胁迫均可通过调节miRNA来影响烟草(*Nicotiana tabacum*)植株体内*ADH*和抗坏血酸过氧化物酶(ascorbate peroxidase)基因*APX*的表达水平(Frazier等2011)。烟草芽和根中*ADH*转录水平受干旱胁迫诱导强烈, 植株可维持相对正常的形态, 而*ADH*的RNAi沉默植株的细胞存活率降低了25%, 表现为轻微萎蔫, 证明烟草植株中ADH是参与抗旱机制之一, 可有效提高细胞的生存能力(Senthil-Kumar等2010)。当苜蓿(*Medicago sativa*)受轻微(–1.3 MPa)和中等(–2 MPa)程度的水分胁迫时, 茎节处的ADH活性上升, 同时乙醇含量随之上升, 亦证明ADH在抵御干旱胁迫中起作用(Irigoyen等2000); 而菜豆(*Phaseolus vulgaris*)种子萌发过程中受短期(6 h)和长期(12 h)干旱胁迫时, ADH活性却轻微降低了, 作者猜测ADH可能不是菜豆体内抗旱的成员(Stephan和Laval-Martin 2000)。这种ADH的功能差异可能与物种有关, 因此ADH在抵御干旱胁迫中的作用机制仍有待于进一步的探究。

1.3 ADH在抗盐胁迫中的作用

高盐胁迫(亦称渗透胁迫)是又一种常见胁迫, 可显著影响大多数植物的生长, 并严重降低农作物产量(Munns和Tester 2008)。玉米幼苗遭受渗透胁迫时, 根系和嫩梢中ADH活性均上升, 且根中ADH的活性是嫩梢中的2倍(Noguchi 2000b), 说明ADH在根系中的作用更加显著, 通过加强根部代谢来抵御渗透胁迫。低浓度(100 mmol·L^{-1})盐胁迫下, 水稻幼苗体内*ADH3*的表达量低于对照, 而高浓度(300和400 mmol·L^{-1})盐胁迫下, *ADH3*的表达量呈升高-下降-升高-下降的规律, 两个峰值均高于对照, 最低值均低于对照, 说明水稻体内的*ADH3*对高盐胁迫更加敏感(梁燕等2012)。通过负调控拟南芥中*ADH*的基因表达, 发现*ADH*缺失的拟南芥突变体种子在遇到渗透、冷害或水涝等胁迫时, 其发芽率均降低(Conley等1999)。但目前为止, 关于ADH和盐胁迫之间关系的研究还很少, 而且机制仍不明确。

1.4 醇脱氢酶在抗冷害胁迫中的作用

低温胁迫也叫冷害胁迫, 当温度降到0~12 ℃之间时, 会使细胞膜脂过氧化, 甚至致使某些对低温敏感的植物细胞死亡, 即发生低温胁迫, 而乙醇可抑制植物细胞膜脂的分解, 增加膜脂流动性, 提高低温耐受能力(Jennings和Saltveit 1994; Saltveit 1994; Frenkel和Erez 1996; Saltveit等2004), 如前所述, ADH是乙醇生成途径中的关键酶, 因此有很多研究报道了ADH与冷害胁迫的关系。

拟南芥受4 ℃冷害胁迫时, 虽然*ADH* mRNA的表达量升高并积累到较高水平, 可是ADH的酶

活性改变不大, 因此认为ADH活性并不是拟南芥耐低温能力的必然要求(Jarillo等1993), 但低温胁迫(5、7.5和10 ℃)会显著提高水稻幼苗中ADH和PDC的活性, 增强乙醇发酵途径, 并且可快速诱导水稻和小麦(*Triticum aestivum*)体内*ADH*基因的表达(Russell等1990; Christie等1991; Noguchi和Yasuda 2007; 严建萍等2011)。水稻幼苗中乙醇浓度在低温胁迫下升高, 喷施4-甲基吡唑(4-methylpyrazole, ADH合成抑制剂)后ADH酶活性和乙醇浓度均降低, 水稻幼苗根系的生长受到抑制, 而该抑制作用可通过施用外源乙醇来解除, 足以说明乙醇对水稻抗寒作用明显, 即证明ADH对水稻抗寒非常重要(Noguchi 2008)。当玉米幼苗受低温胁迫时, 也发现ADH的活性增强, 而*Adh1*$^{-}$*Adh2*$^{-}$双缺失突变体中膜脂过氧化加剧, 膜损伤严重, 导致细胞严重受损(Peters和Frenkel 2004)。用乙醇溶液处理黄瓜幼苗可提高幼苗对低温的耐受能力(Saltveit 1994; Frenkel和Erez 1996), 类似地, 将黄瓜胚根培养在乙醇溶液中, 亦可提高胚根耐受低温的能力(Saltveit等2004)。以上研究充分证明ADH及乙醇在植物耐低温方面的重要性, 但其在耐低温中的具体机制尚不明确。

少量研究表明, CAD也在抗冷害胁迫中发挥作用。甘薯(*Ipomoea batatas*)的*IbCAD1*启动子含有冷害胁迫的响应元件, 经证实, CAD参与到受脱落酸(abscisic acid, ABA)调节的冷害胁迫响应中, 对甘薯抗寒起到积极作用(Kim等2010)。对芒草(*Miscanthus sinensis*)的研究中发现, 不抗寒品种较抗寒品种含有较高的纤维素和木质素含量, 但是受冷害胁迫时, 两个品种的芒草体内CAD活性均升高, 而抗寒品种体内CAD活性升高的更多(Domon等2013)。大麦(*Hordeum vulgare*)经冷害和冻害处理后, 通过转录组水平分析发现, 叶片中CAD活性和基因表达量均受诱导而升高, 促进了木质素单体的合成, 可能是木质素单体的聚合可降低活性氧的破坏作用, 因为木质素是细胞壁的重要成分, 木质素含量或成分所发生的任何改变均会使细胞壁的物理特性发生变化, 而这种变化可能使细胞避免受到胞内形成冰晶所带来的机械胁迫(Takahama和Oniki 1997; Janská等2011)。

目前关于FDH在抗冷害胁迫方面的报道还较少。冷害胁迫可诱导豌豆体内SNOs的含量升高, FDH的活性受诱导而剧烈升高(Corpas等2008)。低温和高温胁迫均可诱导黄瓜和甜瓜(*Cucumis melo*)体内FDH活性和蛋白量的升高, 且低温的诱导作用更加强烈(Kubienova等2013)。

1.5 醇脱氢酶对机械损伤及信号分子的响应

机械损伤是一种很普遍又很严重的胁迫, 会激发植物体内多种信号途径, 导致基因表达和蛋白合成发生剧烈变化(Carrera和Prat 1988; Dammann等1997)。在番茄体内至少存在4种不同的信号途径: ABA、乙烯(ethylene, ETH)、活性氧(reactive oxygen species, ROS)和电信号(Ryan 2000; Leon等2001; Gatehouse 2002; Sagi等2004)。莴苣(*Lactuca sativa*)和玉米受机械损伤12 h后, ABA含量分别升高4.9和4.7倍, ADH活性分别升高1.7和1.5倍(Noguchi 2001), 说明ABA可诱导ADH活性升高。拟南芥的ADH活性也受机械损伤的强烈诱导(Kursteiner等2003; Delessert等2004)。ADH是受ABA诱导的诸多抗逆因子之一(Guy 1990; Hetherington和Quatrano 1991; Hildmann等1992; Robertson等1994; Herde等1996)。1 μmol·L^{-1}的ABA可使莴苣体内ADH活性在6 h开始升高, 100 μmol·L^{-1}的ABA对ADH酶活性的诱导更强烈, 可达对照的3.2倍(Noguchi 2000a)。100 μmol·L^{-1}的ABA处理玉米根尖部位, 可使幼苗在水涝胁迫下的存活率由8%提高到87%, *ADH*的基因表达和活性均升高(Hwang和VanToai 1991)。正常的拟南芥植株体内, *Adh*基因受ABA、高盐、低温和干旱胁迫诱导, 而ABA缺失的拟南芥突变体即使受外源ABA或干旱的作用, *Adh* mRNA含量也降低(de Bruxelles等1996; Ishitani等1998)。Abe等(2003)指出, ABA先诱导*AtMYC2*和*AtMYB2*这两个基因的表达, 然后这两个基因分别编码合成AtMYC2和AtMYB2两个转录因子, 接着AtMYC2和AtMYB2分别结合到*AtADH*和*rd22*启动子的G-box-1和MBS-1元件上, 诱导*AtADH*和*rd22*的表达, 诱导关系如图1所示。

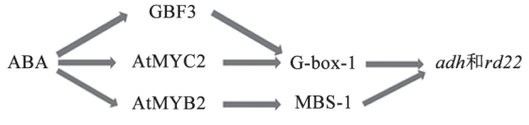

图1 ABA诱导*Adh*和*rd22*基因的表达

Fig.1 ABA induces the expression of *Adh* and *rd22*

参考Abe等(2003)文献, 有所修改。

CAD家族成员亦受机械损伤和激素的诱导。苜蓿中的*CAD*基因在损伤部位表达量增高(Brill等1999)。甘薯的*IbCAD1*能够参与到茉莉酸(jasmonate acid, JA)和水杨酸(salicylic acid, SA)调节的机械损伤响应中, 在甘薯幼苗受创伤胁迫时, 观测到根中*IbCAD1*基因转录本含量升高(Kim等2010), 可能通过增强根系的转导功能来保证上部的矿质营养和水分供应, 从而在根本上增强抗逆能力。茶树(*Camellia sinensis*)叶子中的*CsCAD1*和*CsCAD3*均受ABA的诱导, 而*CsCAD2*不受ABA诱导, 但同时, *CsCAD1*和*CsCAD2*均受JA和SA的诱导(Deng等2013), 表明不同*CAD*成员受不同的信号诱导。苔藓(*Plagiochasma appendiculatum*)中*PaCAD1*和*PaCAD2*的表达受JA的诱导(Sun等2013); 丹参(*Salvia miltiorrhiza*)的*SmCAD*表达水平受茉莉酸甲酯(methyl jasmonate, MeJA)的诱导和赤霉素(gibberellins, GA_3)的抑制(葛茜等2013)。

FDH是维持植物体内NO、SNOs和亚硝基谷胱甘肽(GSNO)平衡的关键酶。研究认为, SNOs、NO和ROS是植株抵御病菌侵染和扩张的重要分子基础(Mlickova等2004; Tomankova等2006; Chaki等2009; Piterkova等2009)。植物利用NO和SNOs作为信号分子来激活防御机制(Durner等1998; Diaz等2003), GSNO是沟通NO和SNOs的中间体, 根据环境条件, FDH不可逆地将GSNO还原为氧化型谷胱甘肽(glutathione oxidized, GSSG)、NH_3和羟胺等物质(严金平等2010), 如图2所示。

在拟南芥*AtFDH1*缺失突变体*gsnor1-3*体内检测不到*FDH* mRNA, FDH酶含量及活性均降低约50%, 但NO和SNOs含量均高于对照(Feechan等2005; Rustérucci等2007; Lee等2008), 尤其在根部的NO信号更加强烈, 耐受除草剂paraquat的能力提高, 增强了基础抗性和获得性系统抗性(systemic acquired resistance, SAR) (Chen等2009); 而*FDH*完全缺失和超表达的拟南芥植株却表现出更易感病的性状(Feechan等2005; Rustérucci等2007)。拟南芥体内FDH活性下降一半和完全缺失的突变体抗性差别如此之大, 可能是因为体内SA含量的差异, 在FDH活性下降50%的突变体内, SA含量未发生变化(Rustérucci等2007; Espunya等2012), 而在*FDH*完全缺失的突变体内, SA含量剧烈下降, 且对外源SA无响应(Feechan等2005; Yun等2011), 说明FDH的调控途径和SA的信号途径相互依赖。拟南芥和烟草体内的FDH活性受JA的下调, 而受SA的上调

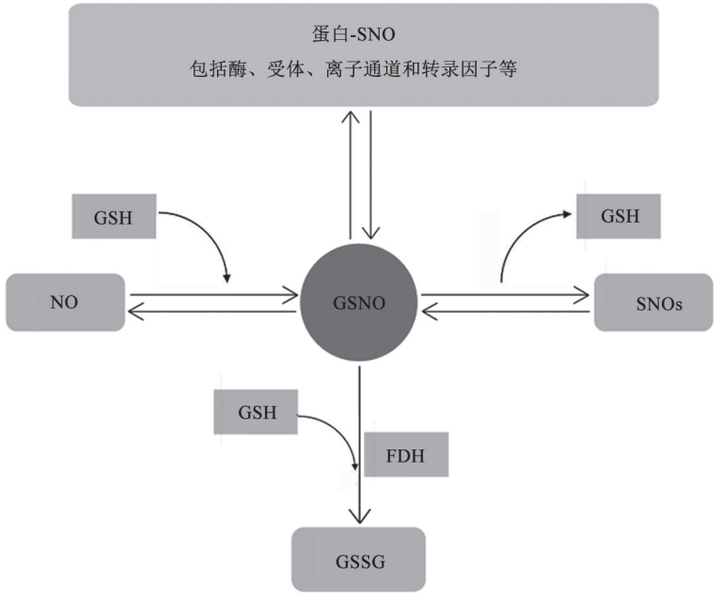

图2 FDH在植物体内起到维持NO和SNOs平衡的作用

Fig.2 FDH balances the contents of NO and SNOs in plants

参考严金平等(2010)文献, 有所修改, 图中GSH: 谷胱甘肽; GSNO: *S*-亚硝基谷胱甘肽; 蛋白-SNO: 蛋白质类亚硝基硫醇; SNOs: 亚硝基硫醇; FDH: 甲醛脱氢酶; GSSG: 氧化型谷胱甘肽。

(Diaz等2003; Espunya等2012), 这种机制可能是因为病原菌侵染后诱发植物体内NO和H_2O_2的迸发, 两者可协同诱发过敏反应和其他防御应答机制, 从而将病原菌侵染信号放大, 有效地激活SA信号途径下游编码病程相关蛋白(pathogenesis-related protein1, PR1)和谷胱甘肽-*S*-转移酶(glutathione *S*-transferase, GST)等防御基因的表达(Rustérucci等2007)。

FDH在机械损伤胁迫下的作用机制目前仍不明确。Delessert等(2004)认为FDH不受机械损伤的诱导, 而Diaz等(2003)认为*FDH*受机械损伤的下调, 在烟草和向日葵(*Helianthus annuus*)体内的*FDH*表达水平受机械损伤下调, 向日葵体内的FDH活性亦有所下降(Diaz等2003; Chaki等2011), 而豌豆和黄瓜(不抗病和抗病品种)体内的FDH活性却均受机械损伤诱导(Corpas等2008; Kubienova等2013), 这种响应机械损伤的差异性可能与物种有关。

机械损伤、ABA、JA和SA对ADH、CAD及FDH调控诱导关系的研究应进一步深入, 更利于全面了解醇脱氢酶家族在植物抗逆中的作用及机制。

2 CAD在植物木质素合成中的作用

木质素是一种广泛存在于植物体中的无定形的、分子结构中含有氧代苯丙醇或其衍生物结构单元的芳香性高聚物, 其具有重要的生物学功能: 为植物器官提供机械支撑, 通过维管单元木质化来提高运输能力, 构建防御体系(Boudet 2000)。木质素的生物合成受两个信号转导途径调控, 其一是涉及到维管组织的发育, 其二是植物的胁迫响应途径(Mitchell等1994)。在木质素合成过程中, 醇脱氢酶家族中只有CAD家族参与催化前体物质的合成, 许多研究证明, CAD在生成木质素单体的最后一步中, 催化羟肉桂醛(主要包括香豆醛、松柏醛和芥子醛)转变为对应的醇(香豆醇、松柏醇和芥子醇), 促进木质素合成前体物质的形成(龚琰和许梦秋2010; Möller等2005; Sun等2013), 并且CAD在植物体内的基因表达、合成及作用部位均具有组织特异性(Sattler等2009; Ma等2011)。本部分主要介绍CAD亚家族在植物木质素生物合成中的作用。

玉米的*COMT*缺失突变体(*bm*)内CAD活性下降, 在叶脉、叶柄髓部及其他含有木质素的组织中, 大量积累红褐色色素, 木质素含量降低, 韧性变差, 防御能力降低(Vignols等1995; Halpin等1998; Piquemal等2002)。高粱(*Sorghum bicolor*) *bmr6-3*突变体*SbCAD2*基因的191位置发生突变, 致使突变体内大量积累松柏醛和芥子醛, 无法转变成对应的醇, 木质素含量降低(Saballos等2009; Sattler等2009)。在水稻*gh2*缺失突变体中, CAD活性和木质素均缺失, 松柏醇和芥子醇这两个木质素合成前体物质均显著降低(Zhang等2006)。在辐射松(*Pinus radiata*)和亚麻(*Linum usitatissimum*)体内, 尽管木质素的合成前体物质积累到一个很高的水平, 却由于CAD活性剧烈下降, 导致植物体内的木质素含量下降(Möller等2005; Wrobel-Kwiatkowska等2007)。在其他一些改变CAD活性或含量的植株体内, 也观察到了类似的结果, 如火炬松(*Pinus taeda*)、烟草、白杨(*Populus tomentosa*)和苜蓿(Halpin等1994; Baucher等1996, 1999; Stasolla等2003)等, 由此可见CAD是木质素合成中不可或缺的重要酶。

植物不同组织的木质素含量不同, 即木质素合成具有组织特异性, *CAD*的基因表达和CAD酶活部位必然具有组织特异性, 对不同植物CAD的研究也证实了这一点。在黑麦草(*Lolium perenne*)的木质化组织中检测到了较高的*LpCAD1*和*LpCAD3*转录水平, 而只在茎组织中检测到了*LpCAD2*转录本(Lynch等2002)。GUS染色法分析拟南芥中*AtCAD-C*和*AtCAD-D*基因的启动子活性发现, *AtCAD-D*在纤维组织和木质部中的表达均较高, *AtCAD-C*在维管素组织的染色深于木质部区域, 并证明*AtCAD-C*和*AtCAD-D*均是花柱内木质素合成的初始基因(Sibout等2003, 2005), 也说明了CAD功能的组织特异性。甘薯的*IbCAD1*主要在根中表达, 在叶子和子叶中均未检测到(Kim等2010)。*CAD*的这种组织特异性表达形式在小白菜(*Brassica rapa*)、上海青(*Brassica chinensis*) (Zhang等2010)、甘蔗(*Saccharum*) (Selman-Housein等1999)、银合欢(*Leucaena leucocephala*) (Pandey等2011)、棉花(*Gossypium hirsuturm*) (倪志勇等2011)上均得到证实。正是这种组织特异性地表达, 使植物获得了较为合理的形态结构, 从而保证了植物的生长, 乃至对胁迫的耐受和抵抗。

木质素是植物防止或减轻受病菌和害虫侵害

的重要屏障, 这在辐射松(Möller等2005)、栓皮栎(*Quercus suber*) (Coelho等2006)、茶树(Deng等2013)等植物中均有报道, 这些研究既证明CAD参与木质素合成, 又为对*CAD*突变体的研究寻找到了实际应用价值, 通过降低或完全去除CAD的活性, 降低植物体内的木质素含量, 使植物组织更易分解, 来提高秸秆的利用率和利用价值。D' Yvoire等(2013)通过沉默*BdCAD1*基因获得了更易糖解的二穗短柄草(*Brachypodium distachyon*), 即更利于饲料的加工处理, 类似的成果在小麦(Saballos等2009)和玉米(Barriere和Argillier 1993; Barriere等1994)上也有报道。

3 ADH在香气物质合成中的作用

香气物质属于植物的次级代谢产物, 是由脂肪酸、氨基酸、碳水化合物、类胡萝卜素和萜类等作为前体物质, 在植物生长发育过程中经过一系列酶促反应而形成的(Schwab等2008; Gonda等2010)。醇脱氢酶家族广泛参与直链醛、支链醛、芳香醛等与对应醇之间的转化, 部分以抗逆功能为主的成员如CAD在响应逆境胁迫的同时, 也可因为转化芳香醛为相应的醇而参与香气物质合成(Goffner等1998), 但ADH作为植物香气物质合成的脂氧合酶(lipoxidase, LOX)等途径中关键酶之一, 是醇脱氢酶家族在植物香气物质合成中最重要的酶。

3.1 植物香气物质合成的主要途径

果实的芳香特征是由几种特定的挥发性物质决定的, 尽管不同果实中含有的芳香成分很多, 但是只有含量超过味感阈值的少数物质对果实的风味起独特作用, 即"特征效应化合物"(刘明池等2008)。果蔬正因为不同的特征效应化合物和比例而呈现迥异的气味(Gonda等2010)。植物香气物质主要有酯类、醇类、醛类和萜类等。植物挥发物质合成途径主要有: (1)脂肪酸代谢途径: 具体包括α氧化、β氧化、γ氧化和LOX途径, 其中以LOX途径和β氧化为主。脂肪酸(饱和或不饱和)经过LOX和β氧化分别生成相应的直链脂肪族醛, 醛在ADH作用下生成相应的醇, 最后生成相应的酯类等(Dixon和Hewett 2000)。(2)氨基酸代谢途径: 以氨基酸为前体的香气物质合成可以通过合成和降解途径合成部分脂肪族、支链或芳香族的醛类, 再在ADH作用下生成相应的醇类, 最后合成羰基化合物、酸类和酯类。部分果实中的酯香型、果香型特征香气成分是通过氨基酸代谢途径产生, 如番木瓜(*Carica papaya*)、草莓(*Fragaria ananassa*)和香蕉(*Musa nana*) (Myers等1970; Tressl和Drawert 1973; Perez等1993)。单糖经无氧代谢产生丙酮酸, 在ADH的作用下氧化脱羧形成乙酰辅酶A, 进而合成某酸乙酯或是乙酸某酯(陈美霞2006)。

3.2 ADH为香气物质的合成提供前体

植物挥发性物质合成的最后两步研究极为广泛(Beekwilder等2004; Larkov等2008), 不同的合成途径均与ADH和醇酰基转移酶(alcohol acyltransferases, AAT)将挥发醛转化为对应的醇或酯的作用有关(Beaulieu 2006)。醇类物质是酯类合成的前体, 被认为是酯类合成的限制因子之一。草莓外源提供丁醇, 其乙酸丁酯和丁酸酯类合成增加, 而乙醇和己醇的供应则促进乙酯和己酯的合成(Ueda等1992); 薄皮甜瓜(*Cucumis melo*)中应用外源乙醇促进了ADH活性的升高, 同时也促进了乙酯类挥发物的合成(Liu等2012)。甜瓜果实的孵育实验也表明, 外源醇可以迅速转化为对应的酯(48 h内), 促进相应酯水平的提高, 充分说明了前体物质对酯类合成的重要意义(Khanom和Ueda 2008)。

3.3 在果实中特异表达的ADH基因家族成员可能参与香气物质合成

研究表明, *ADH*基因家族具有在植物果实中的表达特异性, 暗示了ADH在果实香气物质合成中发挥功能。Sarni-Manchado等(1997)在分析欧洲葡萄成熟相关的*GvADH1*时, Northern分析表明, 该基因主要在成熟果实中表达。根、嫩叶、卷须中几乎不表达, 未成熟果实中含量也很低, 而在葡萄果实成熟初期的表达量迅速增长, 暗示该基因参与成熟果实香气的合成。Tesniere和Verries (2000)从葡萄果实中分离出3个*Adh*基因, RT-PCR结果显示3个基因果期表达模式不同。*Adh1*与*Adh3*在未成熟的果实中表达, 而*Adh2*在果实的发育后期表达。进一步分析发现, *Adh2*是主要表达基因, 且ADH总活性变化与*Adh2*的表达模式一致。*Adh2*和*3*经原核表达纯化后对底物乙醛和乙醇的偏好程度不同。Van Der Straeten等(1991)发现番茄(*Lycopersicon esculentum*)果实绿熟和粉熟阶段均有*ADH* mRNA的出现, 但只在后熟末期高丰度表达。人工催熟处理可以诱导与绿熟期相比至少50倍的表达

丰度, 也推测其参与成熟时的香气合成。Longhurst等(1994)从番茄成熟果实中分离鉴定出4个编码ADH2的cDNA, 经Northern分析验证, *ADH2* mRNA在后熟的末期表达。Moummou等(2012)不但发现短链*SlscADH1*的果实表达特异性, 还发现该重组蛋白偏好以NAD作为辅因子, 在众多底物中对己醛和苯乙醛的亲和力最强。在甜瓜中分离的中链*CmAdh1*基因和短链*CmADH2*也是在甜瓜生长发育阶段, 尤其是果实成熟阶段特异表达。在根茎叶等营养器官中含量较低。CmADH1偏好NADPH作为辅因子, 而CmADH2在NADH作为辅因子时转化效率更高, 两者都可催化直链脂肪醛转化为相应的醇, 且对醇的亲和力为相应醛的10倍, 但只有CmADH1可以催化3-甲基丁醛等支链醛的还原, 尽管活性会比乙醛作为底物时低4~70倍。果期表达、不同的底物和辅因子选择暗示它们可能在为下游AAT提供特异醇底物, 参与果实风味的形成(Manriquez等2006)。

3.4 ADH参与果实香气合成的功能验证

目前, ADH在果实香气合成途径中的位置与醇醛转化作用已经得到广泛认同, 有关ADH在香气物质合成中的功能也得到了部分验证。1998年Speirs等分别获得了番茄*LeADH2*果实特异性表达植株和生长发育持续表达植株, 对转基因植株果实的ADH水平、活性及与特征香气相关的某些醇醛物质进行测定, 结果显示, 植株果实内ADH活性除了在部分植株中被抑制外多数是增强的, 最高可达对照组的2倍; 果期特异表达的众多转化植株中的ADH基因活性均有所提高。成熟果实中变化的ADH活性影响了与香气物质合成相关的醛醇的平衡, 其中己醇和3-*z*-己烯醇的含量升高, 而其他醛类的浓度基本不变。在初步的风味测定中, 发现转基因植株的果实在ADH活性增强同时, 醇浓度水平较高, 果实更具有“成熟”的风味。但是, Moummou等(2012)沉默番茄果实特异表达的短链*SlscADH1*基因后发现, 该基因在番茄果实中的下调除了意外的促进了LOX途径C_5、C_6挥发物的高浓度积累, 其它的香气成分却没有变化。C_6挥发物的积累在拟南芥ADH功能缺失突变体的叶片上也曾出现(Bate等1998)。Moummou等(2012)提出可能是源于LOX途径的C_5、C_6挥发物对*SlscADH1*产生了抑制, 直接影响了酯类分解代谢。在薄皮甜瓜中, ADH在不同品种的果实中表达水平及活性不同, 较高ADH活性和基因表达量对醇类增加影响较大, 果实表现为清香型(徐晓飞等2012)。

3.5 ADH参与香气物质合成的影响因素

多数研究表明果实成熟度、乙烯、一氧化氮(NO)、吡唑类化学物质及低氧(气调贮藏)条件等都是植物ADH活性的影响因素。

3.5.1 果实成熟度对ADH及基因表达的影响 果实成熟度对ADH及基因表达的影响在番茄、葡萄、油橄榄(*Olea europaea*)、芒果(*Mangifera indica*)、柿(*Diospyros kaki*)和甜瓜等众多植物中得到验证。番茄*ADH2* mRNA在未成熟的果皮中表达而且丰度随果实的成熟而升高(Longhurst等1994)。葡萄未成熟果实的*ADH1* mRNA含量很低, 果实成熟初期mRNA含量明显增加(Sarni-Manchado等1997)。不同品种油橄榄中*ADH*的丰度和酶活性均在花后200 d的‘Black ripe’期达到高峰, 除了个别时期外, *ADH*的表达量随果实的成熟而逐渐上升(Iaria等2012)。芒果中的3个*MiADH*在果实生长发育中的表达模式不同: *MiADH1*和*MiADH2*在果实成熟初期积累, *MiADH3*在果实发育早期就开始增加(Singh等2010)。ADH活性在柿果实中的活性伴随果实成熟出现先升高后下降的趋势, 在果实发育初期并没有检测到ADH活性(刘朝蓬等2008)。厚皮甜瓜中发现2个ADH基因(*CmADH1*和*CmADH2*)的表达均在花后39 d, 果实成熟时迅速升高(Manriquez等2006)。总体上, *ADH*的表达伴随植物果实的成熟呈增加趋势。

3.5.2 乙烯对ADH及香气物质的影响 Manriquez等(2006)的研究证明, 果实成熟度对果实内*Adh*基因表达的影响很大程度上是通过乙烯的诱导作用而体现的。对野生型和反义ACO (1-aminocyclopropanecarboxylic acid oxidase)型(AS, 氨基环丙烷羧酸合成受阻)厚皮甜瓜不同成熟阶段果实的乙烯含量和*CmAdh*基因表达水平进行测定, 结果表明*CmAdh1*与*CmAdh2*的丰度与果实内乙烯变化趋势一致; 在AS果实中两种*CmAdh*基因的丰度也受到强烈抑制, 说明乙烯是*CmAdh1*与*CmAdh2*表达水平的主要调控因素。乙烯处理香蕉果实, 可使其乙烯释放高峰提前, 同时ADH活性同步提前上升。1-MCP处理香蕉果实, ADH活性受到抑制(宁文彬等2009)。黄金梨(*Pyrus pyrifolia* Nakai cv.

Whangkeumbae)经1-MCP熏蒸后, 果实中ADH显著低于对照(田长平等2010)。乙烯有可能通过对LOX和ADH活性的调控来影响苹果(*Malus domestica*)果实酯类合成(Li等2006)。但在番茄中研究表明, *LeAdh2*在果实成熟中参与香气物质合成, 不受乙烯调控(Speirs等1998, 2002)。Defilippi等(2005)研究也表明, 1-MCP处理可抑制采后苹果AAT活性, 但对ADH活性似乎影响不大。同时, 作为非呼吸跃变型果实, 葡萄中的ADH活性也随果实成熟而迅速上升。因此, 并不能排除在呼吸跃变果实中存在非乙烯依赖型的ADH (李岩2012)。

3.5.3 NO、4-MP对ADH及香气物质的影响 NO可抑制黄金梨贮藏期间果实硬度下降和乙烯释放, 同时有效抑制了醛类、醇类总量的下降以及酯类总量的增加, 降低ADH活性(田长平等2010)。吡唑类可以抑制不同类ADH的同工酶, 但效果有差异(鲍文娜2007), 4-甲基吡唑(4-methylpyrazole, 4-MP)是ADH的抑制剂, 在动物中可特异性阻止乙醇向乙醛的代谢(Chen等1995), 在植物中可通过抑制ADH的活性以阻碍乙醇生成(Noguchi和Yasuda 2007)。

3.5.4 低氧环境对植物果实ADH及香气物质的影响 气调贮藏是延长果蔬保质期的重要手段之一, 果实中的ADH同样响应低氧环境, 引起香气物质的改变。Longhurst等(1994)研究番茄果实过程中发现, 低氧(3%体积比)环境可促使果皮*ADH* mRNA水平的上升; 将果皮置于正常空气中mRNA含量又可恢复到正常水平。果皮中mRNA水平对空气中的氧气含量变化反映敏感, 在12%氧气的空气中增加量约为20倍, 在含3%氧气的空气中增量可达100倍。Chervin等(1999)在研究梨果实的过程中也发现, 低氧处理的样品中ADH活性增强, 约为对照的2倍, 而且处理组恢复到正常条件后保持较高的活性水平长达18 d。气调储藏可延长梨果实的贮藏期, 但会降低挥发性酯水平, 而酯是梨香气的重要成分, 所以气调储藏一定程度上会影响梨的品质。Lara等(2003)对梨采后气调贮藏后得到结论, 经低氧条件处理, 果实挥发物产量下降。暗示了与酶活的抑制或促进相比, 低氧条件限制相关酶底物浓度才是抑制挥发物合成的主要影响因素。

4 展望

有关醇脱氢酶的研究兴起于20世纪60年代, 起初研究集中在水淹和冷害等逆境响应方面, 随着研究的深入, 其在木质素及香气合成中的作用也逐渐受到重视。CAD是植物木质素生物合成的关键酶, 木质素具有为植物器官提供机械支撑, 通过维管单元木质化来提高运输能力, 构建防御体系等重要功能。因此, CAD与植物的生长发育及对生物和非生物胁的响应迫密切相关。进一步研究CAD在木质素合成中作用和抗逆机制, 通过调控CAD的活性改变植物木质素的含量和生物、非生物胁迫抗性, 提高秸秆利用率, 提升营养器官的利用价值、增强植物抗性等课题都值得深入探讨。

FDH是维持植物体内NO、SNOs和GSNO的平衡的关键酶, 可激活植物防御体系, 抵御病菌侵染和扩张。FDH在不同植物中的调控机理不尽相同, 相关报道较少, 其逆境响应的调控机制有待完善。

尽管ADH是果实香气物质调控的关键环节, 但鉴于其多样的底物、复杂的影响机制, 具体的香气物质调控机理仍未明确。在对高品质果蔬需求日益扩大的今天, 利用酯类合成的相关前体和抑制剂研究该酶及其基因家族成员在香气合成途径中的作用; 对于鉴定出的基因家族成员, 运用生物信息学进行相关分析, 进行时空表达分析以及在不同风味品种上表达验证; 结合色谱、质谱及转基因等分子生物学手段, 进一步研究其在果实香气物质合成中的功能是迫切需求的。深入探讨醇脱氢酶家族在逆境胁迫和果实香气合成中的作用及调控, 对于通过基因工程手段改善作物的品质和提高抗逆(病)性具有重要意义。

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