综 述 Reviews

植物中抗坏血酸的生物学功能研究进展

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摘要: 抗坏血酸是水溶性抗氧化有机小分子, 在植物中广泛存在, 并可作为某些氧化还原酶的辅酶。本文主要综述了抗坏血酸在植物中的合成、转运和所参与的多种生理作用, 如细胞周期调控、成花诱导、光合结构保护、碳代谢和胁迫响应等, 并对今后植物中抗坏血酸的相关研究提出展望。

关键词: 抗坏血酸; 生理功能; 细胞周期; 成花诱导; 光合作用

Research Advance of Biological Function of Ascorbic Acid in Plants

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Abstract: Ascorbic acid (AsA) is a widespread potent water-soluble antioxidant in plants, and acts as co-factor of some reductase. This review focused on the biosynthesis, transportation and plant physiological functions of ascorbic acid in plants, such as cell cycle regulation, floral induction, photo-protection, carbon metabolism and stress response et al., and provided prospect for future research.

Key words: ascorbic acid; physiological function; cell cycle; floral induction; photosynthesis

抗坏血酸(ascorbic acid, AsA)也称维生素C, 是植物中广泛存在的一种水溶性抗氧化有机小分子。随着抗坏血酸在医疗方面的价值得到重新确认, 如增强颈动脉小球的抗缺氧能力(Del Rio等2010)、治疗哮喘(Misso等2005)、抗肿瘤(Fritz等2014)), 植物中AsA的含量控制(合成和降解)及其在植物中的生理功能也受到再次关注。一直以来, 人们认为AsA在植物中的功能主要是清除细胞内的氧化物和超氧化物, 或作为某些还原酶的辅因子参与还原反应。但最新研究表明, AsA参与植物的衰老调控(Fotopoulos和Kanellis 2013)、光合结构保护(Toth等2013)、糖代谢(Garchery等2013)、成花诱导(Barth等2006)和胁迫响应等多种生理调控过程, 并为植物中的离子转运机制(Grillet等2014)和胁迫耐受性研究提供了新的视角。本文即对相关内容做一综述。

1 抗坏血酸的合成和降解

1.1 抗坏血酸的合成

植物体内有5条AsA合成途径(图1) (Ishikawa和Shigeoka 2008)。分别是L-半乳糖途径、古洛糖途径、D-半乳糖醛酸酯途径、依赖肌醇的合成途径和补救途径。其中, L-半乳糖途径最先被发现(Wheeler等1998), 大致途径如下: D-葡萄糖转化为GDP-D-甘露糖, 在GDP-甘露糖-3,5-差向异构酶(GDP-mannose-3',5'-epimerase, GME)的催化下生成GDP-L-半乳糖, 而后转化为L-半乳糖酸-1,4-内酯(L-galactono-1,4-lactone, GalL), 最后在L-半乳糖酸-1,4-内酯脱氢酶(GalL dehydrogenase, GalLDH)的催化下生成AsA (Cruz-Rus等2011)。古洛糖途径由GDP-L-古洛糖起始, 经由L-古洛糖酸-1,4-内酯生成AsA。D-半乳糖醛酸酯途径在草莓中被发现(Agius等2003), D-半乳糖醛酸酯经D-半乳糖醛酸酯还原酶还原产生L-半乳糖酸, 经醛内酯酶催化生成L-半乳糖酸-1,4-内酯合成AsA。随后在拟南芥中又发现了依赖肌醇的合成途径(Lorence等2004)。

L-半乳糖途径是植物合成AsA的主要途径(Wheeler等1998), 通过RNAi技术抑制番茄中的GalLDH, 导致添加25 mmol·L^{-1} L-GalL后, 转基因

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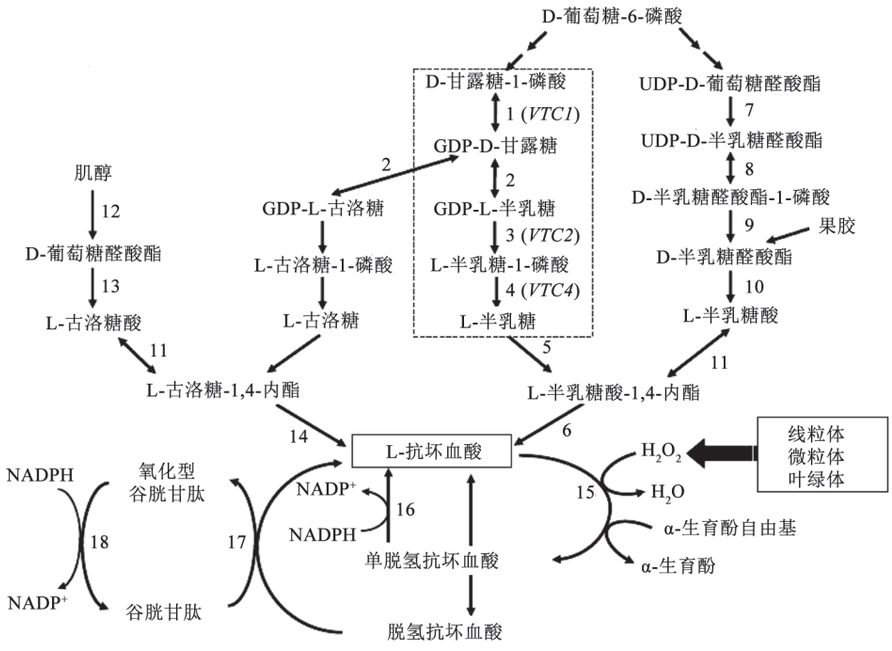

图1 植物中的AsA合成途径

Fig.1 Biosynthetic pathway of ascorbic acid in plants

虚线框里是L-半乳糖途径, 括号里是相对应的拟南芥突变株(*VTC*)。1: GDP-D-甘露糖焦磷酸化酶; 2: GDP-甘露糖-3,5-差向异构酶; 3: GDP-L-半乳糖磷酸化酶; 4: L-半乳糖-1-磷酸酯酶; 5: L-半乳糖脱氢酶; 6: L-半乳糖酸-1,4-内酯脱氢酶; 7: UDP-葡糖醛酸-4-差向异构酶; 8: UDP-半乳糖焦磷酸化酶; 9: D-半乳糖醛酸-1-磷酸酯酶; 10: D-半乳糖醛酸酯还原酶; 11: 醛内酯酶; 12: 肌醇氧化酶; 13: D-葡糖醛酸还原酶; 14: L-古洛糖内酯脱氢酶; 15: 抗坏血酸氧化酶; 16: 单脱氢抗坏血酸还原酶; 17: 脱氢抗坏血酸还原酶; 18: 谷胱甘肽还原酶。本图参考Ishikawa和Shigeoka (2008)一文修改。

植株中AsA的合成显著降低(Alhagdow等2007); 敲除半乳糖途径的关键酶GME, 也导致番茄叶和果实中AsA含量显著降低(Gilbert等2009)。通过基因敲除或插入突变技术, 在拟南芥中也获得了一批AsA缺乏(deficient)突变体, 并被广泛用于AsA的功能研究。其中*VTC1*基因编码GDP-D-甘露糖焦磷酸化酶(GDP-D-mannose pyrophosphorylase, GMPase) (Conklin等1999); *VTC2*和*VTC5*基因编码GDP-L-半乳糖磷酸化酶; *VTC4*编码L-半乳糖-1-磷酸酯酶(Laing等2004); 这些突变株中AsA的含量仅相当于野生型的20%~50% (Conklin 2001)。

补救途径(Yin等2010)是指被氧化的AsA, 也即脱氢抗坏血酸(dehydroascorbate, DHA)或单脱氢抗坏血酸(monodehydroascorbate, MDHA), 在DHA还原酶(DHA reductase, DHAR)或MDHA还原酶(MDHA reductase, MDHAR)的催化下, 重新还原为AsA。补救途径也参与AsA水平的调节(Yin等2010), 并和其他AsA合成途径一起, 在植物对逆境的响应和适应中发挥重要作用(Stevens等2008)。

与植物中具有多条AsA合成途径不同, 动物中仅有一条AsA合成途径, 也即古洛糖途径(Bánhegyi等1997)。由尿苷二磷酸葡萄糖(UDPG)开始, 经由UDP葡萄糖酸、D-葡萄糖醛酸酯、L-古洛酸、古洛糖酸-1,4-内酯, 最后合成抗坏血酸。古洛糖酸内酯脱氢酶催化最后一步反应, 由于基因突变, 在人、几内亚猪和多种动物中缺乏该酶(Linster和Van Schaftingen 2007)。这可能是动物需要大量从植物中摄取抗坏血酸的主要原因。

1.2 抗坏血酸的降解

目前有关AsA降解的研究不多。已发现在大多数植物中, AsA在C2和C3之间断裂, 生成草酸(C1-C2)和L-苏阿糖酸(C3-C6)(Davey等2000; Green和Fry 2005a)。但在葡萄中, AsA还可在C4和C5之间断裂, 由C1-C4生成苏阿糖酸, 苏阿糖酸进一步氧化形成酒石酸(DeBolt等2004)。从AsA到苏阿糖酸的降解过程在细胞内外均可发生, 用*Rosa*悬浮细胞研究发现, 胞外AsA氧化为DHA后(图2, 步骤1), 在DHA氧化酶的催化下生成环化草酰二

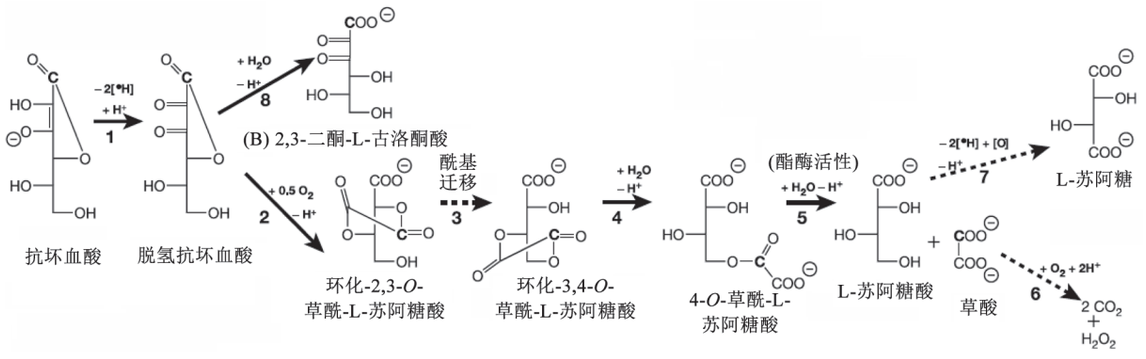

图2 AsA的胞外降解途径

Fig.2 Apoplastic degradation pathway of ascorbic acid

1: 抗坏血酸氧化酶或非酶促反应等; 2: DHA氧化酶; 3: 未知酶; 4和5: 草酰酯酶; 6和7为非酶促反应; 8: DHA氧化酶。本图参考Green和Fry (2005b)一文修改。

酯L-苏阿糖酸(图2, 步骤2和3), 而后由草酰酯酶催化生成4-*O*-草酰-L-苏阿糖酸(图2, 步骤4), 再经非酶促反应生成苏阿糖和草酸(图2, 步骤5) (Green和Fry 2005b)。

值得注意的是, AsA的降解途径产生5个H_2O_2 (图2, 步骤1、2、6和7)。降解途径第一步通过非酶促反应($AH_2+O_2 \rightarrow A+H_2O_2$)生成$H_2O_2$; 第二步通过氧化酶催化产生$H_2O_2$; 第六步在草酸氧化酶的催化下生成$H_2O_2$; 还有2个$H_2O_2$在苏阿糖酸生成苏阿糖的过程中出现, 可能通过未知氧化酶的催化产生(Green和Fry 2005b)。AsA胞外降解产生的H_2O_2, 通过芬顿反应生成羟自由基(OH^-), 再经由非酶促反应裂解细胞壁中的果胶和纤维素, 从而有助于促进细胞膨大或果实软化(Fry 1998)。这一发现印证了AsA不仅可以作为抗氧化物, 在某种条件下, 还可以作为前氧化物(pro-oxidant)的观点(Podmore等1998)。

2 抗坏血酸的转运

AsA主要在源叶(source leaf)中合成, 并经过韧皮部运输至库组织(Franceschi和Tarlyn 2002)。根中也可以合成AsA (Liso等2004), 将^{14}C标记的AsA处理杨树成熟叶片后, 发现^{14}C AsA主要出现在根尖中; 切断韧皮部则导致杨树根尖里AsA含量显著下降, 证明源叶合成的AsA经韧皮部转运对于维持杨树根中的AsA含量起到关键作用(Herschbach等2010), 也说明源叶是根和其他库组织中AsA的主要来源。在半乳糖途径中, GalLDH催化合成AsA的最后一步反应, 且定位于线粒体内(Wheeler等1998)。但植物的细胞质、叶绿体和胞外环境中都含有AsA, 且浓度达到毫克级(Foyer和Lelandais 1996), 意味着植物中存在高效的AsA跨膜和长距离转运系统。

目前对植物中的AsA转运系统知之甚少(Pignocchi等2006)。通过对基因组数据库的筛选, 在拟南芥中发现12个编码膜蛋白的基因与碱基/抗坏血酸转运体(nucleobase/ascorbate transporter, NAT)同源, 并与哺乳动物中的2个依赖钠的抗坏血酸转运体(sodium-dependent vitamin C transporter, SVCT1和SVCT2)相似(Maurino等2006)。*AtNAT*的双突变体(*nat1nat2*、*nat4nat9*、*nat4nat10*和*nat9nat10*)甚至三缺失突变体(*nat1nat2nat3*)都未见表型变化, 表明NAT功能在植物中高度冗余(Maurino等2006)。

也有报道指出, NAT并不负责AsA的转运。迄今为止, NAT家族中仅少数成员的功能较清楚。构巢曲霉(*Aspergillus nidulans*)中的2个NAT (UapA和UapC)只转运黄嘌呤、尿酸和少量其他嘌呤, 抗坏血酸仅结合但不转运(Diallinas等1995)。事实上, 植物中仅鉴定一个玉米的NAT, 且和曲霉中的UapA和UapC一样, 负责转运黄嘌呤和尿酸(Argyrou等2001)。因此, 植物中负责AsA转运的载体还有待深入研究。

AsA向胞外的转运对植物还有其他意义。铁是植物生长所必须的营养元素之一, 但双子叶植物仅转运亚铁离子。因此, 三价铁还原为二价铁是双子叶植物吸收铁的必经途径。在豌豆(*Pisum*

sativum)胚中发现, 三价铁的还原并非由质膜上结合的三价铁还原酶催化, 而是依赖细胞外流的AsA; 而AsA介导的还原反应是离体胚吸收^{55}Fe的必须步骤(Grillet等2014)。此外, 在拟南芥突变体*vtc2-4*和*vtc5-1*中, 铁含量也都显著低于野生型植株(Grillet等2014)。证明AsA外流还具有促进铁离子吸收的功能。

已发现植物细胞优先吸收AsA的氧化形式——DHA (Horemans等2003); 植物中DHA的转运被认为和动物细胞一样, 经由膜上的葡萄糖转运体或己糖转运体(Szarka等2004)。近年研究发现, 植物中吸收DHA和葡萄糖的载体性质不同, 如细胞松弛素B, 根皮素和染料木黄酮显著抑制DHA的吸收, 但对葡萄糖的吸收影响不大(Horemans等2008)。意味着植物中DHA和葡萄糖分别经由不同的载体, 但具体基因尚未得到分离鉴定。

3 抗坏血酸的功能

3.1 促进细胞分裂

在玉米根尖和烟草悬浮培养细胞(BY-2)中, AsA的含量和细胞增值率之间呈正相关, 高含量的DHA阻止细胞从G_1期向S期的转变, 而高含量的AsA能加速分裂(Kerk和Feldman 1995)。对烟草BY-2细胞添加外源DHA, 虽然影响G_1期向S期转变, 但内源DHA含量并没有受到影响, 而AsA含量增加, 表明DHA在细胞内被还原(Potters等2000)。有趣的是, 缺乏GSH的烟草BY-2细胞依然可以摄取并还原外源DHA; 用1 mmol·L^{-1} GSH和DHA同时处理BY-2细胞, 并不能阻止外源DHA对细胞分裂的抑制; 证明生物体内除了GSH驱动的DHA还原外, 还有不依赖GSH的DHA还原, 它们共同且独立作用影响细胞分裂(Potters等2004)。

3.2 影响细胞壁合成

AsA在内质网内催化产生细胞膨大和分裂所必需的富含羟脯氨酸的糖蛋白(Kärkönen和Fry 2006)。ASA还参与了苯氧自由基介导的细胞壁成分的交联, 从而导致细胞壁固化(Smirnoff 2000)。逆境条件下, GME调节ASA合成和细胞壁单糖合成之间的平衡。GME可能同分子伴侣Hsp70互作以增加酶活性, 和(或)更倾向于催化GDP-L-古洛糖的合成, 因为GDP-L-古洛糖只用于合成AsA以响应环境胁迫, 而GDP-L-半乳糖除了用于合成AsA外, 还用于合成细胞壁单糖(Wolucka和Van Montagu 2003)。Gilbert等(2009)发现P35S:Slgme^{RNAi}的番茄茎和果实的细胞壁中甘露糖含量升高, 半乳糖含量降低, 鼠李糖和糠醛酸含量在果实中增加。结合P35S:Slgme^{RNAi}的番茄中AsA含量被有效降低这样的事实, Gilbert等(2009)认为AsA和非纤维化细胞壁多糖的合成之间密切相关。

3.3 保护光合作用和影响碳代谢

AsA不仅能作为电子供体维持光合作用, 还在保护光合器官方面起到重要作用(Ivanov 2014)。叶黄素循环是光保护(photoprotection)机制的重要组成部分, 其组分紫黄质去环氧酶(violaxanthin de-epoxidase, VDE)通过消耗AsA催化紫黄质生成玉米黄质, 从而将过剩光能以非光化学淬灭(non-photochemical quenching, NPQ)的叶绿素荧光释放, 保护光合器官(Bratt等1995)。降低AsA含量影响拟南芥中的VDE的活性, 并导致叶片中NPQ水平降低(Müller-Moulé等2002)和活性氧的增加(Foyer和Noctor 2003), 从而降低光合保护能力。环化电子传递(cyclic electron transport, CET)在植物光保护机制中起到重要作用, 而NAD(P)H脱氢酶复合物是维持CET运转的主要组分(Kouřil等2014)。抗坏血酸缺乏导致类囊体中NAD(P)H脱氢酶表达水平降低(Queval和Foyer 2012), 从而干扰植物中的光合保护能力。定位于叶绿体基质或类囊体膜的抗坏血酸过氧化物酶能利用AsA作为电子受体还原H_2O_2; 但在AsA缺乏条件下钝化, 并影响光合速率(Ishikawa和Shigeoka 2008)。

植物体内的碳分配受环境和发育过程影响(Hermans等2006)。正常条件下, AsA缺乏突变体*vtc-1*和*vtc-2*的CO_2同化率与野生型相近(Pastori等2003), 但在逆境胁迫下, *vtc*突变株中叶黄素循环和CO_2同化率都降低(Müller-Moulé等2002)。抑制番茄中*SlGalLDH*的表达, *P$_{35S}$:Slgalldh^{RNAi}*转化株生长缓慢, 叶片中TCA循环也被明显抑制, 多数中间代谢物的含量显著降低(Alhagdow等2007)。通过RNAi技术抑制番茄中的*AO*活性, 导致叶片和果实中AsA含量和糖含量的增加, 并促进了糖从源叶向库组织的转运(Garchery等2013)。虽然已发现AO作为多种信号途径的载体(De Tullio等2013), 而糖不仅是碳同化的产物, 也作为信号分子(蔗糖和葡

萄糖) (Zhou等2008)响应多种环境胁迫, 但AsA与碳代谢之间的crosstalking还有待深入探讨。

3.4 提高抗逆性

植物中的AsA含量与抗逆性呈正相关, 提高AsA含量或促进氧化态AsA的还原都能提高植物的抗逆性(Gallie 2013)。拟南芥*vtc-1*突变株中的AsA含量只有野生型的30%, 对臭氧非常敏感(Veljovic-Jovanovic等2001), 且在盐胁迫下H_2O_2积累则明显高于野生型(Huang等2005)。过表达*AO*的转基因烟草的AsA含量低于野生型, 盐胁迫下细胞内外的H_2O_2积累较野生型高(Yamamoto等2005)。在水培萝卜的培养液中添加AsA合成的底物GalL, 有效增加了萝卜对臭氧的抗性(Maddison等2002)。在大麦中也检测到相似的结果(Mächler等1995)。盐和臭氧等多种非生物胁迫都导致植物中的H_2O_2积累(Mittler 2002), 而提高AsA含量或促进氧化态AsA的还原, 都能使细胞内的AsA含量维持较高水平, 从而通过酶促(抗坏血酸过氧化物酶等)或非酶促途径有效清除胁迫产生的H_2O_2, 这可能是AsA提高植物抗逆性的重要原因之一。

3.5 影响开花时间和衰老

开花是植物从营养生长向生殖生长转变的关键事件。拟南芥中存在4条途径调控开花: 春化途径、自主途径、赤霉素途径和光周期途径(Boss等2004)。近年发现AsA也参与开花时间调控。

叶面喷施AsA合成的前体L-半乳糖增加植物体内的AsA含量, 延迟开花(Wheeler等1998)。拟南芥AsA缺乏突变体在长日条件下都较野生型提前开花, 且所有已知的开花途径(包括光周期途径、自主途径和赤霉素途径)的基因都出现表达差异, 并与观察到的开花表型相符(Kotchoni等2009)。但用*vtc1-1*与光周期途径晚花突变体*gi-1*、*co-2*、*ft-1*和自主途径晚花突变体*fca-1*杂交产生的双突变体*vtc1-1gi-1*、*vtc1-1co-2*、*vtc1-1ft-1*和*vtc1-1fca-1*, 同*vtc-1*相比, 双突变体开花较晚, 表明*gi-1*、*co-2*、*ft-1*和*fca-1*对于*vtc1*具有上位性(epistatic) (Kotchoni等2009)。已发现拟南芥*vtc1-1*中的ABA含量高于野生型(Barth等2004), 在烟草中过表达抗坏血酸氧化酶降低AsA含量, 也导致ABA含量增加(Fotopoulos等2008), 这是否与AsA对开花时间的影响有关, 还有待进一步研究(Barth等2004; Pastori等2003)。

开花是衰老的表现之一, 而衰老的拟南芥中具有高含量的水杨酸(salicylic acid, SA) (Morris等2000)。Barth等(2004)研究发现拟南芥*vtc-1*突变株中衰老相关基因*SAG13*、*SAG15*和*SAG27*的表达明显上调, 且具有较高SA含量。已知降低AsA水平增加植物中的ABA含量(Pignocchi等2006), *SAG27*受SA诱导(Quirino等1999), 而*SAG15*和*SAG13*分别受ABA和乙烯诱导(Nakashima等1997; Weaver等1998)。这些结果表明*vtc-1*通过诱导ABA、SA和乙烯等含量的升高, 进而促进*SAG*表达的上调, 导致衰老(Barth等2004)。但在黑暗条件下, AsA不足植株的衰老速度却慢于野生型(Fotopoulos和Kanellis 2013)。在烟草中过表达抗坏血酸氧化酶, 导致转基因植株中的AsA含量低于野生型, H_2O_2含量高于野生型; 但在暗条件下衰老的速度较野生型延迟。认为可能是低水平的AsA诱导植物产生了较高的H_2O_2耐受性, 从而比野生型烟草具有更好的暗适应性(Fotopoulos和Kanellis 2013)。这也说明AsA在复杂的衰老信号途径中的具体作用仍有待深入研究。

3.6 影响植物发育

AsA含量变化影响果实发育。抑制番茄中*GalLDH*的表达, P_{35S}:$Slgalldh^{RNAi}$转化株出现AsA含量降低、植株矮化、生长缓慢、果实变小等特征(Alhagdow等2007)。沉默番茄中的*GME*, 发现*P35S:*$Slgme^{RNAi}$转化株具有AsA含量降低、幼苗生长减慢、茎脆弱和果实发软等表型, 补充外源AsA不能改变这些现象。推测抑制GME造成的生长障碍不仅和AsA含量降低有关, 还可能与细胞壁多糖成份的改变有关(Gilbert等2009)。而降低番茄中的抗坏血酸氧化酶活性, 增加叶片中AsA的含量, 促进了蔗糖从源叶向库组织的转运, 从而提高转化株番茄果实的直径和产量(Garchery等2013)。这种AsA含量与番茄果实产量呈正相关的现象, 可能与AsA能维持光合作用和呼吸作用有关(Nunes-Nesi等2008)。

AsA含量还影响植物体细胞胚的诱导分化过程(Becker等2014)。拟南芥突变体*vtc2-5*中的抗坏血酸含量仅有野生型的30%, 但诱导出的体细胞胚的数量和细胞分裂速度大约是野生型材料的6倍; 分析转录组数据发现, 抗坏血酸不足改变了基因

网络调控方式, 促进了愈伤组织对IAA产生积极响应, 导致每个外植体所产生胚的数量增加(Becker等2014)。可见AsA在植物发育的不同组织和不同阶段, 所执行的生理或分子功能也有差别。

4 展望

综上所述, AsA在植物生长发育过程中的作用已远远超出通常所理解的“抗氧化”范畴, 不仅涵盖碳代谢、细胞分裂和生长、植物开花调控、逆境响应和离子吸收等生理功能, 还影响植物的氮代谢(Balestrini等2012; Barth等2010), 并与DNA的去甲基化(Dickson等2013; Minor等2013)有关。但其中仍有许多问题有待深入研究: (1)植物中AsA的外流和DHA的吸收转运体究竟是什么? (2)胞外AsA是否还影响其他离子的吸收或转运? (3) AsA含量的差异如何影响植物对IAA的响应? (4)植物如何通过AsA含量波动响应外界胁迫并调控基因表达? 与动物中仅存的一条抗坏血酸合成途径相比, 植物中多条复杂的AsA合成途径暗示了AsA对植物生长的重要性, 也意味着植物中抗坏血酸的功能还有待更深入的研究和发现。

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