

• 农业生物技术 •

大豆 *GmGolS2-2* 提高转基因烟草耐旱性

于海伟¹, 邱爽¹, 张军², 李珊珊¹, 孙天国¹, 马天意¹, 赵艳¹, 赵旭³, 翟莹^{1*}

1 齐齐哈尔大学生命科学与农林学院, 黑龙江 齐齐哈尔 161006

2 黑龙江省农业科学院畜牧兽医分院, 黑龙江 齐齐哈尔 161005

3 吉林省产品质量监督检验院, 吉林 长春 130022

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YU Haiwei, QIU Shuang, ZHANG Jun, LI Shanshan, SUN Tianguo, MA Tianyi, ZHAO Yan, ZHAO Xu, ZHAI Ying. Soybean *GmGolS2-2* improves drought resistance of transgenic tobacco[J]. Chinese Journal of Biotechnology, 2023, 39(7): 2762-2771.

摘 要: 肌醇半乳糖苷合成酶(galactinol synthase, *GolS*)基因在植物应对非生物胁迫时发挥重要作用。本研究构建大豆 *GmGolS2-2* 基因植物表达载体并转化烟草, 对转基因烟草的耐旱性进行鉴定。通过逆转录-聚合酶链式反应(reverse transcription-polymerase chain reaction, RT-PCR)从大豆叶片中克隆了 975 bp 的 *GmGolS2-2* 基因编码序列。通过限制性内切酶位点 *Nde* I 和 *Eco*R I 将 *GmGolS2-2* 基因与植物表达载体 pRI101 相连, 通过叶盘法转化烟草。基因组 DNA PCR 和实时荧光定量 PCR 结果显示, 共获得 3 棵 *GmGolS2-2* 转基因烟草。干旱胁迫下 *GmGolS2-2* 转基因烟草的生长状态好于野生型烟草。干旱胁迫处理后, 转基因烟草的电解质渗透率和丙二醛含量低于野生型烟草, 脯氨酸含量和可溶性糖含量则高于野生型烟草。实时荧光定量 PCR 结果显示, *GmGolS2-2* 的异源表达提高了转基因烟草中抗逆相关基因 *NtERD10C* 和 *NtAQPI* 的表达量。以上结果表明, *GmGolS2-2* 提高了转基因烟草的耐旱性。

关键词: 大豆; 肌醇半乳糖苷合成酶; 抗旱基因; 遗传转化; 烟草

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*Corresponding author. E-mail: fairy39809079@126.com

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Soybean *GmGolS2-2* improves drought resistance of transgenic tobacco

YU Haiwei¹, QIU Shuang¹, ZHANG Jun², LI Shanshan¹, SUN Tianguo¹, MA Tianyi¹, ZHAO Yan¹, ZHAO Xu³, ZHAI Ying^{1*}

¹ College of Life Science and Agroforestry, Qiqihar University, Qiqihar 161006, Heilongjiang, China

² Branch of Animal Husbandry and Veterinary of Heilongjiang Academy of Agricultural Sciences, Qiqihar 161005, Heilongjiang, China

³ Jilin Province Institute of Product Quality Supervision and Inspection, Changchun 130022, Jilin, China

Abstract: Galactinol synthase (GolS) genes play important roles in plant response to abiotic stress. In this research, the plant expression vector of soybean *GmGolS2-2* gene was constructed and transformed into tobacco to study the drought tolerance of transgenic tobacco. A *GmGolS2-2* gene with 975 bp coding sequence was cloned from soybean leaves by reverse transcription-polymerase chain reaction (RT-PCR). *GmGolS2-2* was linked to the plant expression vector pRI101 by restriction enzyme sites *Nde* I and *Eco*R I, and transformed into tobacco by leaf disc method. Genomic DNA PCR and real-time PCR showed that three *GmGolS2-2* transgenic tobacco plants were obtained. The growth status of *GmGolS2-2* transgenic tobacco under drought stress was better than that of wild-type tobacco. After drought stress treatment, the electrolyte leakage and malondialdehyde content of transgenic tobacco were lower than those of wild-type tobacco, but the proline content and soluble sugar content were higher than those of wild-type tobacco. The results of real-time PCR showed that the heterologous expression of *GmGolS2-2* increased the expression of stress-related genes *NtERD10C* and *NtAQPI* in transgenic tobacco. The above results indicated that *GmGolS2-2* improved drought resistance of transgenic tobacco.

Keywords: soybean; galactinol synthase; drought resistant gene; genetic transformation; tobacco

大豆作为我国主要经济作物之一，是重要的蛋白质和食用油来源，在农业生产中占有非常重要的地位。大豆和其他作物一样易受低温、干旱和高盐等非生物胁迫的危害，不仅影响大豆的生长发育，还影响其产量和品质^[1-3]。

当受到非生物逆境胁迫时，不同种类的渗透调节物质可以在植物体内快速合成并积累，以此来增强植物细胞的渗透调节能力，维持细胞正常生理机能，进而提高植物的抗逆能力。棉子糖系列寡糖(raffinose family oligosaccharides, RFOs)属于可溶性糖，它们是植物体内典型的渗透调节物质之一，在高等植物中的含量仅次于

蔗糖^[4-5]。研究发现，植物遭受逆境胁迫后，RFOs 含量的迅速升高既可以提高植物细胞的保水能力，也可以协助清除自由基，稳定细胞膜的结构^[6-7]。

RFOs 的生物合成始于肌醇半乳糖苷合成酶(galactinol synthase, GolS)催化肌醇和尿苷二磷酸半乳糖转化为肌醇半乳糖苷^[8]。作为第一个将碳转化为 RFOs 的生物合成酶，GolS 对 RFOs 的积累有显著影响，因此 GolS 的编码基因往往与植物的非生物胁迫耐受性密切相关^[9]。在植物中，GolS 基因一般以多基因家族形式存在，但不同植物中的 GolS 基因数目差异较大^[6]。

作为模式植物,拟南芥中已发现 7 个 *GolS* 基因,其中 *AtGolS1* 和 *AtGolS2* 响应干旱和高盐胁迫,*AtGolS3* 响应低温胁迫^[10]。后续鉴定表明,*AtGolS1* 和 *AtGolS2* 的过表达可以促进肌醇半乳糖苷和棉子糖的积累,从而提高转基因植株对非生物胁迫的耐受性^[7,11]。花生中存在 10 个 *GolS* 基因,干旱条件下它们的表达量大多上调。其中,*AdGolS3* 作为 *AtGolS2* 的同源基因,可以抑制转基因拟南芥的代谢紊乱和氧化损伤,提高对干旱和盐胁迫的耐受性^[12]。研究发现,WRKY 转录因子可以调控 *GolS* 基因的表达。例如,旋蒴苣苔 *BhWRKY1* 可能通过脱落酸(abscisic acid, ABA)依赖的信号通路来调控 *BhGolS1* 的表达,从而导致 RFOs 在叶片中的积累^[13]。同样,在水稻中过表达 *OsWRKY11* 可以使 *OsGolS1* 和 *OsGolS2* 的表达显著上调,进而导致棉子糖过度积累,赋予转基因植株脱水耐受性^[14]。此外,在水稻中非生物胁迫也可以通过保留内含子来对 *GolS* 基因的表达进行转录后调控,表明 *GolS* 基因表达调控的复杂性^[15]。

本课题组前期研究发现,大豆 *GmGolS2-2* 的表达能够被干旱、高盐和低温胁迫诱导,尤其在干旱胁迫下 *GmGolS2-2* 的表达量升高近 200 倍^[16]。为此,本研究将 *GmGolS2-2* 构建植物表达载体,转化烟草后进行抗旱性鉴定,为植物抗旱基因工程育种提供优良的候选基因资源。

1 材料与方法

1.1 材料

供试植物材料为‘北豆 9 号’大豆和‘NC89’烟草,均由本实验室保存。

1.2 RNA 提取及 cDNA 反转录

取 0.1 g 大豆嫩叶,使用 RNAiso Plus 试剂(TaKaRa)提取大豆叶片总 RNA 并反转录成第一链 cDNA (cDNA 反转录试剂盒购自

Innovagene 公司)。

1.3 基因克隆

根据 *GmGolS2-2* (GenBank 登录号:XM003555744)基因编码序列设计 PCR 扩增引物,上游引物 5'-GGAATTCCATATGATGGCTCCTAATATCACCACC-3', (下划线代表 *Nde* I 酶切位点),下游引物 5'-GGAATTCCTAAGCAGCAGATGGGGC-3' (下划线代表 *Eco*R I 酶切位点)。通过 PCR 扩增 *GmGolS2-2*,退火温度设置为 58 °C,模板为合成的第一链 cDNA。PCR 扩增产物连接 pMD18-T 克隆载体(TaKaRa)后转化大肠杆菌(*Escherichia coli*) DH5 α 感受态细胞。pMD18-T-*GmGolS2-2* 重组载体质粒先经 *Nde* I 和 *Eco*R I 进行双酶切验证,后送生工生物工程(上海)股份有限公司进行测序验证。*GmGolS2-2* 基因核苷酸和蛋白质氨基酸序列的比对使用 DNAMAN 软件。

1.4 植物表达载体的构建及烟草遗传转化

pMD18-T-*GmGolS2-2* 重组载体质粒经 *Nde* I 和 *Eco*R I 双酶切,回收目的基因片段。使用 DNA Ligation Kit (TaKaRa)将 *GmGolS2-2* 与带有 *CaMV35S* 启动子的植物表达载体 pRI101 (TaKaRa)连接,转化大肠杆菌 DH5 α 感受态细胞。使用 *Nde* I 和 *Eco*R I 双酶切 pRI101-*GmGolS2-2* 重组载体质粒,经验证后的质粒转化根癌农杆菌 EHA105 感受态细胞。通过农杆菌菌液 PCR 对含 pRI101- *GmGolS2-2* 的转基因工程菌进行验证。烟草的遗传转化采用农杆菌侵染烟草叶盘法^[17]。在 MS 培养基中添加 50 mg/L 卡那霉素筛选 T₀ 代和 T₁ 转基因烟草。

1.5 转基因烟草鉴定

使用基因组提取试剂盒(TaKaRa)提取 T₀ 和 T₁ 代转基因烟草叶片基因组 DNA。以转基因烟草基因组 DNA 作为模板,野生型(wild-type, WT)烟草基因组 DNA 和 pRI101-*GmGolS2-2* 重组载

2.2 *GmGolS2-2* 转基因烟草筛选及鉴定

在培养中通过添加卡那霉素筛选抗性愈伤组织(图 2A), 筛选出的抗性芽如图 2B 所示, 抗性芽生根如图 2C 所示。

T₀ 代转基因烟草基因组 DNA PCR 扩增结果显示(图 3A), 获得的 3 棵烟草抗性苗中均克隆出目的基因条带, 将这 3 棵 *GmGolS2-2* 转基因烟草分别命名为 OE1、OE2 和 OE3。qPCR 检测结果同样显示(图 3B), 在转基因烟草(OE1、OE2 和 OE3)中均检测到 *GmGolS2-2* 的表达。

GolS 活性检测结果如图 4 所示, GolS 活性在 3 个 *GmGolS2-2* 转基因烟草植株中(OE1、

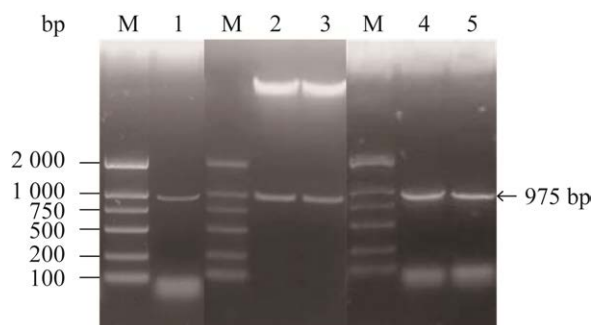


图1 *GmGolS2-2*克隆及植物表达载体构建 M: DL2000分子量标记物; 1: *GmGolS2-2* PCR; 2-3: pRI101-*GmGolS2-2*质粒双酶切; 4-5: 转化农杆菌PCR

Figure 1 Cloning and plant expression vector construction of *GmGolS2-2*. M: DL2000 marker; 1: PCR of *GmGolS2-2*; 2-3: Double digestion of pRI101-*GmGolS2-2* plasmid; 4-5: PCR of transformed *Agrobacterium tumefaciens*.

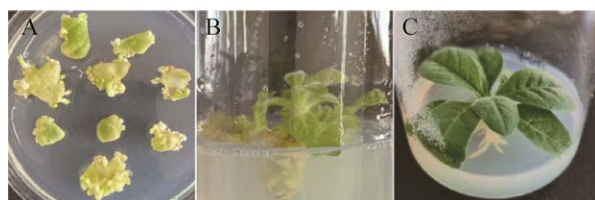


图2 *GmGolS2-2*转基因烟草的筛选 A: 愈伤组织. B: 抗性苗. C: 生根

Figure 2 Screening of *GmGolS2-2* transgenic tobacco. A: Callus. B: Resistant seedling. C: Rooting.

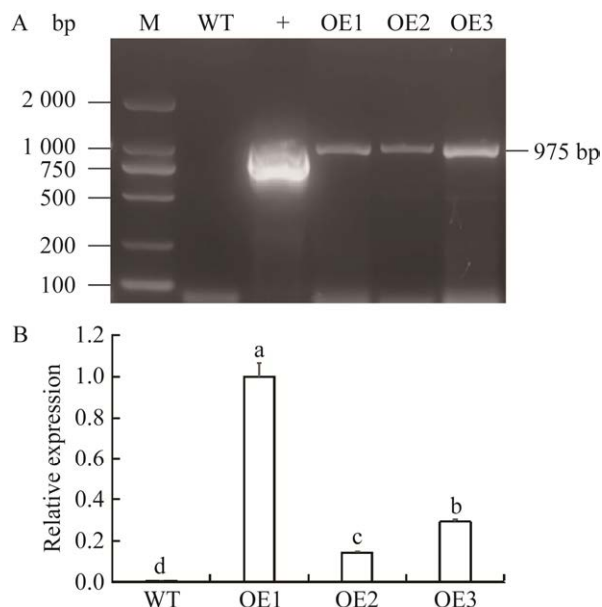


图3 *GmGolS2-2* 转基因烟草鉴定 A: 转基因烟草基因组 DNA PCR 检测. B: *GmGolS2-2* 在转基因烟草中的表达量; M: DL2000 分子量标记物; WT: 野生型烟草; +: pRI101-*GmGolS2-2* 阳性质粒; OE1-OE3: T₀ 代转基因烟草植株. 不同小写字母表示差异显著性($P<0.05$)

Figure 3 Identification of *GmGolS2-2* transgenic tobacco. A: Genomic DNA PCR detection of transgenic tobacco. B: Expression levels of *GmGolS2-2* in transgenic tobacco plants; M: DL2000 marker; WT: Wild-type tobacco plants; +: pRI101-*GmGolS2-2* positive plasmid; OE1-OE3: T₀ transgenic tobacco plants. Different lowercase letters indicate significant difference ($P<0.05$).

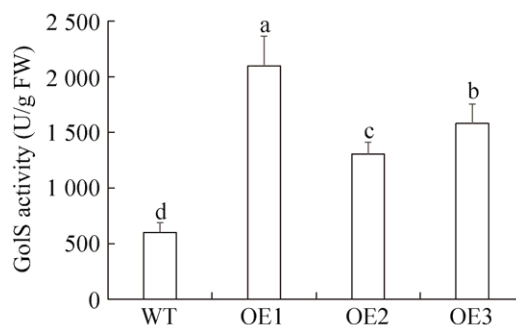


图4 *GmGolS2-2*转基因烟草GolS活性 不同小写字母表示差异显著性($P<0.05$)

Figure 4 GolS activity in *GmGolS2-2* transgenic tobacco. Different lowercase letters indicate significant difference ($P<0.05$).

OE2 和 OE3)均显著高于 WT 烟草,此结果表明 *GmGolS2-2* 在转基因烟草中的转录产物具有 *GolS* 催化活性。

2.3 *GmGolS2-2* 转基因烟草抗旱性鉴定

GmGolS2-2 转基因烟草停止浇水 16 d 内

的状态如图 5 所示,WT 烟草脱水速度明显快于转基因烟草。此外,PEG8000 溶液浇灌 *GmGolS2-2*转基因烟草 36 h 后的状态(图 6)同样显示,WT 烟草萎蔫比较严重,而转基因烟草状态变化不大。



图5 *GmGolS2-2*转基因烟草停止浇水后状态 WT: 野生型烟草; OE1–OE3: T₁代转基因烟草植株
Figure 5 State of *GmGolS2-2* transgenic tobacco after stopping watering. WT: Wild-type tobacco plants; OE1–OE3: T₁ transgenic tobacco plants.

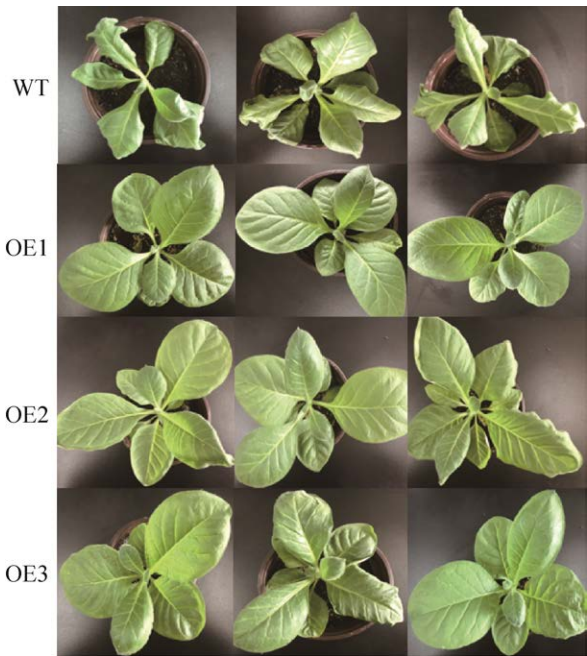


图6 *GmGolS2-2*转基因烟草在PEG8000溶液浇灌36 h后状态 WT: 野生型烟草; OE1–OE3: T₁代转基因烟草植株

Figure 6 Status of *GmGolS2-2* transgenic tobacco after 36 h of PEG8000 watering. WT: Wild-type tobacco plants; OE1–OE3: T₁ transgenic tobacco plants.

对 PEG8000 模拟干旱胁迫处理过程中 WT 烟草和转基因烟草叶片的电解质渗透率、丙二醛含量、脯氨酸含量及可溶性糖含量进行检测。如图 7A 和 7B 所示,干旱胁迫处理前(0 h),转基因烟草和 WT 烟草的电解质渗透率和丙二醛含量不存在显著差异;干旱胁迫处理后,转基因烟草和 WT 烟草的电解质渗透率和丙二醛含量均显著升高,但转基因烟草始终低于 WT 烟草。如图 7C 和 7D 所示,干旱胁迫处理前和处理 12 h 时,转基因烟草和 WT 烟草的脯氨酸含量和可溶性糖含量不存在显著差异;干旱胁迫处理 24 h 后,转基因烟草脯氨酸含量和可溶性糖含量均显著高于 WT 烟草。综合以上结果表明, *GmGolS2-2* 的异源表达可以促进转基因烟草在干旱胁迫下积累更多的渗透调节物质,提高细胞渗透压,减轻对细胞的破坏,进而提高转基因烟草的耐旱性。

2.4 *GmGolS2-2* 转基因烟草抗逆相关基因表达量分析

为探究 *GmGolS2-2* 异源过表达后对转基因

烟草中抗逆相关基因的表达是否存在影响,对 *GmGolS2-2* 表达量最高的 OE1 烟草中 4 个抗逆相关基因 *NtERD10B*、*NtERD10C*、*NtSOD* 和 *NtAQP1* 的表达量进行检测。qPCR 结果显示(图 8), *NtERD10C* 在转基因烟草中的表达量上调了 3 倍, *NtAQP1* 在转基因烟草中的表达量

上调了近 10 倍;而 *NtERD10B* 和 *NtSOD* 的表达量在转基因烟草和 WT 烟草中差异不显著。以上结果表明, *GmGolS2-2* 在烟草中异源表达可以上调部分烟草抗逆相关基因的表达,这些抗逆相关基因表达量的升高也有助于提高 *GmGolS2-2* 转基因烟草的耐旱性。

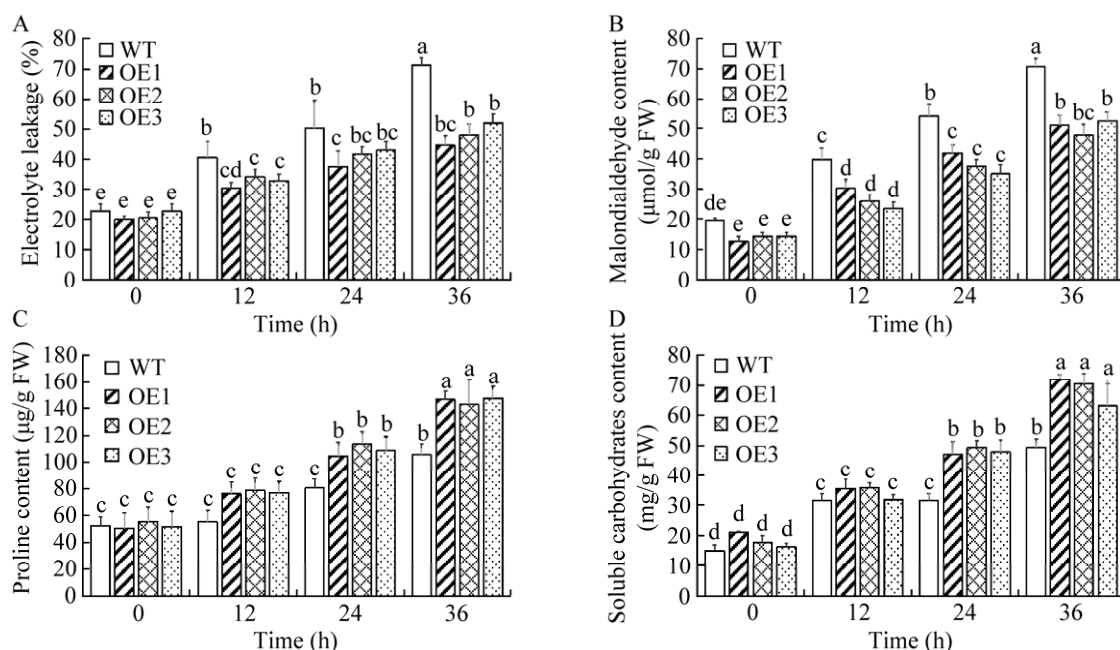


图 7 *GmGolS2-2* 转基因烟草干旱处理下的生理参数 A: 电解质渗透率. B: 丙二醛含量. C: 脯氨酸含量. D: 可溶性糖含量. WT: 野生型烟草; OE1–OE3: T_1 代转基因烟草植株. 不同小写字母表示差异显著性($P < 0.05$)

Figure 7 Physiological parameters of *GmGolS2-2* transgenic tobacco under drought treatment. A: Electrolyte leakage. B: Malondialdehyde content. C: Proline content. D: Soluble carbohydrates content. WT: Wild-type tobacco plants; OE1–OE3: T_1 transgenic tobacco plants. Different lowercase letters indicate significant difference ($P < 0.05$).

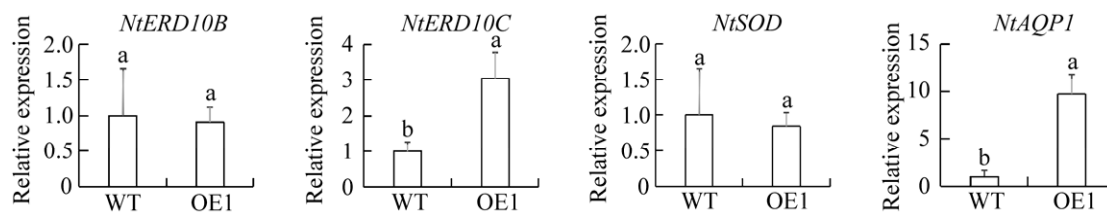


图 8 *GmGolS2-2* 转基因烟草抗逆相关基因表达量 WT: 野生型烟草; OE1: T_0 代转基因烟草植株. 不同小写字母表示差异显著性($P < 0.05$)

Figure 8 Expression of stress-related genes in *GmGolS2-2* transgenic tobacco. WT: Wild-type tobacco plants; OE1: T_0 transgenic tobacco plants. Different lowercase letters indicate significant difference ($P < 0.05$).

3 讨论与结论

干旱是严重限制作物生长和产量的主要非生物胁迫条件之一。干旱胁迫下可溶性糖类(蔗糖、RFOs 和海藻糖等)的积累是植物进化出的用于减少干旱胁迫危害的一种策略^[21-22]。在前期研究中,我们发现了 2 个能够被干旱胁迫强烈诱导的基因 *GmGolS2-1* 和 *GmGolS2-2*,其中 *GmGolS2-1* 已经被证明可以提高转基因植株的耐旱性^[16]。前人的研究也表明,基因的表达模式往往与基因的功能密切相关,例如拟南芥 *AtGolS2*、芝麻 *SiGolS6*、鹰嘴豆 *CaGolS1* 和 *CaGolS2* 等的表达均能够被干旱胁迫诱导,后续的研究也证实这些基因的确能够在干旱胁迫中发挥重要作用,它们的过表达提高了转基因植株的抗旱性^[10-11,23-25]。

GolS 基因的过表达有利于肌醇半乳糖苷和棉子糖的积累,研究发现,它们能够作为活性氧(reactive oxygen species, ROS)的清除剂,为细胞提供保护,避免由干旱胁迫造成的氧化损伤^[7,25]。在干旱胁迫下, *GmGolS2-2* 转基因烟草的脯氨酸含量和可溶性糖含量显著高于 WT 烟草,表明转基因烟草在干旱胁迫下可以积累更多的渗透调节物质,提高细胞的保水能力,还有利于清除活性氧;同时,转基因烟草的电解质渗透率和丙二醛含量则显著低于 WT 烟草,说明转基因烟草在干旱胁迫下活性氧的积累较少,受到的氧化损伤相对较低,细胞膜的破坏程度小于 WT 烟草^[26-27]。这些实验结果与干旱胁迫下 *GmGolS2-2* 转基因烟草和 WT 烟草的生长状态相符合,也支持 *GmGolS2-2* 提高了转基因烟草耐旱性的观点。

研究发现,由于 *AtGolS3* 的过表达造成了转基因植物中部分基因表达量显著上调,进而导致代谢发生变化^[28]。同样, *GmGolS2-2* 在

烟草中的异源过表达对转基因烟草中的部分抗逆相关基因的表达也产生了影响,例如, *NtERD10C* 和 *NtAQP1* 在 *GmGolS2-2* 转基因烟草中的表达量显著升高。相似的实验结果也出现在我们前期鉴定的 *GmGolS2-1* 转基因烟草中^[16]。研究表明, *ERD10* 基因编码早期脱水响应蛋白, *AQP1* 基因编码水通道蛋白,它们都具有保护植物免受各种胁迫的功能^[29-31]。*NtERD10C* 和 *NtAQP1* 在转基因烟草中表达量的提高可能与转基因烟草对干旱耐受性的提高有关。然而,目前尚不清楚 *GmGolS2-2* 通过何种机制来影响 *NtERD10C* 和 *NtAQP1* 的表达,是通过蛋白质间相互作用,还是间接地通过其他转录因子或信号转导途径来调控,这些推测都需要进一步的实验证明。此外,推测 *GmGolS2-2* 能够影响的抗逆相关基因的数量不仅限于此,后续可以通过转录组测序进行进一步的鉴定。综上所述, *GmGolS2-2* 提高了转基因烟草耐旱性,可以作为大豆抗旱分子育种的候选基因资源。

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