

番茄耐旱和耐盐遗传改良的研究进展及展望

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摘要: 就番茄耐旱和耐盐遗传改良的研究进展进行了综述。主要包括番茄耐旱和耐盐的野生资源及其遗传进化, 基于基因组的 QTL 挖掘, QTL 的遗传与累加效应, 以及利用基因工程转入包括离子运输和区室化、渗透调节、活性氧清除、转录因子、胁迫蛋白等在内的外源基因, 提高番茄的耐旱和耐盐性。就目前番茄耐旱和耐盐遗传改良过程中存在的问题与未来的应对策略, 包括番茄耐旱和耐盐表型鉴定、全基因组关联分析、基因工程、基因的协同调控、小 RNA、表观遗传学等, 进行了探讨与展望。

关键词: 番茄; 非生物胁迫; 耐盐性; 耐旱性; 遗传改良

中图分类号: S 641.2

文献标志码: A

文章编号: 0513-353X (2012) 10-2061-14

A Review and Perspectives on Genetic Improvement of Salt and Drought Tolerance in Tomato

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Abstract: The genetic improvement of tomato tolerance to drought and salt stress was reviewed in this paper. The contents mainly included the potential wild tomato species for drought and salt tolerance and the evolution, QTL exploration, the genetic and stacking effects of the identified QTLs, and the genetic engineering with exogenous genes, including ion transport and compartmentalization, osmotic regulation, antioxidant protection, transcription factor, stress related proteins, for tomato genetic improvement with drought and/or salt tolerance. Meanwhile, the present problems and future strategies, mainly including phenotype determination of drought and salt tolerance in tomato, genome wide association study (GWAS), genetic engineering, cross-talking, small RNA and epigenetics, were also discussed.

Key words: tomato; abiotic stress; salt tolerance; drought tolerance; genetic improvement

多数栽培番茄 (*Solanum lycopersicum*) 品种对干旱敏感, 对盐中度敏感。本文中对干旱和盐胁迫两种重要的非生物胁迫因子, 就目前番茄遗传改良的研究进展进行了综述, 并对目前存在的主要问题及未来相关的应对技术与策略进行了探讨。

收稿日期: 2012-05-16; 修回日期: 2012-06-12

基金项目: 国家高技术研究发展计划(‘863’计划)项目(2012AA100104)

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1 番茄耐旱和耐盐的遗传资源

经过人类长期高压选择，现代番茄栽培种的遗传背景已变得十分狭窄（Shirasawa et al., 2010）而分布在起源地南美洲的番茄野生种或野生近缘种，却蕴藏着极其丰富的优良基因资源。一些野生种如 *S. sitiens*、潘那利番茄 (*S. pennellii*)、*S. corneliomulleri*、智利番茄 (*S. chilense*) 等生长在极度干旱的西安第斯高海拔沙漠地区 (Chetelat et al., 2009; Xia et al., 2010)，尤其是 *S. sitiens* 和智利番茄 (*S. chilense*)，其生长地年降雨量仅为 0.9~1.5 mm，是世界上最干旱的地区之一 (Chetelat et al., 2009)。而另外一些野生种，如醋栗番茄 (*S. pimpinellifolium*)、秘鲁番茄 (*S. peruvianum*)、契斯曼尼番茄 (*S. cheesmaniae*)、潘那利番茄 (*S. pennellii*)，可在盐碱含量较高的土壤中生存 (Tal, 1971; Tal & Shannon, 1983)。这些野生种为了应对周围的逆境，自身演化形成了适应干旱和盐碱的特异机制。如潘那利番茄 (*S. pennellii*) 叶片表皮具有较多气孔，可吸收和利用空气中的水分；智利番茄 (*S. chilense*) 具有发达的根系，可摄取深层土壤水分；在盐胁迫下，秘鲁番茄 (*S. peruvianum*)、潘那利番茄 (*S. pennellii*)、契斯曼尼番茄 (*S. cheesmaniae*) 仍能维持较高的相对水分含量，而且对不同离子具有选择吸收的能力 (Tal, 1971; Rush & Epstein, 1981)。这些野生种为番茄耐旱和耐盐遗传改良提供了十分宝贵的资源材料 (Chetelat et al., 2009; Xia et al., 2010)。

2 番茄野生资源耐旱和耐盐性的遗传进化

考古发现，早期人类对作物后代的选择通常是无意识的，而且最后仅固定驯化了很少的性状 (Tang et al., 2010)。野生种的地区适应机制及其进化历史，为研究生物进化提供了极具价值的证据：(1) 它们的生长区域跨越大范围的气候、生物地理和环境差异，从温热带雨林一直到极度干旱的沙漠；(2) 栽培种具有大量而丰富的基因组信息 (Fischer et al., 2011)。通过对 12 个番茄野生种或野生近缘种的分布研究发现，环境因子，尤其是温度和降雨梯度等非生物生态条件是这些野生种适应多样性形成的主要驱动力 (Moyle & Muir, 2010)。现已发现，存在于大多数种子植物中的 *Asr* (ABA/water stress/ripening induced) 基因家族协同调控干旱和盐胁迫下的 ABA 响应 (Maskin et al., 2001)。Fischer 等 (2011) 对智利番茄 (*S. chilense*) 和秘鲁番茄 (*S. peruvianum*) 两个番茄野生近缘种群体的 *Asr* 基因家族的分子变异进行分析，发现 *Asr1* 经过了高强度的选择，*Asr2* 没有发生正向选择，*Asr4* 表现出与智利番茄 (*S. chilense*) 在极端干旱条件下相一致的适应性模式，并发现一个新的成员 *Asr5*，诸多 *Asr* 基因组成一个动态的基因家族，这些串联排列基因在植物适应性中起到重要作用。干旱胁迫下，*Asr2* 编码的转录因子在番茄叶片和根部上调，对多毛番茄 (*S. hirsutum*)、契斯曼尼番茄 (*S. cheesmaniae*)、樱桃番茄 (*S. esculentum* var. *cerasiforme*)、智利番茄 (*S. chilense*)、矮生秘鲁番茄 (*S. peruvianum* var. *humifusum*) 和 *S. peruvianum* f. *glandulosum* 等 6 个番茄野生种家系的 *Asr2* 同源基因的编码序列进行分析，并对番茄野生种的适应进化进行比较，发现智利番茄 (*S. chilense*) 和矮生秘鲁番茄 (*S. peruvianum* var. *humifusum*) 对干旱环境的适应性与其它野生种有显著区别，智利番茄 (*S. chilense*) 和矮生秘鲁番茄 (*S. peruvianum* var. *humifusum*) 内 *Asr2* 基因的氨基酸替换速率加速是其适应干旱环境的机制 (Framkel et al., 2003)。*Asr2* 编码区的错义替换和同义替换是智利番茄 (*S. chilense*) 和 *S. arcanum* 适应干旱和湿润环境的主要原因 (Giombini et al., 2009)。串联重复可能导致 *Asr* 基因家族成员发生不同的进化途径，最终参与形成了植物的特异适应性 (Fischer et al., 2011)。研究和明确野生种的耐旱和耐盐机制及其进化选择，将为番茄耐旱耐盐遗

传改良提供必要的科学依据。

3 番茄野生资源耐旱和耐盐基因的挖掘

3.1 不同生长发育阶段耐旱和耐盐 QTL 定位

研究表明, 番茄与其它作物一样, 耐旱和耐盐性受 QTL 控制和不同生长发育阶段的影响(Foolad, 2004)。到目前为止, 从醋栗番茄 LA0722、潘那利番茄 LA0716、类番茄茄 LA2951、契斯曼尼番茄 L2 等野生资源中, 分别定位了不同生长发育阶段的 QTL (Breto et al., 1994; Foolad & Chen, 1999; Monforte et al., 1999; Villalta et al., 2007; Xu et al., 2008; Gong et al., 2010; Li et al., 2011; 刘磊等, 2011; Uozumi et al., 2012), 结果表明, 控制番茄耐旱、耐盐性的 QTL 遗传复杂, 受多个位点影响(表 1)。一些研究者对耐盐的潘那利番茄 LA0716 进行了盐胁迫条件下生理和生长发育相关因子的分析, 定位了 125 个氧化胁迫相关 QTL 和 311 个生理响应相关 QTL (Frary et al., 2010, 2011)。综合上述研究可以发现, 一些 QTL 在染色体上成簇分布, 并表现高度遗传连锁, 而一些 QTL 同位, 推测其来自野生种的特定染色体渐渗片段, 这些片段可能为番茄耐旱或耐盐遗传改良提供一定的基因资源。目前对于抗性相关的 QTL 研究相对较多, 而产量相关 QTL 的研究还十分有限。此外, 由于存在评价方法的差异, 如干旱胁迫程度、盐浓度等不同, 一些结果也难以比较。

表 1 不同生长发育阶段定位的耐旱或耐盐 QTLs
Table 1 QTLs identified for salt or drought tolerance in different stages

性状 Trait	发育阶段 Development stage	QTL 数 Number of QTL	染色体 Chromosome	群体 Population	处理/(mmol·L ⁻¹) Treatment	调查性状 Investigated trait	参考文献 Reference
耐盐 Salt tolerance	芽期 Germination stage	5	1, 3, 7, 8, 12	(<i>S. lycopersicum</i> UCT5 × <i>S. pennellii</i> LA0716) F ₂	150 NaCl + 15 CaCl ₂ 200 NaCl + 20 CaCl ₂	达到 50% 发芽率的 天数 Days to 50% germination rate	Foolad & Jones, 1993
	芽期 Germination stage	8	1, 2, 3, 7, 8, 9, 12	(<i>S. lycopersicum</i> UCT5 × <i>S. pennellii</i> LA0716) F ₂	175 NaCl + 17.5 CaCl ₂	达到 50% 发芽率的 天数 Days to 50% germination rate	Foolad et al., 1997
	芽期 Germination stage	7	1, 2, 5, 7, 9, 12	(<i>S. lycopersicum</i> NC84173 × <i>S. pimpinellifolium</i> LA0722) BC ₁ , BC ₁ S ₁	150 NaCl + 15 CaCl ₂	发芽指数 Germination index	Foolad et al., 1998
	芽期 Germination stage	8	1, 3, 5, 6, 8, 9	(<i>S. lycopersicum</i> UCT5 × <i>S. pennellii</i> LA0716) F ₂	175 NaCl + 17.5 CaCl ₂	发芽率 Germination rate	Foolad & Chen, 1998
	芽期 Germination stage	6	4, 5, 7, 9, 11, 12	(<i>S. lycopersicum</i> NC84173 × <i>S. pimpinellifolium</i> LA0722) BC ₁	150 NaCl + 15 CaCl ₂	达到 50% 发芽率的 天数 Days to 50% germination rate	Foolad et al., 2007
	苗期 Seedling stage	6 & 4	4, 6, 9, 12 & 6, 7, 11	<i>S. lycopersicoides</i> LA2951IL, <i>S. pennellii</i> LA0716IL	700 NaCl + 70 CaCl ₂	耐盐级数 Salt tolerance scale	Li et al., 2011
	营养生长期 Vegetative stage	5	1, 3, 5, 9	(<i>S. lycopersicum</i> NC84173 × <i>S. pimpinellifolium</i> LA0722) BC ₁ , BC ₁ S ₁	700 NaCl + 70 CaCl ₂	耐盐级数 Salt tolerance scale	Foolad & Chen, 1999
	营养生长期 Vegetative stage	5	1, 3, 5, 6, 11	(<i>S. lycopersicum</i> NC84173 × <i>S. pimpinellifolium</i> LA0722) BC ₁ , BC ₁ S ₁	700 NaCl + 70 CaCl ₂	耐盐级数 Salt tolerance scale	Foolad et al., 2001
	营养生长期 Vegetative stage	125	1~12	M82, <i>S. pennellii</i> LA0716, <i>S. pennellii</i> LA0716IL	150 NaCl	抗氧化剂活性 Antioxidant activity	Frary et al., 2010
	营养生长期 Vegetative stage	311	1~12	M82, <i>S. pennellii</i> LA0716, <i>S. pennellii</i> LA0716IL	150 NaCl	生长参数、离子 含量 Growth parameters, ion concentration	Frary et al., 2011

续表 1

性状 Trait	发育阶段 Development Stage	QTL 数 Number of QTL	染色体 Chromosome	群体 Population	处理 Treatment	调查性状 Investigated Trait	参考文献 Reference
	芽期和开花期 Germination, flowering stage	1 & 1	8 & 8	<i>S. pennellii</i> LA716 IL8-3	150 NaCl	发芽率/顶腐病发病率 Germination rate /blossom-end rot disease incidence	Uozumi et al., 2012
	结果期 Fruit production stage	8	10, 11, 12	(<i>S. lycopersicum</i> × <i>S. pimpinellifolium</i>) F ₂	171.1 NaCl	总果质量、结果数、单果质量 Total fruit weight, fruit number, average fruit weight	Breto et al., 1994
	结果期 Fruit production stage	12	4, 5, 12	(<i>S. lycopersicum</i> × <i>S. pimpinellifolium</i>) F ₂	171.1 NaCl	总果质量、结果数、单果质量 Total fruit weight, fruit number, average fruit weight	Monforte et al., 1996
	结果期 Production stage	1	5	(<i>S. lycopersicum</i> E9 × <i>S. cheesmaniae</i> L2) F ₂	171.1 NaCl	早熟性、产量 Earliness, yield	Monforte et al., 1999
	全生育期 Whole development stages	15 & 16	1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12	(<i>S. lycopersicum</i> × <i>S. pimpinellifolium</i>) F ₇	200 NaCl	生长参数、产量、离子含量 Growth parameters, yield, ion concentration	Villalta et al., 2007
耐旱 Drought tolerance	芽期 Germination stage	4	1, 8, 9, 12	(<i>S. lycopersicum</i> NC84173 × <i>S. pimpinellifolium</i> LA0722) BC ₁ , BC ₁ S ₁	14% PEG	发芽率、发芽天数 Germination rate, germination days	Foolad et al., 2003
	芽期 Germination stage	9	1, 4, 5, 8, 9, 12	(<i>S. lycopersicum</i> NC84173 × <i>S. pimpinellifolium</i> LOA722) BC ₁	14% PEG	达到50%发芽的天数 Days to 50% germination rate	Foolad et al., 2007
	苗期 Seedling stage	1	5	<i>S. pennellii</i> LA0716 IL	碳同位素测量 Carbon isotope measurement	碳同位素组分 Carbon isotope composition	Xu et al., 2008
	苗期 Seedling stage	2	2, 9	<i>S. pennellii</i> LA0716 IL	15% PEG	叶片电导率 Leaf electric conductivity	Gong et al., 2010
	苗期 Seedling stage	3 & 11	3, 6, 12 & 1, 2, 3, 4, 5, 8, 9, 10, 12	<i>S. lycopersicoides</i> LA2951IL, 3 次干旱胁迫 <i>S. pennellii</i> LA0716IL	Three times drought stress	耐旱级数 Drought tolerance scale	刘磊等, 2011

3.2 耐旱和耐盐 QTL 的遗传及累加效应

目前, 对于从野生资源鉴定出的 QTL 的遗传及互作效应仍有不同的结论。通过对来自醋栗番茄 LA0722 的 F₂ 和 BC₁ 群体的研究认为, 芽期和苗期的耐旱、耐盐性受少数主效 QTL 和几个微效 QTL 控制, 且主效 QTL 效应较大, 部分耐盐的 QTL 在群体和种间是保守的, QTL 间仅存在很小或者无上位效应 (Foolad, 2004); 而其它研究表明, 番茄耐旱和耐盐性受众多 QTL 控制, 受环境影响较大, 每个 QTL 的效应较小, 互作明显 (Monforte et al., 1999; Villalta et al., 2007; Li et al., 2011; 刘磊等, 2011; Uozumi et al., 2012)。产生上述不同结论的主要原因可能是由于鉴定方法、作图群体及资源材料等不同。通过对来自耐旱和耐盐的潘那利番茄渐渗系片段的遗传分析发现, 一些 QTL 呈明显的显性效应, 互作呈典型的小于加性的效应 (Gur & Zamir, 2004; Li et al., 2011)。通过将耐旱的 IL7-5-5 (显性效应, 纯合体和杂合体在相对干旱条件下较对照 M82 增产 12%~22%)、IL8-3 (纯合体产量较对照 M82 低 34%, 杂合体增产 25%)、IL9-2-5 (典型的加性效应) (Gur & Zamir, 2004) 聚合, 育成了美国加州主栽品种 AB2 (Lippman et al., 2007)。虽然该成果为番茄耐旱和耐盐新品种选育展示了一定前景, 但其遗传效应更多受益于杂种优势, 耐旱和耐盐性只是相对而言。因此, 如何进一步利用这些鉴定出的 QTL 育成真正耐旱或耐盐的番茄品种, 仍然是一项庞大的工程和十分艰巨的任务。

4 基于基因工程的遗传改良

过去 20 年期间, 利用具有离子运输和区室化、渗透调节、活性氧清除、转录因子、胁迫蛋白等功能的基因, 进行番茄耐旱和耐盐基因工程的遗传改良, 已不同程度地提高了番茄的抗逆性(表 2)。其抗逆性提高的主要机制包括: (1) 离子运输和区室化: 主要通过位于质膜和液泡膜的两种逆向转运蛋白(Na^+/H^+ antiporter)限制 Na^+ 的内流、增强 Na^+ 的外排、维持较高的 K^+/Na^+ 以及将 Na^+ 区室化, 从而降低细胞质内 Na^+ 的浓度, 消除 Na^+ 对植物的毒害作用, 使植物在逆境下维持正常的生长发育; (2) 渗透调节: 甜菜碱、甘露醇、海藻糖、多胺、渗透蛋白等渗透调节物质在细胞内大量积聚, 稳定胞内的渗透平衡, 增强细胞的保水能力。通过渗透调节, 不仅提高了植物的耐旱和耐盐性, 而且提高了耐寒、耐高温、抗氧化胁迫等非生物胁迫的能力; (3) 活性氧清除: 草酸氧化酶、抗坏血酸过氧化酶等抗氧化剂可以清除活性氧和自由基对细胞的损伤, 维持离子平衡和膜的稳定, 从而抵抗非生物胁迫对植物造成的氧化伤害; (4) 转录因子: 也称为反式作用因子, 能够与真核基因启动子区域中顺式作用元件发生特异性的相互作用, 通过它们之间以及与其它相关蛋白之间的相互作用, 激活或抑制转录。将调控抗逆相关基因的转录因子转入植物, 可以启动多个胁迫相关基因的表达, 提高植物对非生物胁迫和生物胁迫的抗性; (5) 胁迫蛋白: 受 ABA、渗透或低温胁迫诱导产生, 具有很高的亲水性和热稳定性, 可以重新定向细胞内的水分子, 束缚盐离子, 以减轻脱水对细胞造成的不良影响; 促进水分的长距离运输和细胞内外的跨膜水分运输; 一些蛋白还可以维持细胞膜和蛋白质结构及功能的稳定性。

表 2 利用基因工程转入番茄的耐旱和(或)耐盐相关基因

Table 2 The transformed genes conferring salt and/or drought tolerance by genetic engineering in tomato

分类 Catalog	基因 Gene	功能或编码蛋白质 Function/ Coding protein	作用机理 Mechanism	抗性 Resistance	参考文献 Reference
离子运输和 区室化 Ion transportation and compart- mentalization	<i>AtNHX1</i>	液泡膜 Na^+/H^+ 逆向转运蛋白 Tonoplast Na^+/H^+ antiporter	Na^+ 和 H^+ 反向运输, 使细胞质多余 的 Na^+ 排入液泡 Reverse transportation of Na^+ and H^+ , extra Na^+ flowing into vacuole	耐盐 Salt tolerance	Zhang & Blumwald, 2001
	<i>PgNHX1</i>	液泡膜 Na^+/H^+ 逆向转运蛋白 Tonoplast Na^+/H^+ antiporter	Na^+ 和 H^+ 反向运输, 使细胞质多余 的 Na^+ 排入液泡 Reverse transportation of Na^+ and H^+ , extra Na^+ flowing into vacuole	耐盐 Salt tolerance	Bhaskaran & Savithramma, 2011
<i>LeNHX2</i>		K^+/H^+ 逆向转运蛋白 K^+/H^+ antiporter	促进 K^+ 在细胞内区室化 Facilitating K^+ compartmentalization	耐盐 Salt tolerance	Rodríguez-Rosales et al., 2008
	<i>HAL1</i>	耐盐的胞质蛋白 Salt tolerant cytoplasm protein	促进 K^+/Na^+ 的选择, 细胞内 K^+ 的积 累和 Na^+ 含量的降低 Facilitating K^+/Na^+ selectivity and intracellular K^+ accumulation, decreasing intracellular Na^+ concentration	耐盐 Salt tolerance	Gisbert et al., 2000; Rus et al., 2001
<i>HAL2</i>		二磷酸 - 3 - 核苷酸酶 Bisphosphate-3-nucleotidase	将 PAP 和 PAPS 去磷酸化 Dephosphorylating PAP and PAPS	耐盐 Salt tolerance	Arrillaga et al., 1998
<i>AVP1</i>		液泡内 H^+ - 焦磷酸酶 Vacuolar H^+ -PPase	促进离子积累; 降低液泡渗透势; 促进根系生长 Facilitating ion accumulation, reducing vacuolar solute potential, promoting root development	耐旱 Drought tolerance	Park et al., 2005
<i>MdVHP1</i>		液泡内 H^+ - 焦磷酸酶 Vacuolar H^+ -PPase	促进离子积累; 维持较高相对水分 含量; 降低液泡渗透势 Facilitating ion accumulation, maintaining high RWC and low solute potential	耐盐、耐旱 Salt and drought tolerance	Dong et al., 2011

续表 2

分类 Catalog	基因 Gene	功能或编码蛋白质 Function/ Coding protein	作用机理 Mechanism	抗性 Resistance	参考文献 Reference
渗透调节 Osmotic regulation	<i>BADH</i>	甜菜碱醛脱氢酶 Betaine aldehyde dehydrogenase	催化甜菜碱醛转化为甜菜碱 Converting betaine aldehyde into glycinebetaine	耐盐 Salt tolerance	Moghaieb et al., 2000; Jia et al., 2002
	<i>betA</i>	胆碱脱氢酶 Choline dehydrogenase	催化胆碱生成甜菜碱 Converting choline into glycinebetaine	耐盐 Salt tolerance	王淑芳等, 2001
	<i>CodA</i>	胆碱氧化酶 Choline oxidase	催化胆碱生成甜菜碱 Converting choline into glycinebetaine	耐盐、耐旱、耐寒、抗氧化 Salt, drought and cold tolerance, antioxidation	Park et al., 2007; Goel et al., 2011
	<i>TPS1</i>	海藻糖 - 6 - 磷酸合成酶 Trehalose-6-phosphate synthase	参与糖类代谢和渗透调节 Participating in sugar metabolism and osmoregulation	耐盐、耐旱、抗氧化 Salt and drought tolerance, antioxidation	Cortina & Culianez-Macia, 2005
	<i>Atlpk2β</i>	肌醇多磷酸盐 6-3-激酶 Inositol polyphosphate 6-3-kinase	参与胞内信号转导和维持 Ca^{2+} 平衡 Cellular signal transduction and Ca^{2+} homeostasis maintenance	耐旱、耐寒、抗氧化 Drought and cold tolerance, antioxidation	Zhang et al., 2009
	<i>MtlD</i>	甘露醇 - 1 - 磷酸脱氢酶 Mannitol-1-phosphate dehydrogenase	参与甘露醇的形成 Involving in biosynthesis of mannitol	耐盐、耐旱、耐寒 Salt, drought and cold tolerance	Khare et al., 2010
	<i>Osmotin</i>	24-kD 多功能胁迫响应蛋白 24-kD multifunction stress response protein	参与细胞渗透调节; 维持蛋白质结构稳定 Cell osmoregulation and protein structure stability maintenance	耐盐、耐旱 Salt and drought tolerance	Goel et al., 2010
	<i>OXO</i>	草酸氧化酶 Oxalate oxidase	降解草酸的毒性, 参与胁迫防御 Oxalate toxicity degradation and stress defense	耐盐 Salt tolerance	Dessalegne et al., 1997
	<i>cAPX</i>	细胞质抗坏血酸过氧化酶 Cytosolic ascorbate peroxidase	清除活性氧的毒害 Eliminating ROS toxicity	耐盐、耐寒、耐高温、抗 UV-B Salt, cold, heat and UV-B tolerance	Wang et al., 2005, 2006
	<i>SIGMEs</i>	GDP - 甘露糖 3',5' - 表异构酶 GDP-Mannose 3',5'-epimerase	参与抗坏血酸的生物合成 Involving in biosynthesis of ascorbic acid	耐盐、耐寒、抗氧化 Salt and cold tolerance, antioxidant stress	Zhang et al., 2011
活性氧清除 Active oxygen elimination	<i>MdSPDS1</i>	亚精胺合成酶 1 Spermidine synthase 1	促进亚精胺和精胺等多胺积累; 提高抗坏血酸过氧化酶的活性 Facilitating spermidine and spermine accumulation, enhancing APX activity	耐盐 Salt tolerance	Neily et al., 2011
	<i>PtADC</i>	精氨酸脱羧酶 Arginine decarboxylase	促进多胺的积累, 清除活性氧的毒害 Facilitating polyamines accumulation and eliminating ROS toxicity	耐旱 Drought tolerance	Wang et al., 2011
	<i>Samdc</i>	S - 腺苷甲硫氨酸脱羧酶 S-adenosylmethionine decarboxylase	参与多胺的合成; 自由基清除 Involving in biosynthesis of polyamines; radicals scavenging	耐盐、耐旱、耐寒、耐高温、抗病 Salt, drought, cold and heat tolerance; disease resistance	Hazarika & Rajam, 2011
	<i>CBF1</i>	转录因子 Transcription factor	转录激活因子, 对低温和水分胁迫产生响应 Transcription activated factor, response for low temperature and water stress	耐盐、耐旱、耐寒 Salt, drought and cold tolerance	Hsieh et al., 2002; Lee et al., 2003
转录因子 Transcription factor	<i>Osmyb4</i>	MYB 转录因子 MYB transcription factor	诱导胁迫相关基因的表达 Inducing expression of stress related genes	耐旱、抗病毒病 Drought tolerance; viral disease resistance	Vannini et al., 2007

续表 2

分类 Catalog	基因 Gene	功能或编码蛋白质 Function/ Coding protein	作用机理 Mechanism	抗性 Resistance	参考文献 Reference
	<i>LeERF1</i>	转录因子 Transcription factor	可溶性糖、脯氨酸在胞内积累; 启动胁迫相关基因的表达 Facilitating soluble sugar and praline accumulation, inducing expression of stress related gene	耐旱 Drought tolerance	Lu et al., 2010
	<i>SIAREB</i>	类 AREB 转录因子 AREB-like transcription factor	在 ABA 和非生物胁迫下诱导相关基因的表达 Inducing expression of stress-responsive genes under ABA and abiotic stress	耐盐、耐旱 Salt and drought tolerance	Hsieh et al., 2010
	<i>SIERF3△RD</i>	EAR 结构域缺失的转录因子 EAR motif deletion transcription factor	降低膜的脂质过氧化作用; 诱导病原相关蛋白基因的表达 Reducing membrane lipid peroxidation, inducing pathogen related protein gene expression	耐盐、抗病 Salt tolerance, disease resistance	Pan et al., 2010
	<i>SIERF1</i>	AP2/ERF 转录因子 AP2/ERF transcription factor	可溶性糖、脯氨酸等在胞内积累; 启动胁迫相关基因的表达 Facilitating soluble sugar and praline accumulation, inducing expression of stress related gene	耐盐 Salt tolerance	Lu et al., 2011
	<i>SIERF5</i>	AP2/ERF 转录因子 AP2/ERF transcription factor	诱导胁迫相关基因的表达 Inducing expression of stress related genes	耐盐、耐旱 Salt and drought tolerance	Pan et al., 2012
胁迫蛋白 Stress related proteins	<i>bspA</i>	66-kD 热稳定蛋白 66-kD boiling stable protein	在 ABA 和非生物胁迫下, 促进热稳定蛋白在细胞内积累 Facilitating boiling stable protein accumulation under ABA and abiotic stress	耐旱 Drought tolerance	Roy et al., 2006
	<i>HVA1</i>	胁迫诱导 LEA 蛋白 Stree induced LEA protein	LEA 蛋白积累, 增强植株抵抗胁迫的能力 LEA protein accumulation to increase plants stress resistance	耐盐 Salt tolerance	陈火英 等, 2006

5 问题与展望

5.1 番茄耐旱和耐盐的表型鉴定

随着科学技术的快速发展, 尤其是不同物种基因组测序的完成, 获得了大量的基因组、转录组、代谢组的数据, 而对这些数据的注释, 需要准确的表型数据以研究和揭示其功能。标准的表型数据或者表型组学结果, 不仅可用于不同地点、不同研究者、不同年份的比较分析, 而且可用于对受效应较小的多基因控制的基因组进行选择 (Genome Selection, GS) (Cabrera-Bosquet et al., 2012)。近几年, 高通量和标准化表型分析或表型组学备受关注 (Volk, 2010; Cabrera-Bosquet et al., 2012)。标准化表型数据将有助于基因型和表型分析, 对作物的产量、品质及抗逆性进行改良 (Volk, 2010)。Famoso 等 (2010) 建立了新的表型鉴定体系, 对耐铝性差异较大且适应不同营养液生长的谷物和水稻进行比较分析。番茄的耐旱和耐盐性受众多 QTL 控制, 不同研究者采用不同的处理和鉴定方法, 如耐旱时间、耐盐浓度、离子组分等不一致, 导致一些结果无法比较分析。譬如, Katerji 等 (2000) 将番茄划分为中度盐敏感作物; Dasgan 等 (2002) 将 $200 \text{ mmol} \cdot \text{L}^{-1}$ NaCl 胁迫下的番茄基因型生长期盐害级数 (Salinity Scale Classes) 分为 1~5 级; 而为了鉴定芽期、苗期、营养生长期、开花结果期的耐盐 QTL, 采用了不同的盐处理浓度 ($\text{mmol} \cdot \text{L}^{-1}$) 和方法: $150 \text{ NaCl} + 15 \text{ CaCl}_2$, $175 \text{ NaCl} + 17.5 \text{ CaCl}_2$, $200 \text{ NaCl} + 20 \text{ CaCl}_2$, $700 \text{ NaCl} + 70 \text{ CaCl}_2$, 150 NaCl , 171.1 NaCl , 200 NaCl 等 (表 1)。就海水而言, 大约含有 3% 的 NaCl, 其中 Na^+ 浓度为 $460 \text{ mmol} \cdot \text{L}^{-1}$, Mg^{2+} 浓度为 $50 \text{ mmol} \cdot \text{L}^{-1}$,

Cl⁻浓度为 540 mmol·L⁻¹ (Chen, 1946), 而不同类型的土壤盐渍化则存在更大的差异。对于番茄耐旱性鉴定, 则采用 ABA 和 PEG 处理, 一次干旱胁迫和多次干旱胁迫等不同的处理方法 (Karaba, 2007; Gong et al., 2010; 刘磊 等, 2011)。因此, 耐盐和耐旱标准鉴定方法的确立, 已成为深入挖掘番茄野生资源优异目标基因和培育耐盐、耐旱新品种亟待解决的问题。

5.2 基于全基因组关联分析的野生资源基因的挖掘

近几年, 基于脊椎动物的不相关个体基因型和表型连锁分析的关联作图 (Association Mapping), 为作物遗传改良提供了新的思路 (Rafalski, 2010)。随着第二代测序技术的飞速发展, 大量物种全基因组测序的完成, 关联作图更显示出明显的优势。与传统的连锁作图相比, 关联作图具有以下优点: (1) 利用不相关的个体, 不需要构建作图群体, 省时、省费用; (2) 可以进行多点、多次重复试验; (3) 可获得高分辨率的图谱, 检测多个等位点; (4) 广泛的参考群体。目前, 该策略已应用于水稻、玉米、大豆、大麦、甜菜等作物遗传资源的挖掘与改良 (McMullen et al., 2009; Stich, 2009; Rosenberg et al., 2010)。最典型的是利用巢式关联作图 (Nested Association Mapping) 对玉米的遗传组成进行分析 (McMullen et al., 2009; Tian et al., 2011)。实践证明, 关联作图是深入挖掘作物有益基因和 QTL 的有效途径 (Stich, 2009)。番茄作为经典的双子叶模式植物, 对其研究极其深入而广泛。但利用关联分析挖掘现有资源和野生资源的报道却十分有限, 仅对少数栽培种和野生种进行了初步探讨 (Arunyawat et al., 2007; van Berloo et al., 2008; Labate et al., 2009), 其主要原因是栽培种遗传背景狭窄 (Shirasawa et al., 2010)。野生种秘鲁番茄和智利番茄自然群体的连锁不平衡 (Linkage Disequilibrium, LD) 衰减极其迅速, 为未来进行关联作图, 分析其功能变异展示了十分可观的前景 (Arunyawat et al., 2007)。Causse 等 (2011) 成功利用 *S. lycopersicum* var. *cerasiforme* 群体, 对影响番茄品质的性状进行了关联分析。另外, 由于野生资源长期通过昆虫授粉, 重组度非常高, 为高精度作图和精细定位功能位点提供了十分有利的条件 (Rosenberg et al., 2010)。番茄栽培种基因组测序已经完成, 野生种醋栗番茄 LA1589 和潘那利番茄 (*S. pennellii*) LA0716 的测序正在进行, 这不仅为开发大量覆盖全基因组的 SNP 标记奠定了基础, 也为利用全基因组关联分析, 深入、广泛地挖掘野生资源耐旱和耐盐基因提供了可能。

5.3 基因工程

大规模植物基因组测序以及宏基因组学工程的开展, 为作物抗逆基因工程提供了更为丰富的基因来源 (Mittler & Blumwald, 2010)。目前, 通过基因工程将基因聚合以提高番茄耐旱或耐盐性的报道不多, 在两个基因的转化方面, 刘晶等 (2005) 将 *BADH* 导入转 *AtNHX1* 的番茄中得到二价转基因植株, 其耐盐性及各项生理指标均优于野生型和单基因植株; *AVP1* 和 *PgNHX1* 在番茄中共同表达使植株的耐盐性均优于野生型和单基因植株 (Bhaskaran & Savithramma, 2011)。对于多基因的转化, 由于多个外源酶蛋白的掺入, 应注意转基因表达的位置、水平及时间, 并确保每种酶反应所需要的条件, 避免复合基因转化可能带来的副作用 (Holmberg & Bulow, 1998)。另外, 通过转入单个转录因子启动多个目标基因的表达也是一种十分有效的策略。到目前为止, 虽然得到了不同作物的大量转基因后代, 但获得的耐旱和耐盐品种却极少。因此, 如何利用基因工程技术进一步提高作物的耐旱和耐盐性, 仍值得思考。

5.4 耐旱和耐盐基因协同和特异调控

植物在生长发育过程中, 一般要同时应对多种生物或非生物胁迫, 尤其是干旱和盐碱通常重叠

交叉发生。在适应环境的过程中, 植物演化形成了应对不同逆境的相同或特异的响应机制 (Verslues et al., 2006)。番茄对干旱和盐胁迫响应的基因包括转录因子、胁迫防御、信号转导、ROS清除、细胞壁代谢、激素代谢等 (Gong et al., 2010; Sun et al., 2010)。植物具有多个胁迫识别和信号转导途径, 一些途径交互发生, 一些途径特异调控 (Zhu, 2002), 如植物对干旱和高盐引发的渗透胁迫的调控存在着显著差异, 干旱诱导的渗透胁迫可通过降低气孔开度和表皮水分散失来提高水分利用率; 而盐害产生的渗透胁迫则通过渗透调节保持离子平衡。现已发现, 植物对多种胁迫和一种胁迫的反应并不相同, 应对多种胁迫受一系列复杂和特异的调控网络的调控 (Atkinson & Urwin, 2012), 因此, 利用单一胁迫技术对多重胁迫进行鉴定可能不适合 (Mittler & Blumwald, 2010)。耐旱和耐盐基因的协同和特异调控机制的明确, 将为番茄耐旱和耐盐遗传改良提供必要的依据。

5.5 小 RNA 分子

小 RNA (Small RNAs) 包括 microRNAs (miRNAs) 和小干扰 RNA (siRNAs), 其中最保守、最典型的为 miRNAs。近几年, 大量文献报道 20 多种 miRNAs 参与植物的耐旱和耐盐调控, 其中 miR393、miR160、miR167、miR169 等在受到干旱或盐胁迫时, 均被上调或下调, 这类 miRNAs 在植物抗逆过程中发挥着重要作用 (Sunkar et al., 2007; Naqvi et al., 2008)。但相关研究在番茄中还较少, Sly-miR169c 在番茄中过表达, 可减少气孔张开, 降低蒸腾速率, 提高耐旱性 (Zhang et al., 2011)。目前, 对于小 RNA 的调控机制还不十分清楚, 而且其鉴定主要依赖于测序谱, 存在一定偏差, 需逐个鉴定 (Sunkar et al., 2007), 但其为明确植物抗逆的复杂调控提供了新的技术手段, 也为利用小 RNA 介导基因调控, 提高植物的耐旱和耐盐性提供了可能。

5.6 表观遗传调控

表观遗传学是指基于非基因序列改变所引起的基因表达水平的变化。环境信号诱导基因组的表观修饰, 如 DNA 甲基化、组蛋白修饰、染色体重塑等, 可改变染色体的结构, 影响基因的表达, 并可遗传给下一代, 提高植物后代的抗逆性 (Chinnusamy & Zhu, 2009)。现已发现, 几种核染色质相关蛋白, 包括组蛋白修饰酶、链接组蛋白 H1 和核染色质重塑复合体, 影响胁迫响应的基因调控 (Kim et al., 2010)。目前, 已鉴定出的与番茄耐旱有关的链接组蛋白包括 H1-D 和 H1-S, 其中 H1-S 具有多个功能, 包括参与气孔电导的负调控、在不同时间点控制植株的水分状态等 (Wei & O'Connell, 1996; Scippa et al., 2004)。Gonzalez 等 (2011) 证明了跨越番茄 *Asr1* 基因 (一种非转座的蛋白编码基因) 的 CNN - 甲基化等位基因 (CNN-methylated epialleles) 的存在, 另外, 为对表观遗传修饰和作物对非生物胁迫的适应性存在相关性的假设进行验证, 对叶片内 *Asr1* DNA 的胞嘧啶甲基化状态进行了研究, 发现干旱胁迫导致所分析的 110 个非对称位点中的 75 个位点甲基化标记去除, 并伴随着抑制性 H3K27me3 表观遗传标记的减少和 RNA 水平表达的大幅度降低, 这些去甲基化大多数发生在内含子区域。非生物胁迫诱导表观遗传变异, 使植物发生适应性响应, 有利于植物生存, 而且还可传给后代, 即植物具有胁迫记忆性 (Chinnusamy & Zhu, 2009)。这种跨代表观遗传变异 (transgenerational epigenetic variation) 有可能产生新的改变转录的等位点, 从而影响植物表型的多样性, 在植物进化过程中发挥重要作用 (Turner, 2011), 也可利用其最大程度地挖掘作物后代的产量等潜力 (Chinnusamy & Zhu, 2009)。另外, 人们还发现, 植物小 RNA 也介导表观遗传修饰 (Simon & Meyers, 2011)。番茄 12 个野生或野生近缘种, 具有丰富的表型变异, 分布极其广泛, 适应不同的生态地理区域。应用表观遗传学, 比较分析栽培种和野生种对干旱和盐碱的适应性差异, 有望为深入了解野生资源的耐旱和耐盐性并为番茄遗传改良提供新的线索。

综上所述,虽然通过从野生资源中挖掘抗性基因或 QTL 以及利用基因工程来提高番茄的耐旱和耐盐性已取得一些进展,但与实际生产需要仍存在较大差距。过去几十年,由于技术条件限制,从野生资源挖掘出的耐旱和耐盐基因十分有限。随着不同物种全基因组测序的完成,全基因组关联分析、小 RNA、表观遗传学等新技术和新策略的产生及利用,特别是对耐旱和耐盐物种遗传进化机制的明确,将为深入挖掘抗逆相关基因和理解抗逆机制提供新的线索与途径。挖掘出的大量野生资源及基因经过进化选择,在所确定的栽培种基因组水平上,构建庞大而复杂的耐旱耐盐调控体系,仍需对作物生长发育的调控网络进行深入了解。另外,在作物耐旱和耐盐遗传改良的同时,进一步提高现有作物栽培种的产量,是应对干旱和盐碱环境的双重举措。

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