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·研究报告·

异源表达异子蓬*SaPEPC2*基因提高烟草抗旱性和光合特性

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摘要 磷酸烯醇式丙酮酸羧化酶(PEPC)是C_4光合关键酶, 有助于植物在非生物胁迫下抵御逆境。异子蓬(*Suaeda aralocaspica*)是一种无须Kranz结构即可在单细胞中高效执行C_4光合作用的荒漠盐生植物, 在C_3作物遗传改良方面具有天然优势。以转异子蓬*SaPEPC2*基因烟草(*Nicotiana tabacum*)为材料, 探讨了其抗旱功能和光合性能。结果表明, 过表达*SaPEPC2*提高了烟草叶片持水能力, 可保持叶绿素稳定; 积累更多的渗透调节物质, 增强了抗氧化酶活性, 进而降低了植株体内的活性氧水平, 减轻膜损伤程度; 同时还增强了烟草抗旱相关基因和内源光合基因的表达, 提高了PEPC活性和净光合速率, 可能是促进了烟草体内的“类C_4微循环”途径所致。研究结果为进一步利用异子蓬单细胞C_4途径PEPC基因培育高光效抗逆农作物品种奠定了基础。

关键词 单细胞C_4植物, 异子蓬, PEPC, 抗旱性, 光合作用

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C_3植物主要依靠1,5-二磷酸核酮糖羧化酶/加氧酶(Ribulose-1,5-bisphosphate carboxylase/oxygenase, Rubisco)固定大气中的CO_2, 由于光呼吸过程中O_2和CO_2竞争Rubisco活性位点, 导致固定的CO_2损失, 且损失率高达50%, 严重影响了CO_2同化效率(Ogren, 1984; Chen and Spreitzer, 1992)。然而, 大多数粮食作物如小麦(*Triticum aestivum*)、水稻(*Oryza sativa*)、大豆(*Glycine max*)和马铃薯(*Solanum tuberosum*)都是C_3植物, 光合速率低会直接影响作物产量。相比之下, 经典的C_4植物如玉米(*Zea mays*)、高粱(*Sorghum bicolor*)、甘蔗(*Saccharum officinarum*)和谷子(*Setaria italica*), 经过长期的适应性进化, 叶片形成花环状Kranz解剖结构。磷酸烯醇式丙酮酸羧化酶(phosphoenolpyruvate carboxylase, PEPC)分布于叶肉细胞, 起到高效固定CO_2的作用(CO_2泵); Rubisco分布于维管束鞘细胞, 由于PEPC显著提高了周围环境中的CO_2浓度, 导致Rubisco加O_2活性受到抑制, 光呼吸程度降低(Bowes, 2010)。此外, 维管束鞘富含胞间连丝, 有利于光合产物在两种细胞间运输, 从而使C_4植物在高温、强光、干旱条件下也能维持较高的光合效率和水分利用率(Sharpe and Offermann, 2014)。由于干旱胁迫严重影响植物的生长发育, 导致光合效率降低和作物减产等, 对农业生产造成的损失仅次于病虫害(邵瑞鑫等, 2016; 陈征等, 2016)。因此, 长期以来人们都试图将C_4光合途径引入C_3植物来改善光合性能和抗逆能力, 以期最终提高作物产量。与通过常规杂交育种引入复杂的Kranz结构相比, 直接单独或协同转化C_4光合关键酶基因显得更加简便易行。尤其是在发现水生植物黑藻(*Hydrilla verticillate*)、水蕴草(*Egeria densa*)、北美冰兰(*Sagittaria subulata*)、禾本科植物*Orcuttia viscida*以及苋科植物异子蓬(*Suaeda aralocaspica*)、*Bienertia cycloptera*、*B. sinuspersici*和*B. kavirens*具有单细胞C_4光合途径之后(Holaday and Bowes,

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1980; Magnin et al., 1997; Keeley, 1998; Casati et al., 2000; Voznesenskaya et al., 2001, 2002; Bowes, 2010), 人们更加坚信可以在无须Kranz结构的情况下, 在C_3作物中建立起“类C_4光合途径”(Taniguchi et al., 2008)。

PEPC作为C_4和景天酸代谢(crassulacean acid metabolism, CAM)植物光合碳同化途径的关键酶, 是通过分子育种手段导入外源基因以提高C_3作物光合效率的首选基因。当存在Mg^{2+}或Mn^{2+}时, PEPC催化碳酸氢盐(HCO_3^-)与磷酸烯醇式丙酮酸(phosphoenolpyruvic acid, PEP)进行羧化反应, 生成四碳化合物草酰乙酸(oxalacetic acid, OAA) (Izui et al., 2004)。在非光合组织和C_3植物叶片中, PEPC主要负责补充三羧酸(tricarboxylic acid, TCA)循环的中间产物(Driever and Kromdijk, 2013)。此外, PEPC在维持碳氮相互作用(Huppe and Turpin, 1994; Shi et al., 2015)、种子形成与萌发(Ruiz-Ballesta et al., 2016)、果实成熟(O'Leary et al., 2011)以及为豆科根瘤的共生固氮提供苹果酸盐(Suganuma et al., 1997; Nomura et al., 2006)等方面发挥重要作用。近年来, 越来越多的研究证实PEPC还参与调控各种生物与非生物胁迫响应(Wang et al., 2012; Qian et al., 2015; Qi et al., 2017; Waseem and Ahmad, 2019)。特别是在干旱胁迫下, PEPC活性增加促进碳代谢, 进而促进TCA循环, 产生更多苹果酸、可溶性糖和氨基酸等渗透性调节物质以抵御逆境胁迫(Liu et al., 2017b; 石慧敏等, 2022)。将不同来源的*PEPC*基因转入C_3植物, 如拟南芥(*Arabidopsis thaliana*) (Kandoi et al., 2016)、水稻(Zhang et al., 2017; 宋凝曦等, 2020)、小麦(Qi et al., 2017)、大豆(张艳等, 2015)、杨树(*Populus* × *euramericana*) (尹昊, 2009)和油菜(*Brassica napus*) (吴梅, 2011), 大多数转基因株系均表现出PEPC活性和光合速率增加, 水分利用率和产量提高, 特别是在高温、高光强和干旱胁迫下增幅更加明显; 此外, 转基因植株的抗逆性也得到不同程度的提高(刘小龙等, 2015; Tang et al., 2018; Giuliani et al., 2019; 李佳馨等, 2021)。然而, 目前大多数研究都是将玉米、谷子和高粱等传统C_4植物的*PEPC*基因转入C_3植物, 对荒漠C_4植物光合基因资源的应用鲜见报道。

异子蓬是新疆盐化荒漠中一种极端抗逆的一年生草本植物, 在长期进化过程中逐渐演化出单细胞C_4光合途径, 通过将单个叶肉细胞分隔成远端室和近端室2个不同的功能域来区隔四碳循环和三碳循环(Voznesenskaya et al., 2001; Edwards et al., 2004)。其C_4光合结构更加紧凑, 光合作用不受O_2的抑制, 不存在光呼吸, 较经典C_4植物具有更高的光合效率(Smith et al., 2009; Sharpe and Offermann, 2014; Liu et al., 2020)。异子蓬这种独特的生化区隔模式提高了将“类C_4微循环”导入C_3植物而无须建立Kranz双细胞体系的可能性, 这无疑为农作物高光效遗传改良开辟了新途径(Edwards et al., 2008; Miyao et al., 2011)。本课题组前期开展了异子蓬非光合型*SaPEPC2*基因功能的初步研究(Cao et al., 2021), 发现利用*CaMV35S*组成型启动子过表达*SaPEPC2*基因具有增强烟草光合能力、提高耐旱性的潜力, PEPC活性较非转基因植株高1.3–2.6倍(未发表数据)。研究显示, 在玉米*PEPC*自身启动子驱动下的转基因水稻PEPC活性增加14–60倍(Suzuki et al., 2006)。基于此, 为提升外源*PEPC*基因表达效率, 本研究拟利用过表达*SaPEPC2*基因自身启动子驱动编码区(*Pro*$_{SaPEPC2}$*::SaPEPC2*)的转基因烟草, 进一步探究*SaPEPC2*基因对C_3植物光合性能和抗旱性的影响, 以期进一步阐明单细胞C_4光合作用独特的生理过程, 并为利用异子蓬单细胞C_4途径培育抗逆、优质、高产和高效的转基因农作物品种提供理论依据。

1 材料与方法

1.1 植物材料

NC89非转基因烟草(*Nicotiana tabacum* L.)种子经无水乙醇和10%次氯酸钠灭菌后, 用无菌牙签点播在固体MS培养基表面, 置于温度25°C、光周期为16小时光照/8小时黑暗、光照强度为396 μmol·m^{-2}·s^{-1}的培养箱中培养, 获得45–60天苗龄的无菌苗。使用Easy-Geno同源重组克隆试剂盒(Tiangen, Cat No.V1201)将*SaPEPC2*启动子全长(GenBank: MW291560.1)连接到pCAMBIA2300-*SaPEPC2* (编码区序列GenBank: KX009562.1)载体中, 获得pCAMBIA2300-*Pro*$_{SaPEPC2}$*::SaPEPC2*过表达载体, 将阳性质粒转化农杆菌EHA105, 通过叶盘法遗传转化烟草。选择能在400 mg·mL^{-1}卡那霉素抗性培养基上正常生长的T_1代转基因烟草幼苗开展后续实验。

1.2 转基因烟草鉴定及干旱胁迫

选取卡那霉素筛选培养基中的12株阳性植株, 待长出3–4片真叶后移苗。提取叶片DNA, 用*SaPEPC2*启动子和编码区的特异引物进行PCR检测(扩增片段长度为707 bp), 确认$Pro_{SaPEPC2}$::*SaPEPC2*已成功转入烟草。选取3株阳性植株(OE5、OE7和OE13), 以*NtActin*为内参基因进行RT-PCR鉴定, 并采用Go-*Taq*® qPCR Master Mix荧光定量试剂盒(Promega, Cat No.CQP-TM318)检测转基因烟草中*SaPEPC2*基因的表达量。提取非转基因(non-transgenic, NT)和阳性植株(OE5、OE7和OE13)叶片总蛋白, 用SaPEPC2特异性一抗和HRP二抗(BOSTER, Cat No.BST18K-21C19A54)进行Western blot检测。

选择长势一致的阳性幼苗进行干旱胁迫实验。自然干旱处理: 分别挑选4盆非转基因植株和转基因株系5–6叶期幼苗置于同一托盘中。在处理前3天浇足水分, 于温室中自然干旱, 以正常浇水组作为对照。每隔2天更换托盘方向, 去除边际效应, 直至出现明显萎蔫的表型后恢复浇水, 统计存活叶片数, 每天观察拍照。模拟干旱处理: 待处理组植株充分吸水后, 用200 mmol·L^{-1}甘露醇从上至下浇透育苗盆, 确保置换出盆中原有水分后置于盛有相应溶液的托盘, 以正常浇水组作为对照。

1.3 干旱胁迫后转基因烟草生理指标的测定

选择模拟干旱处理48小时和对照组植株的相同位置, 采摘靠近顶端的两层对叶, 立即投入液氮后于–80°C冰箱保存。利用苏州科铭有限公司试剂盒, 分别测定叶绿素(Cat No.CPL-2-G)、丙二醛(malondialdehyde, MDA) (Cat No.MDA-2-Y)、超氧阴离子$O_2^{\cdot-}$ (Cat No.SAQ-2-G)、H_2O_2 (Cat No.H2O2-2-Y)、脯氨酸(Cat No.PRO-2-Y)、甜菜碱(Cat No.TCJ-2-G)和可溶性糖(Cat No.KT-2-Y)含量; 以及超氧化物歧化酶(superoxide dismutase, SOD) (Cat No.SOD-2-Y)、过氧化物酶(peroxidase, POD) (Cat No.POD-2-Y)和过氧化氢酶(catalase, CAT) (Cat No.CAT-2-Y)活性。选择自然干旱处理7天和对照组植株相同位置对叶, 利用苏州科铭有限公司试剂盒, 测定PEPC (Cat No.PEPC-2-Y)和Rubisco (Cat No.RUBPS-2A-Y)酶活性。

1.4 转基因烟草叶片失水率和相对含水量测定

选择对照组植株, 剪取相同位置的叶片立即称量初始鲜重(FW), 在室温下每隔1小时测量叶片失水后的重量(DAW), 连续记录6小时内的变化, 随后置于150°C烘箱烘干至恒重(DW)。计算失水率和相对含水量: 失水率(%)=(FW–DAW)/FW×100, 相对含水量RWC (%)=(DAW–DW)/(FW–DW)×100。

1.5 转基因烟草形态学和光合作用指标测定

将转基因株系和NT植株按照叶龄期不同, 分为大(7–8叶期)、中(6–7叶期)、小(5–6叶期)三种类型, 分别选择每组中长势一致的植株进行形态指标测定。用直尺测量株高、最大叶长和叶宽, 用数显游标卡尺测定茎粗, 用ImageJ软件等比例计算叶面积。利用LI-6400XT便携式光合作用测量系统(LI-COR Biosciences, USA)于上午9:00–12:00, 分别测定不同叶龄植株叶片的净光合速率(P_n)、气孔导度(G_s)、胞间CO_2浓度(C_i)和蒸腾速率(T_r)。使用系统自带LED光源控制光强为1 000 μmol·m^{-2}·s^{-1}, 叶室温度为25°C。选择植株从上至下第2层功能对叶, 避开主叶脉进行测定, 每个株系设置4个生物学重复。

1.6 干旱胁迫下转基因烟草光合基因及胁迫响应基因的表达分析

以*NtActin*为内参基因, 分别检测烟草内源光合基因*NtPEPC*、*NtCA* (编码磷酸酐酶, Carbonic anhydrase)、*NtFBP* (编码果糖-1,6-二磷酸酶, Fructose-1,6-bisphosphatase)、*NtTPT* (编码磷酸丙糖转运器, Triose phosphate translocator); 以及干旱胁迫响应基因*NtP5CS* (编码吡咯烷-5-羧酸合酶, Pyrroline-5-carboxylate synthase)、*NtERD* (脱水诱导早期应答基因, Dehydrin of early response to dehydration)和*NtDREB1* (编码脱水响应元件结合蛋白, Dehydration responsive element binding protein)在正常浇水和模拟干旱胁迫48小时的表达模式。

1.7 数据统计分析

分别用SPSS 20.0和Graphad Prism 9.0软件进行数据分析和作图。采用χ^2检验分析干旱胁迫后叶片存活数, 不同小写字母表示具有显著性差异(P<0.05)。利

用单尾Student's *t*检验分析各转基因株系与NT之间的差异显著性水平。

2 结果与讨论

2.1 转*SaPEPC2*基因烟草过表达株系鉴定

选择12株过表达*SaPEPC2*烟草株系，基因组DNA PCR结果显示，有9个株系扩增出707 bp条带，与阳性对照的条带大小相同，NT植株未扩增出目的条带，初步证明*SaPEPC2*基因已成功转入烟草(图1A)。选择PCR条带较亮的阳性植株(OE5、OE7和OE13)提取总RNA，反转录成cDNA，以*NtActin*作为内参基因进行RT-PCR和qRT-PCR检测，结果显示外源*SaPEPC2*基因在mRNA水平上发生了转录，且表达量存在差异(图1B, C)。采用SaPEPC2特异性抗体对各株系幼苗进行Western blot检测，结果显示转基因株系中外源SaPEPC2蛋白显著积累，在NT植株中未检测到相应信号，说明*SaPEPC2*基因在蛋白水平上成功表达(图1D)。

2.2 转基因烟草自然干旱胁迫表型

对过表达*SaPEPC2* (OE5、OE7和OE13)转基因烟草幼苗进行自然干旱胁迫处理。结果显示，正常浇水条件下，对照组NT植株与各转基因株系生长状况一致；干旱处理22天后，各转基因株系全部萎蔫，此时恢复浇水；复水30天后，转基因株系恢复生长情况较好，存活叶片数显著多于NT，说明过表达*SaPEPC2*能增强受体植物的耐旱性(图2A, B)。测定正常浇水处理下的各株系离体叶片失水率和相对含水量，结果显示随时间的延长失水率逐渐增高，但各转基因株系失水率总体趋势低于NT，相对含水量显著高于NT，说明过表达*SaPEPC2*提高了烟草叶片持水能力，更耐受干旱胁迫(图2C, D)。

2.3 模拟干旱下转基因烟草生理指标及胁迫响应基因的表达变化

正常浇水条件下，转基因株系和NT植株的MDA、H_2O_2、脯氨酸和甜菜碱含量以及SOD活性无显著差异，转基因株系$O_2^{\cdot-}$含量显著低于NT植株，可溶性糖(soluble sugar, SS)含量、POD和CAT活性显著高于NT植株。在甘露醇模拟干旱胁迫下，转基因株系SOD、POD和CAT活性以及脯氨酸、可溶性糖和甜菜碱含量显著升高，MDA、H_2O_2和$O_2^{\cdot-}$含量显著降低(图3)，表明过表达*SaPEPC2*可促进转基因烟草主动

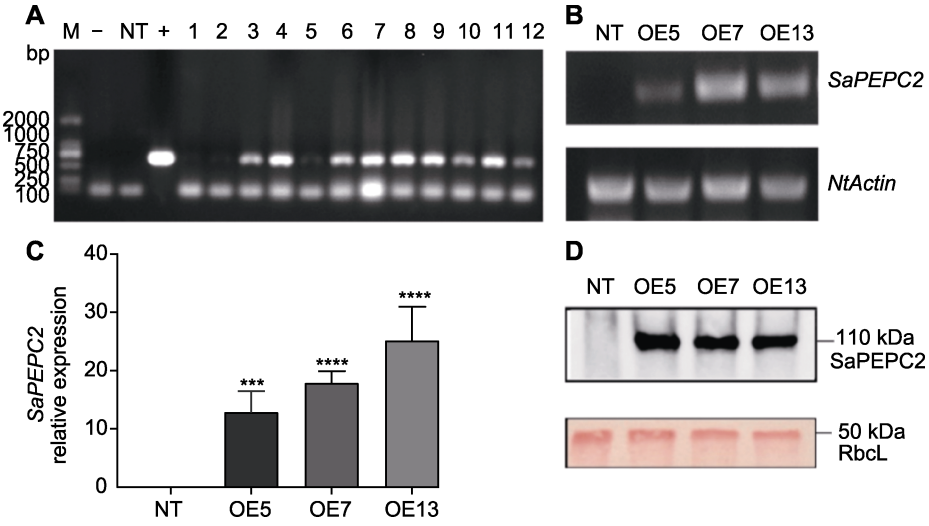

图1 *SaPEPC2*转基因烟草基因组DNA PCR **(A)**、RT-PCR **(B)**、qRT-PCR **(C)**和Western blot **(D)**鉴定

M: DL2000分子量标准; –: 阴性对照; +: 阳性对照; NT: NC89非转基因烟草; 1–12: 转基因株系。*** P<0.001; **** P<0.0001 (Student's *t* test)

Figure 1 Identification of *SaPEPC2* in transgenic tobacco by genomic DNA PCR **(A)**, RT-PCR **(B)**, qRT-PCR **(C)** and Western blot **(D)**

M: DL2000 marker; –: Negative control; +: Positive control; NT: NC89 non-transgenic tobacco; 1–12: Transgenic lines. *** P<0.001; **** P<0.0001 (Student's *t* test)

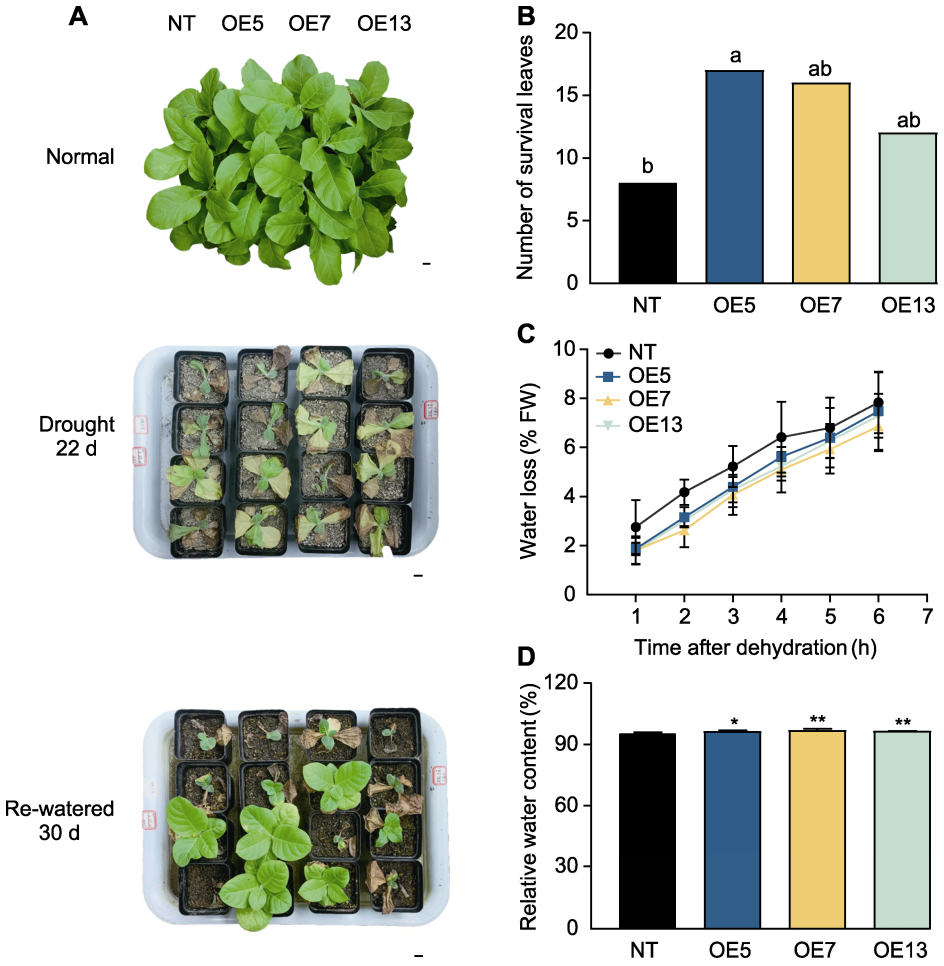

图2 自然干旱条件下过表达*SaPEPC2*转基因烟草表型**(A)**、叶片存活数**(B)**、失水率**(C)**和相对含水量**(D)**

不同小写字母表示不同株系在*P*<0.05水平差异显著。* *P*<0.05; ** *P*<0.01 (Student's *t*检验)。NT: 非转基因。OE5、OE7、OE13为不同转基因株系。Bars=2 cm

Figure 2 Phenotype **(A)**, leaf survival number **(B)**, water loss rate **(C)** and relative water content **(D)** in *SaPEPC2* overexpression transgenic tobacco lines under natural drought conditions

Different lowercase letters indicate significant differences among different lines at *P*<0.05 level. * *P*<0.05; ** *P*<0.01 (Student's *t* test). NT: Non-transgenic. OE5, OE7 and OE13 are different transgenic individuals. Bars=2 cm

积累渗透调节物质，保持较高的抗氧化酶活性，加快分解多余的活性氧(reactive oxygen species, ROS)，降低细胞膜损伤程度。正常条件下，OE5和OE7脱水诱导早期应答基因*NtERD*表达量显著高于NT植株；干旱胁迫下，所有植株胁迫响应基因的表达量均呈上升趋势，但转基因株系对干旱胁迫的响应更积极，特别是脯氨酸合成关键酶编码基因*NtP5CS*表达量升高约2–3倍(图4)，说明过表达*SaPEPC2*可通过调节干旱胁迫响应基因的表达增强植物抗的逆性。

2.4 干旱胁迫下转基因烟草光合特性变化

正常条件下，转基因株系叶绿素*a*和总叶绿素含量以及PEPC和Rubisco酶活性均显著高于NT植株；干旱胁迫下，叶绿素含量和光合酶活性均降低，但转基因植株仍显著高于NT植株，PEPC和Rubisco酶活性分别提高1.5–3倍和2.5–3倍(图5A–E)，表明过表达*SaPEPC2*能提高光合关键酶活性，缓解干旱胁迫造成的叶绿素降解。正常条件下，转基因株系中*NtCA*和*NtFBP*表达量显著高于NT植株；干旱胁迫后，转基因烟草C_3途径相关基因均显著上调表达，表达量平均约为NT植株的2倍(图6A–D)，表明外源*SaPEPC2*的导入可提高受体植物(特别是干旱胁迫下)内源光合基因的表达，推测导入外源*SaPEPC2*可能促进烟草体内建立了类似C_4途径的微循环。在正

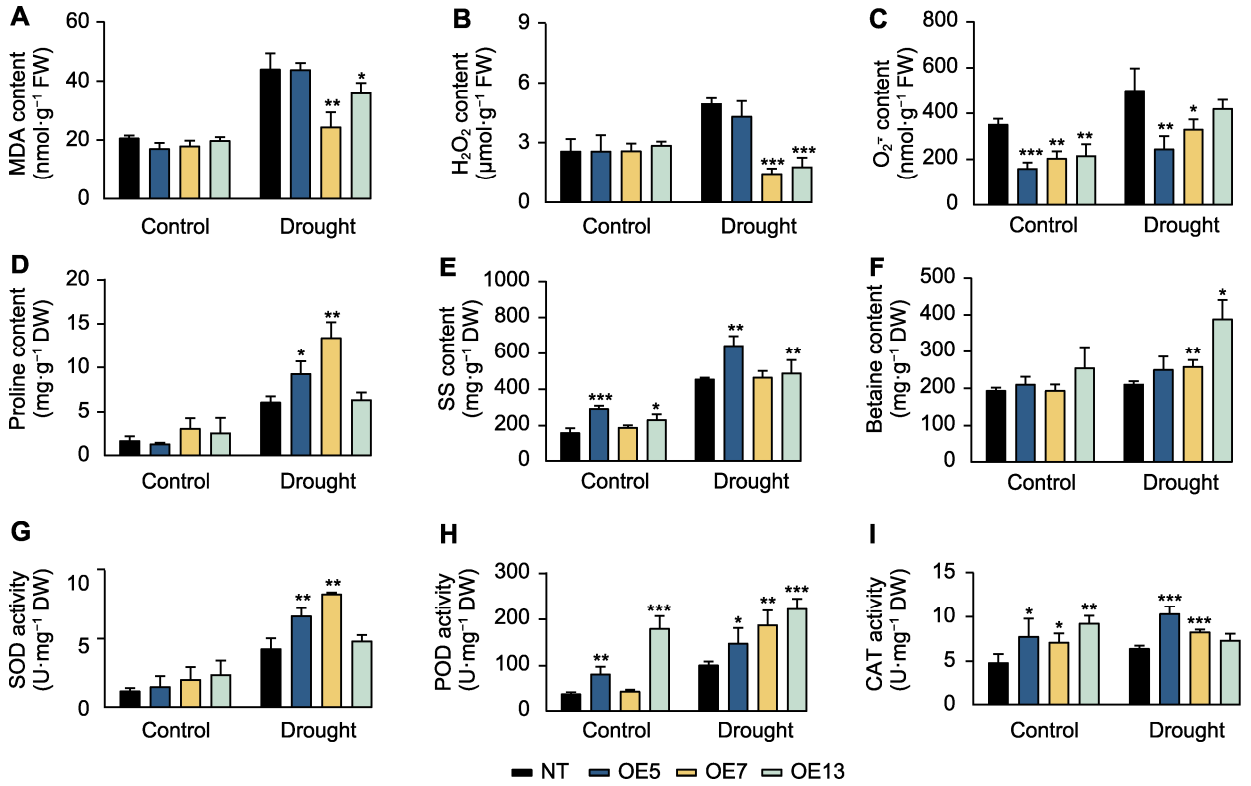

图3 干旱胁迫下过表达*SaPEPC2*转基因烟草的生理指标

(A) 丙二醛(MDA)含量; **(B)** H_2O_2含量; **(C)** $O_2^{\cdot-}$含量; **(D)** 脯氨酸含量; **(E)** 可溶性糖(SS)含量; **(F)** 甜菜碱含量; **(G)** 超氧化物歧化酶(SOD)活性; **(H)** 过氧化物酶(POD)活性; **(I)** 过氧化氢酶(CAT)活性。*、**和***分别表示转基因植株与非转基因(NT)植株在0.05、0.01和0.001水平存在显著差异(Student's *t*检验)。OE5、OE7和OE13同图2。

Figure 3 Physiological indicators in *SaPEPC2* overexpression transgenic tobacco lines under drought stress

(A) Malondialdehyde (MDA) content; **(B)** H_2O_2 content; **(C)** $O_2^{\cdot-}$ content; **(D)** Proline content; **(E)** Soluble sugar (SS) content; **(F)** Betaine content; **(G)** Superoxide dismutase (SOD) activity; **(H)** Peroxidase (POD) activity; **(I)** Catalase (CAT) activity. *, **, and *** indicate significant differences existing between the transgenic line and non-transgenic (NT) plant at 0.05, 0.01, and 0.001 levels, respectively (Student's *t* test). OE5, OE7 and OE13 are the same as shownin Figure 2.

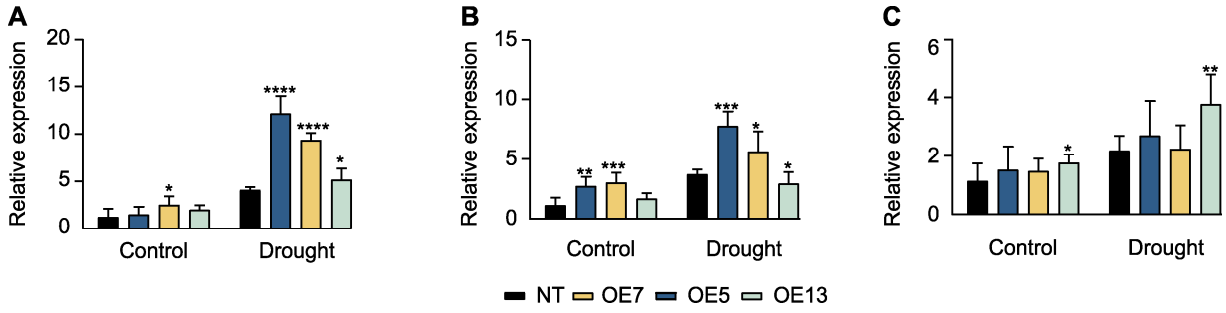

图4 干旱胁迫下过表达*SaPEPC2*转基因烟草干旱胁迫响应基因的表达模式

(A) *NtP5CS* (编码吡咯烷-5-羧酸合酶); **(B)** *NtERD* (脱水诱导早期应答基因); **(C)** *NtDREB1* (编码脱水响应元件结合蛋白)。*、**、***和****分别表示转基因植株与非转基因(NT)植株在0.05、0.01、0.001和0.0001水平存在显著差异(Student's *t*检验)。

Figure 4 Expression patterns of drought stress-responsible genes in *SaPEPC2* overexpression transgenic tobacco lines under drought stress

(A) *NtP5CS* (encoding pyrroline-5-carboxylate synthase); **(B)** *NtERD* (early responsive to dehydration); **(C)** *NtDREB1* (encoding dehydration responsive element binding protein). *, **, ***, and **** indicate significant differences existing between the transgenic line and non-transgenic (NT) plant at 0.05, 0.01, 0.001, and 0.0001 levels, respectively (Student's *t* test).

常浇水条件下，不同叶龄期转基因株系的光合作用指标检测结果显示，转基因株系净光合速率、蒸腾速率和气孔导度均显著高于NT植株，净光合速率增加1.0–2.5倍，胞间CO_2浓度显著降低(图7A–D)，说明过表达*SaPEPC2*烟草具有较高的气孔导度、蒸腾速率以及较强的胞间CO_2利用能力，有利于叶片进行光合作用，进而提高净光合速率。

2.5 转基因烟草生长状态指标

将转基因株系和NT植株分为大(7–8叶期)、中(6–7叶期)、小(5–6叶期)三个类型。大型转基因烟草OE13的株高、叶长、叶宽和叶面积显著高于NT植株，叶面积增大1.3倍；小型转基因烟草OE7叶面积显著高于NT植株，增大1.5倍(图8A–F)。这与叶绿素含量以及净光合速率测定结果相符，说明过表达*SaPEPC2*可能通过增加烟草叶面积提高光合能力。小型转基因烟草OE7鲜重和干重显著高于NT植株，分别增加1.1倍和1.3倍(图8G)。说明过表达*SaPEPC2*能够维持较高的叶片含水量，有助于烟草积累更多有机物质，在一定程度上提高产量。

2.6 讨论

植物光合作用对干旱极为敏感，土壤水分亏缺使叶片含水量减少，进而影响气孔开闭和CO_2吸收，导致光合速率降低。长期干旱胁迫引起植物渗透压失衡，蛋白质合成异常，细胞膜系统受损伤，致使作物减产(Zou et al., 2023)。而C_4光合途径关键酶PEPC能够积极参与抗逆生理反应(Bandyopadhyay et al., 2007; Doubnerová and Ryšlavá, 2011; Ding et al., 2013)，对于提高C_3植物干旱胁迫下的光合能力具有潜在应用价值。*PEPC*属于编码不同异构体的多基因家族，每个亚型都与特定的生理功能相关联(Ku et al., 1996)。在所有植物中都存在组成型表达的非光合*PEPC*亚型，被称为“管家型”或“C_3型”，在C_4植物中通常只有一种亚型参与光合作用，被称为“C_4型”(Chollet et al., 1996)。过表达高粱C_4型*PEPC*的

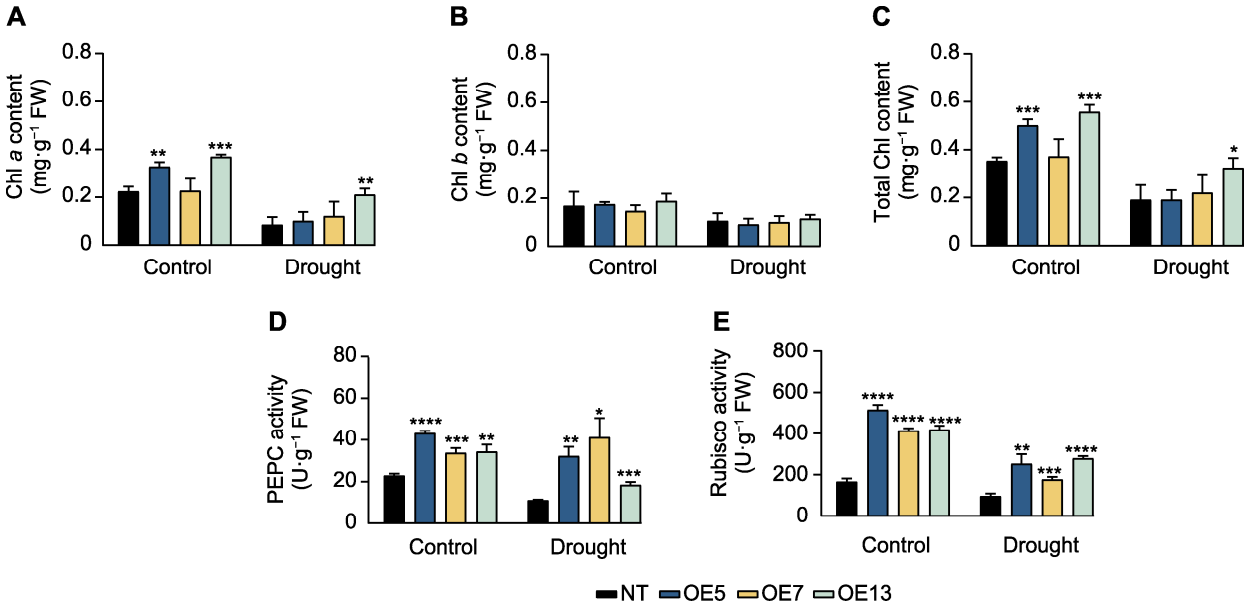

图5 干旱胁迫下过表达*SaPEPC2*转基因烟草叶绿素含量和光合酶活性

(A) 叶绿素*a*含量; **(B)** 叶绿素*b*含量; **(C)** 总叶绿素含量; **(D)** 磷酸烯醇式丙酮酸羧化酶(PEPC)活性; **(E)** Rubisco酶活性。*、**、***和****分别表示转基因植株与非转基因(NT)植株在0.05、0.01、0.001和0.0001水平存在显著差异(Student's *t*检验)。

Figure 5 Chlorophyll content and photosynthetic enzyme activity in *SaPEPC2* overexpression transgenic tobacco lines under drought stress

(A) Chlorophyll *a* content; **(B)** Chlorophyll *b* content; **(C)** Total chlorophyll content; **(D)** Phosphoenolpyruvate carboxylase (PEPC) activity; **(E)** Rubisco activity. *, **, ***, and **** indicate significant differences existing between the transgenic line and non-transgenic (NT) plant at 0.05, 0.01, 0.001, and 0.0001 levels, respectively (Student's *t* test).

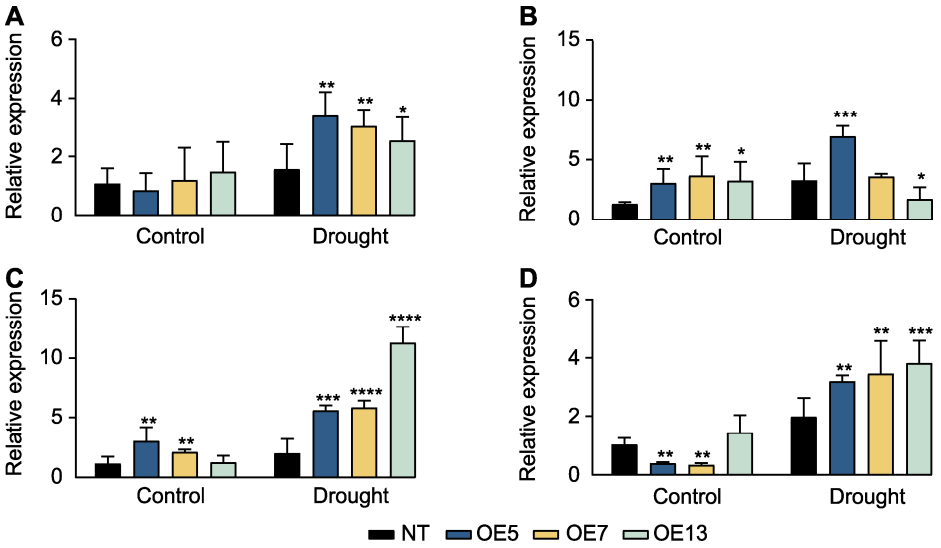

图6 干旱胁迫下过表达*SaPEPC2*转基因烟草内源光合基因的表达模式

(A) *NtPEPC*; **(B)** *NtCA* (编码磷酸酐酶); **(C)** *NtFBP* (编码果糖-1,6-二磷酸酶); **(D)** *NtTPT* (编码磷酸丙糖转运器)。*、**、***和****分别表示转基因植株与非转基因(NT)植株在0.05、0.01、0.001和0.0001水平存在显著差异(Student's *t*检验)。

Figure 6 Expression patterns of endogenous photosynthetic genes in *SaPEPC2* overexpression transgenic tobacco lines under drought stress

(A) *NtPEPC*; **(B)** *NtCA* (encoding carbonic anhydrase); **(C)** *NtFBP* (encoding fructose-1,6-bisphosphatase); **(D)** *NtTPT* (encoding triose phosphate translocator). *, **, ***, and **** indicate significant differences existing between the transgenic line and non-transgenic (NT) plant at 0.05, 0.01, 0.001, and 0.0001 levels, respectively (Student's *t* test).

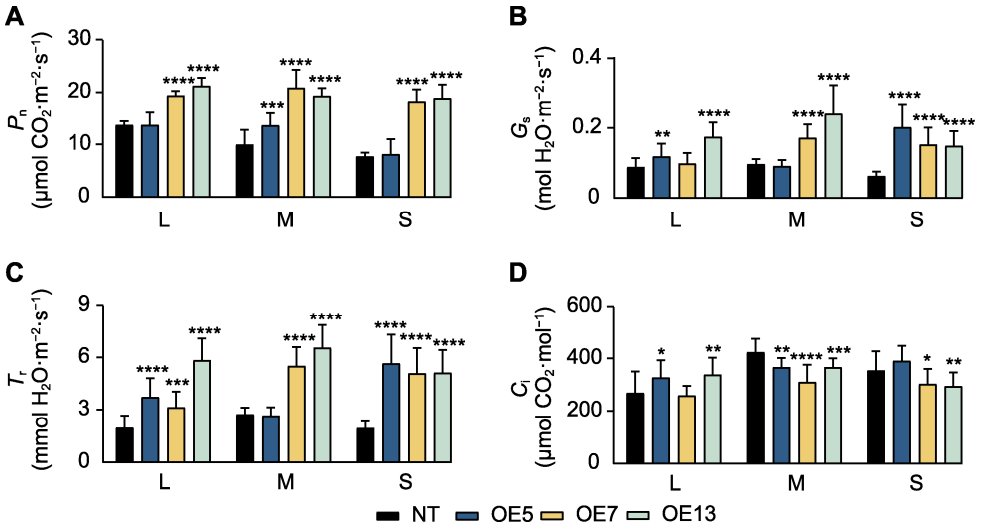

图7 过表达*SaPEPC2*转基因烟草光合指标

(A) 净光合速率(P_n); **(B)** 气孔导度(G_s); **(C)** 蒸腾速率(T_r); **(D)** 胞间CO_2浓度(C_i)。L: 大(7–8叶期)植株; M: 中(6–7叶期)植株; S: 小(5–6叶期)植株。*、**、***和****分别表示转基因植株与非转基因(NT)植株在0.05、0.01、0.001和0.0001水平存在显著差异(Student's *t*检验)。

Figure 7 Photosynthetic indicators in *SaPEPC2* overexpression transgenic tobacco lines

(A) Net photosynthetic rate (P_n); **(B)** Stomatal conductance (G_s); **(C)** Transpiration rate (T_r); **(D)** Intercellular CO_2 concentration (C_i). L: Large (7–8 leaf stage) plants; M: Medium (6–7 leaf stage) plants; S: Small (5–6 leaf stage) plants. *, **, ***, and **** indicate significant differences existing between the transgenic line and non-transgenic (NT) plant at 0.05, 0.01, 0.001, and 0.0001 levels, respectively (Student's *t* test).

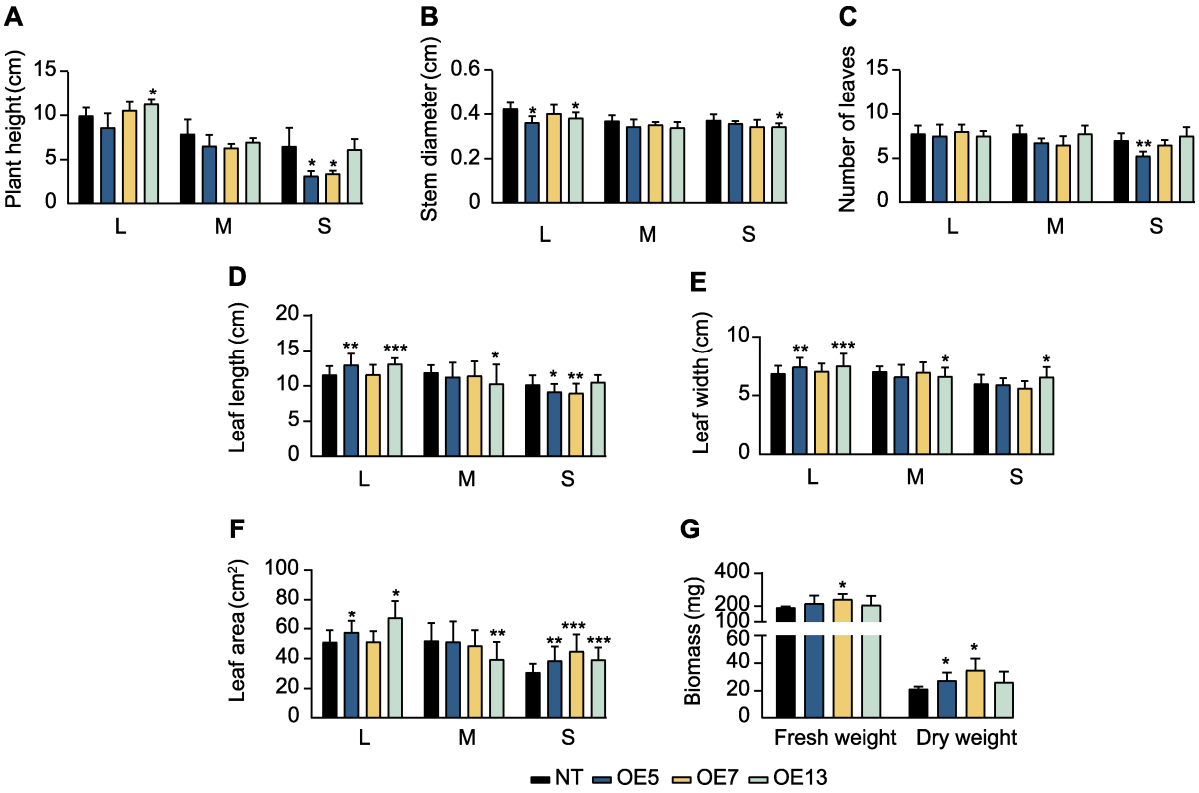

图8 过表达*SaPEPC2*转基因烟草生长指标

(A) 株高; **(B)** 茎粗; **(C)** 叶片数; **(D)** 叶长; **(E)** 叶宽; **(F)** 叶面积; **(G)** 生物量。L、M和S同图7。*、**和***分别表示转基因株系与非转基因(NT)植株在0.05、0.01和0.001水平存在显著差异(Student's *t*检验)。

Figure 8 Growth indexes of *SaPEPC2* overexpression transgenic tobacco lines

(A) Plant height; **(B)** Stem diameter; **(C)** Number of leaves; **(D)** Leaf length; **(E)** Leaf width; **(F)** Leaf area; **(G)** Biomass. L, M, and S are the same as shownin Figure 7. *, **, and *** indicate significant differences existing between the transgenic line and non-transgenic (NT) plant at 0.05, 0.01, and 0.001 levels, respectively (Student's *t* test).

转基因玉米在干旱胁迫下水分利用率和干物质积累量增加(Jeanneau et al., 2002); 过表达玉米C_4型*PEPC* (*ZmPEPC1*)水稻通过NO和钙信号级联途径提高短期耐旱性(Qian et al., 2015; Liu et al., 2017a), 减轻干旱胁迫下转基因水稻的光抑制并提高其耐强光能力(周宝元等, 2011; 丁在松等, 2012); 转*ZmPEPC1*小麦在干旱胁迫下提高了光合效率和单株产量(Qin et al., 2016)。然而, 目前大部分报道均为过表达经典的C_4植物光合型PEPC, 对荒漠C_4植物以及"管家型"PEPC关注较少。

新疆荒漠单细胞C_4盐生植物异子蓬PEPC蛋白质在绿色组织细胞质中广泛分布, 不具备Kranz结构, 而是通过在单个绿色组织细胞内形成极性分布的生化区隔来同时高效完成C_4和C_3循环, 这种无结构分化的机制可能更适合在C_3植物中发挥作用(Sharpe and Offermann, 2014)。本课题组前期获得了异子蓬2种*SaPEPC*基因亚型, 其中*SaPEPC1*在绿叶组织中的表达随发育进程而增强, 对光敏感, 可能参与C_4光合途径, *SaPEPC2*更接近非光合*PEPC*亚型(Cao et al., 2021); 二者在本体中的表达均受盐和干旱胁迫诱导, *PEPC*活性显著升高(Cao et al., 2015); 在大肠杆菌中异源表达能显著促进重组菌在盐、干旱及氧化胁迫下的生长, 说明两种*SaPEPC*均能积极参与各种非生物胁迫响应(Cheng et al., 2016; 程刚, 2017)。基于此, 本研究利用*SaPEPC2*全长启动子驱动该基因cDNA导入烟草, 探讨异子蓬非光合型*PEPC*基因的抗旱功能以及对光合作用的影响。

对转基因烟草的耐旱性分析显示, 各转基因株系较NT植株均表现出更高的存活率和含水量(图2), 表明过表达*SaPEPC2*能维持较高的叶片持水能力, 从

而降低干旱胁迫对光合作用的抑制效应，这与转基因株系叶绿素含量、PEPC和Rubisco酶活性在干旱胁迫下显著高于NT植株的结果相吻合(图5)。进一步分析干旱处理下的生理指标，转基因株系MDA、H_2O_2和$O_2^{\cdot-}$含量更低，抗氧化酶系统(SOD、POD和CAT)活性更高，特别是渗透调节物质脯氨酸和可溶性糖含量显著高于NT植株，表现出更高的ROS清除能力(图3)。推测这可能由于非光合型*SaPEPC2*导入促进TCA循环产生更多的中间产物，如谷氨酸(脯氨酸前体)，进而增加脯氨酸含量(Qin et al., 2016); TCA循环产生的NADPH又进入Calvin循环合成淀粉和蔗糖，增加可溶性糖含量; NADPH也能用于渗透调节物质的生物合成，从甘露糖-6-磷酸合成脯氨酸(Doubnerová and Ryšlavá, 2011)。与之一致的是，干旱胁迫后转基因株系的脯氨酸合成酶基因*NtP5CS*表达量较NT植株显著升高2–3倍(图4A)。研究表明，过表达龙舌兰(*Agave americana*) CAM型*PEPC*促进转基因烟草脯氨酸的生物合成，提高了耐盐性和抗旱性(Liu et al., 2021)。基于此，我们推测过表达*SaPEPC2*烟草可能通过上述复杂的代谢过程，积累更多渗透调节物质，同时促进胁迫响应基因转录活性增强(图4B, C)，从而间接改善受体材料对干旱胁迫的耐受性。

有趣的是，过表达*SaPEPC2*还增高了干旱胁迫下烟草内源*NtPEPC*以及C_3途径相关基因的转录本丰度，并且在正常条件下，虽然转基因株系*NtPEPC*表达量无显著变化，但*NtCA*表达量显著高于NT植株(图6B)。碳酸酐酶(CA)位于PEPC上游，负责催化空气中CO_2发生水合反应，产生HCO_3^- (杨朗, 2015)。推测过表达*SaPEPC2*虽不能直接带动光合循环，但可能通过加速消耗底物，促进*NtCA*上调表达来促进*NtPEPC*行使功能。研究显示，转玉米*ZmPEPC1*水稻在高光强下CA和PEPC活性的提高有利于CO_2传输和同化，从而使其具有初级CO_2浓缩机制(凌丽俐, 2007)。在过表达玉米*ZmPEPC1*小麦中也发现了类似机制(齐学礼, 2016; Qi et al., 2017)。在C_3循环中，光合作用碳固定的最初产物3-磷酸甘油酸(3-PGA)被还原成磷酸丙糖(TP)，磷酸丙糖转运器(TPT)负责催化TP和无机磷酸(Pi)在叶绿体和胞质之间的反向运输(孙金月, 2003)。果糖-1,6-二磷酸酶(FBP)在TPT从叶绿体中输出TP后，调控蔗糖的合成，是糖异生重要的调控位点(郭利娜, 2013)。本研究中，干旱胁迫后转基因株系*NtTPT*和*NtFBP*基因表达量较NT植株显著增高2–6倍(图6C, D)，说明过表达*SaPEPC2*在一定程度上也能推动C_3循环，较NT植株产生更多TP进而转化为蔗糖，这与过表达株系可溶性糖含量显著增加相吻合(图3E)。非光合型*SaPEPC2*虽不能在转录水平上直接调控其它基因的表达，但其过表达可能引起细胞代谢状态改变，导致烟草内源调控网络的重新配置，至于是否建立“类C_4微循环”，未来仍需通过对功能获得和功能丧失型*SaPEPC2*突变体的转录和代谢分析来验证。研究显示，过表达*ZmPEPC1*水稻通过维持气孔导度减缓水分胁迫(刘小龙等, 2015)。本研究中，正常条件下，过表达*SaPEPC2*烟草的气孔导度较NT植株显著增大，进一步引起蒸腾速率显著增高(图7B, C)，这有利于水分在叶片中的快速运输，并为光合作用提供足够的原料和更多的矿质元素(王超等, 2008)，使转基因株系的净光合速率显著升高1.0–2.5倍(图7A)，生物量增加1.3倍(图8G)；胞间CO_2浓度显著低于NT植株，说明转*SaPEPC2*烟草净光合速率的增高不仅与气孔导度增大有关，还可能与其胞间CO_2利用能力增强密切相关。

综上所述，本研究初步阐明异子蓬*SaPEPC2*基因参与调节C_3植物抗旱性和光合性能的机制，通过参与调控细胞代谢网络来提高抗旱相关基因和内源光合基因的表达、增强转基因烟草PEPC酶活性、保持叶绿素稳定、积累更多渗透调节物质以及增强抗氧化酶活性等方式，最终提高转基因烟草的光合效率和抗旱能力。研究结果为后续利用异子蓬单细胞C_4途径高光效*SaPEPC*基因改良C_3作物奠定了基础。

作者贡献声明

曹婧、廖星鑫和牛祎：撰写论文，完成实验和绘制图表；多兴武和阿克也得力·居玛哈孜：完成实验；买热哈巴·阿不都克尤木和热孜瓦尼姑丽·胡甫尔：协助完成实验和数据分析；曹婧和兰海燕：设计实验，修改论文，提供技术支持。所有作者均已阅读并同意论文的出版版本。

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Heterologous Expression of *Suaeda aralocaspica SaPEPC2* Gene Improves Drought Resistance and Photosynthesis in Transgenic Tobacco

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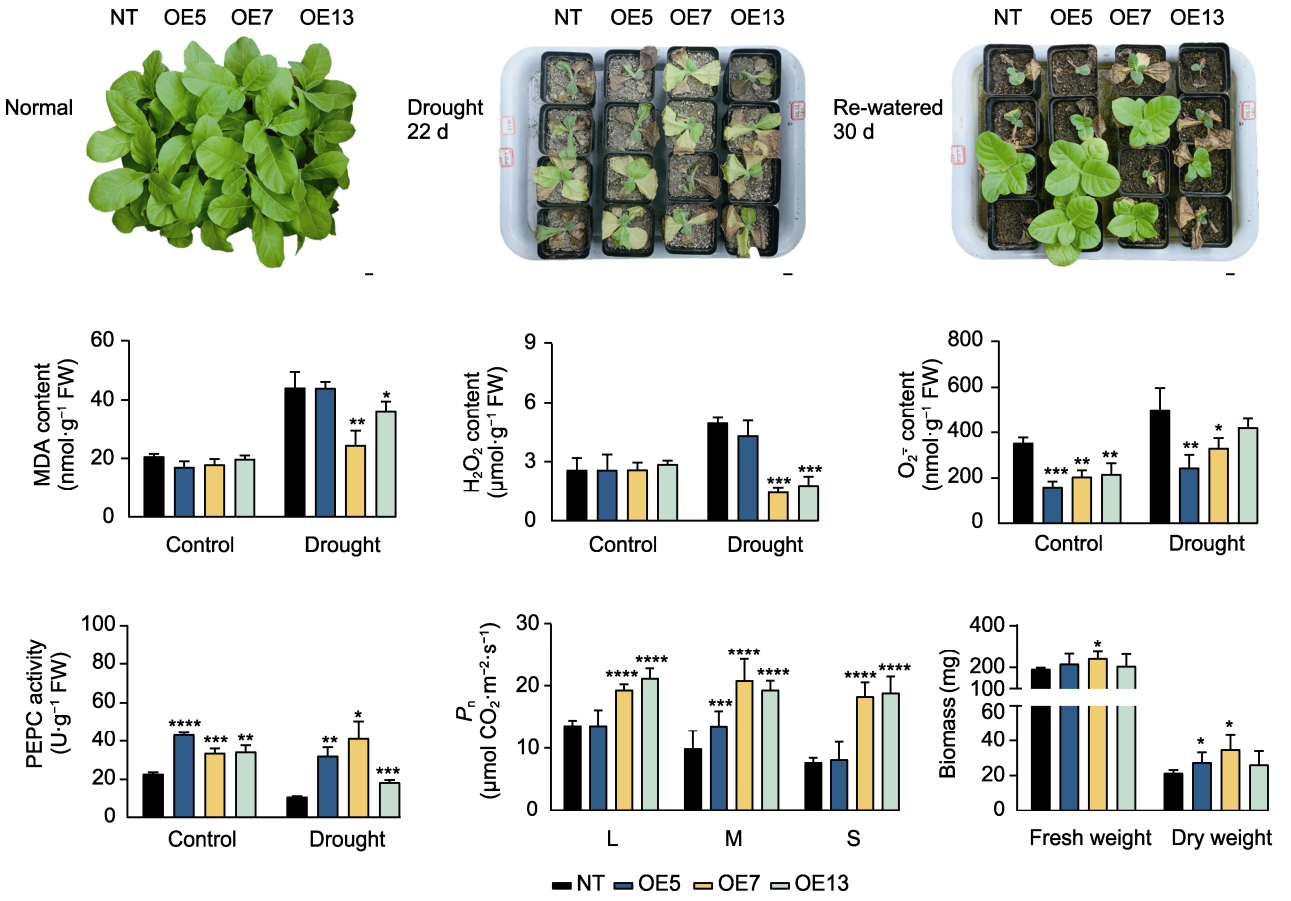
INTRODUCTION: Phosphoenolpyruvate carboxylase (PEPC) is a key enzyme for C_4 photosynthesis that help plants to resist adversity under abiotic stress. *Suaeda aralocaspica*, an annual halophyte, has gradually developed a single-cell C_4 photosynthetic pathway by compartmentalizing a chlorenchyma cell into distal and proximal ends to delineate and form the four-carbon (C_4) and three-carbon (C_3) cycles through long term of evolution. This unique biochemical compartmentation pattern holds promise for introducing "C_4-like microcirculation" into C_3 plants without establishing Kranz anatomy.

RATIONALE: The *PEPC* gene act as an essential component of C_4 photosynthesis, but few studies have reported on the *PEPC* gene in single cell C_4-pathway species. To reveal the impact of the *SaPEPC2* gene from *S. aralocaspica* on the photosynthetic performance and drought resistance of C_3 plants, we evaluated the drought resistance function and photosynthetic performance of transgenic tobacco (*Nicotiana tabacum*) overexpressing the *SaPEPC2* gene driven by its own promoter ($Pro_{SaPEPC2}$::*SaPEPC2*) through physiological measurements and gene expression analysis methods.

RESULTS: Our findings demonstrated that overexpressing the *SaPEPC2* gene in tobacco improved leaf water retention, maintained chlorophyll stability, promoted the accumulation of osmotic adjustment substance, enhanced antioxidant enzyme activities, reduced ROS levels, mitigated the extent of membrane damage, upregulated the expression of drought-related and endogenous photosynthesis genes, and increased PEPC enzyme activity and net photosynthetic rate.

CONCLUSION: In conclusion, overexpressing the *SaPEPC2* gene likely facilitates the formation of a "C_4-like microcirculation" pathway in tobacco. These results may provide the theoretical foundation for the potential utilization of the single-cell C_4 pathway *PEPC* genes from *S. aralocaspica* to breed high light-efficiency and stress-resistant crop varieties.



Overexpression of *SaPEPC2* in tobacco results in stronger drought resistance and higher photosynthesis efficiency compared to non-transgenic plants.

Key words single-cell C_4 plant, *Suaeda aralocaspica*, PEPC, drought resistance, photosynthesis

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