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通过遗传操作提高非典型油料植物作为生产三酰甘油生物工厂的潜力

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摘要

到 2030 年，全球植物油需求量预计将翻番。然而目前的植物油生产平台，包括油棕和温带油籽却难以满足如此的增幅。因此，探索新型植物油来源对弥补未来的植物油的短缺变得越来越重要。植物油的主要形式是三酰甘油（TAG），最近通过基因工程在植物营养组织中生产 TAG 引起了人们极大的兴趣。多学科的“组学”研究也愈发提高了我们对植物脂质生物化学和代谢的理解。鉴于此，生物化学途径鉴定及对脂肪酸生物合成、脂质组装和转换关键的基因的注释已得到有效更新。近年来，通过对 TAG 生物合成涉及的关键基因和调节因子的遗传操作，高生物量植物营养组织和油籽中 TAG 的积累得到了前所未有的迅速发展。本文总结了目前从单基因操作到旨在增加高生物量植物组织中 TAG 积累的多基因叠加基因工程策略，讨论了可能有助于进一步缓解食用油和生物柴油潜在短缺的植物油生产的新方向和建议。

1. 引言

目前，为了满足食品、饲料和工业用途的需要而生产的植物油主要来源于油棕（*Elaeis guineensis*）和几种主要的温带油料作物，其中包括大豆（*Glycine max*）、油菜（*Brassica napus*）、向日葵（*Helianthus annuus*）和花生（*Arachis hypogaea*）[1,2]。但是，由于全球人口激增，植物油消费量已显著增加[3]。近年来，由于对植物油来源的可再生生物柴油替代化石燃料的需求不断增加，植物油的短缺更是日益明显[4–7]。研究表明，植物油和石油的化学结构非常相似，植物油可以通过加工达到生物燃料的适用标准。自2000年以来，世界年生物燃料供应量增长了8%，2015年世界运输燃料消耗的比例惊人地占到了4%[8]。这主要由过去10年间棕榈油产量的急剧增加所致[9]。但由于原始热带雨林中棕榈树的过度种植，以及其潜在的不利生态影响可能造成的环境隐患，长期依赖棕榈油的情况不太可能持续[10–13]。此外，耕地面积减少已经对油菜作物的种植造成了巨大的影响，其结果是在加拿大和欧盟（EU）的油菜主产区很可能发生价格上涨[8]。尽管产量稳步增长，但发展中国家的油籽消费在未来几年依然可能会超过生产水平（图1）[3–8]。因此，开发替代资源或新型植物油生产平台来弥补植物油短缺意义重大。

植物油[主要是三酰甘油（triacylglycerol，TAG）]可以在油籽中积累到很高的水平，从而在通过光合作用进行植物自养之前支持种子发芽和幼苗发育。然而，非种子组织中的TAG含量却低很多，例如在拟南芥（*Arabidopsis thaliana*）中的TAG只占叶片组织干重（DW）

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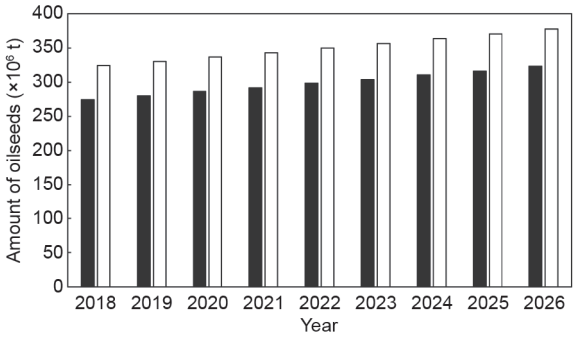

图1. 发展中国家油籽经济的预测。发展中国家油籽植物油的消费和生产预计将在未来几年出现增长趋势。在发展中国家，油籽（大豆和其他油籽）作为植物油原料的消费和生产预计将在未来几年出现上升趋势。黑柱代表油籽产量；白柱代表油籽需求量。据相关预测，油籽产量在未来几年内很可能将较难以满足相应的需求，世界欠发达国家和地区需要更多的植物油[3–8][†]。

的0.04%~0.2% [14,15]。一般来说，除少数植物如油棕、油橄榄（*Olea europaea*）和油莎草（*Cyperus esculentus*）外，植物营养组织中的TAG本质上都是非累积性的[16]。因此，过去提高植物油含量的研究主要集中在油籽而不是植物营养组织上。

近年来，国际上开始探索通过基因工程技术将植物营养组织（包括光合作用的叶组织和诸如块根等非光合作用的组织）作为TAG积累部位的可行性。生物产量高的植物如烟草（*Nicotiana tabacum*）[17,18]、甘蔗（*Saccharum officinarum*）[19]和马铃薯（*Solanum tubersosum*）[20,21]被用于探索在非种子组织中大量生产TAG。由于多学科的组学研究和合成生物学的快速发展，我们对植物脂质代谢的认识已大大增加[22]。特别是转录组学、蛋白质组学和脂质组学，不但为更好地理解典型油料作物油脂代谢提供了更多的机会，而且有助于研究非典型TAG积累植物组织的油脂代谢[23]。有关脂肪酸生物合成、脂质组装和转换关键基因的生物化学途径和基因注释的知识已经迅速增加，从而建立起了相应的植物脂质代谢模型[24–26]。通过对这些知识加以运用，采用各种遗传工程策略提高植物营养组织中TAG的积累是目前获得植物油来源的一个较好方法。

2. 提高植物脂质生产

2.1. 传统的高油植物育种方法

以提高植物油脂产量为代表的传统育种技术主要关注提高种子的脂质储存能力[27]。早期的研究采用杂交和杂种优势等育种方法来改良油料作物的基因型和表型，以扩大植物油的产量[28]。分子标记技术如数量性状基因座（QTL）和单核苷酸多态性（SNP）构建的遗传图谱和关联分析已经被广泛用于研究植物油的生产潜力，特别是第三大油料作物的油菜 [27,29–32]。关于调控油菜含油量的QTL位点已有很多报道[33–37]。此外，通过研究与油生物合成相关的主要QTL综合图谱，目前已确定了决定大豆TAG积累的20个共有QTL[38]。除了传统的油籽作物外，田间育种方法（如轮回选择）也被用于谷类作物含油量的遗传改良。通过103轮选择了籽粒含油量超过20%的玉米（*Zea mays*）群体，其含油量已远远超过其原始种质（仅为4.7%）[39]。同样，在含油量分别为11%和3%的两个栽培品种中，经过9轮选择，培育出含油量为18%的燕麦（*Avena sativa*）新品种[40,41]。

占植物油市场份额最大（47%）的棕榈油也通过育种获得了一系列的改良。尽管存在引起生态失衡和水土流失的争议，油棕仍然是当前最受欢迎的植物油生产来源[42]。增加棕榈油生产力的育种技术已经从传统的表型选择发展到标记辅助选择（MARS），与19年的表型选择周期相比，该技术既经济又高效[43]。而且，高通量分子标记技术也被用来选择改良棕榈的脂肪酸组成，并构建特定的油棕遗传图谱[44,45]。例如，最近通过合性定位（homozygosity mapping）技术发现了一个与撒哈拉以南非洲地区的油棕生产直接相关的重要基因*SEEDSTICK*（*STK*）[46]。

尽管如此，产油量仍然是一个与许多因素关联的数量性状。常规育种技术在很大程度上依赖于费时费力的研究，而基因工程却可以提供快速和直接的手段来操纵脂质代谢，从而将碳源重新引导至脂质的生物合成。这其实与传统的育种并不矛盾；相反，传统育种技术与未来的基因工程相辅相成，在建立高效的植物油生物工厂方面依然将继续发挥重要作用[47,48]。

2.2. 潜在的新型油料作物

2.2.1. 麻风树种子

麻风树（*Jatropha curcas*）种子含油丰富，已被确认为潜在的非食用油源植物[49,50]。由于麻风树的耐旱性非常好，因而可以在生长条件不甚理想的地区广泛种植[51]。除了分子标记选择和转基因技术之外，突变育

[†] 数据来自 OECD 调查：https://www.oecd-ilibrary.org/agriculture-andfood/data/oecd-agriculture-statistics/oecd-fao-agricultural-outlook-edition-2017_d9e81f72-en?parentId=http%3A%2F%2Finstance.metastore.ingenta.com%2Fcontent%2Fcollection%2Fagr-data-en.

种、杂种优势育种和种间杂交都被用于提高麻风树种子油的生产能力[52]。

2.2.2. 乌桕

尽管乌桕（*Triadica sebifera*）目前被一些发达国家视为入侵植物[53]，但其已然成为一个潜在的新型生物柴油来源[54,55]。有研究指出甲醇的酯交换非均相体系可以将乌桕籽油转化为生物柴油标准化产品，其转化效率高达94%[56]。由于其内果皮的乌桕油富含饱和脂肪酸，因而氧化稳定性很高，暗示其作为一种多功能生物燃料原料的应用潜力[57,58]。专门针对乌桕非种子组织中的脂质积累的深度转录组测序也有了相应的报道[59,60]。

2.2.3. 油莎草

油莎草是一种匍匐茎和块茎植物，在澳大利亚和美国被视为一种入侵杂草，然而其块茎干重含有26%~30%油分[61]。欧洲培育的一个黄色油莎草商业品种（被称为“Chufa”）被广泛用于烹饪[62]。诚然，油莎草和其他作物之间的竞争对当地的经济和生态可能带来一些不可预知的影响[63,64]，但油莎草油可以为新的植物油平台提供一个良好的选择。生化研究表明，黄色油莎草块茎的油生物合成通常在较晚的发育阶段才开始，糖分是油合成的主要底物[65]。已有研究开展不同发育阶段黄色油莎草油脂代谢的转录组分析，更有意义的是不仅其本身能够被进一步发展成油用块茎作物，而且还可以作为一种高生物产量地下块茎（如马铃薯）产油的模式系统[66]。

3. 植物营养组织中油脂生物合成的遗传调控

3.1. 植物 TAG 的合成代谢和分解代谢

脂肪酸的生物合成、中性脂肪的组装和代谢降解是植物油脂的基本生化循环。目前对植物脂动态代谢网络的了解主要来自于油料作物含油种子的研究，而对营养器官中的这些网络在很大程度上仍未明了[67,68]。尽管如此，大多数关键脂质通路基因的表达在多种组织中依然可以被检测到，表明植物脂质的生物合成在一定程度上在种子器官和营养组织中很可能是相似的[26]。

脂肪酸的生物合成和TAG组装是一系列高度分工的生化过程（图2）。细胞质内糖酵解途径后由磷酸烯醇丙酮酸（PEP）产生的丙酮酸，是质体脂肪酸“从头”合成中辅酶A（CoA）合成的主要直接碳源。在质体中，含生物素的乙酰CoA羧化酶（ACCase）通过添加羧基将乙酰CoA激活为3碳中间体丙二酸单酰CoA，进而成为催化脂肪酸生物合成中的第一个关键步骤。然后，脂肪酸合成（FAS）复合体将丙二酰基上的CoA转化为酰基载体蛋白（ACP）。质体中脂肪酸“从头”合成的最终产物通常是酰基-ACP的形式，主要包括16:0-ACP、18:0-ACP和18:1-ACP，随后以游离脂肪酸（FFA）的形式穿越质膜被输出到细胞质中，酰基-ACP的硫酯酶家族[酰基-ACP硫酯酶A（FatA）和酰基-ACP硫酯酶B（FatB）]促成了这一过程。在细胞质内质网（ER）中的FFA被重新激活为酰基CoA从而产生临时的“酰基CoA库”作为依赖于酰基CoA的Kennedy途径的酰基供体。在这整个过程中，FFA的水平因其潜在的细胞毒性可被如ACCase这样的限速酶高度调控[16]。

在ER内的经典Kennedy途径中，*sn*-甘油-3-磷酸（G3P）作为甘油脂生物合成的主要甘油骨架。具体而言，*sn*-甘油-3-磷酸酰基转移酶（GPAT）和溶血磷脂酸酰基转移酶（LPAAT）催化G3P与来自胞质“酰基CoA库”的酰基顺序酰化以合成二酰甘油（DAG），后者再通过限速酶二酰甘油酰基转移酶（DGAT）转化成TAG[68]。DGAT是唯一负责TAG产生的关键酶[68]，而参与启动甘油脂合成的其他酶（如GPAT）则在“16:3/18:3”植物的分化以及真核生物和原核生物在脂质代谢途径中产生异同方面发挥重要作用[69–73]。除了肯尼迪通路外，还有一个不依赖酰基CoA的独立途径，通常以运输质体内释放出的FFA的方式直接进入ER，在酰基编辑程序的调控下合成磷脂酰胆碱（PC）库[26]。PC分子因此可以被磷脂酰化甘油酰基转移酶（PDAT）转化为TAG，PDAT被认为是通过不依赖酰基CoA途径而独立调控TAG生物合成的早期限速酶[74]。在依赖/不依赖酰基CoA的两个途径中，DAG是TAG生物合成的基础底物，其来源是多种多样的，除了在肯尼迪通路中经磷脂酸磷酸酶（PAP）催化由磷脂酸（PA）合成外，PC作为DAG的额外供体，由PC:DAG磷酸胆碱转移酶调控[16]。这里值得一提的是，ER中形成的PC库不同于其对应的胞质酰基CoA，它不仅为TAG生物合成提供酰基底物，更有助于维持植物细胞的内脂膜稳态[75]。

在典型的油籽中，TAG以被单层磷脂膜包围的油体（OB）或脂滴（LD）的形式存在。一组LD整合蛋白[以油质蛋白（oleosin）为主，其次是油体钙蛋白（caleosin）和油体固醇蛋白（steroleosin）]在由磷脂单层膜包围的球形脂质体上被专门嵌入[76]。通过这种独特的结构，

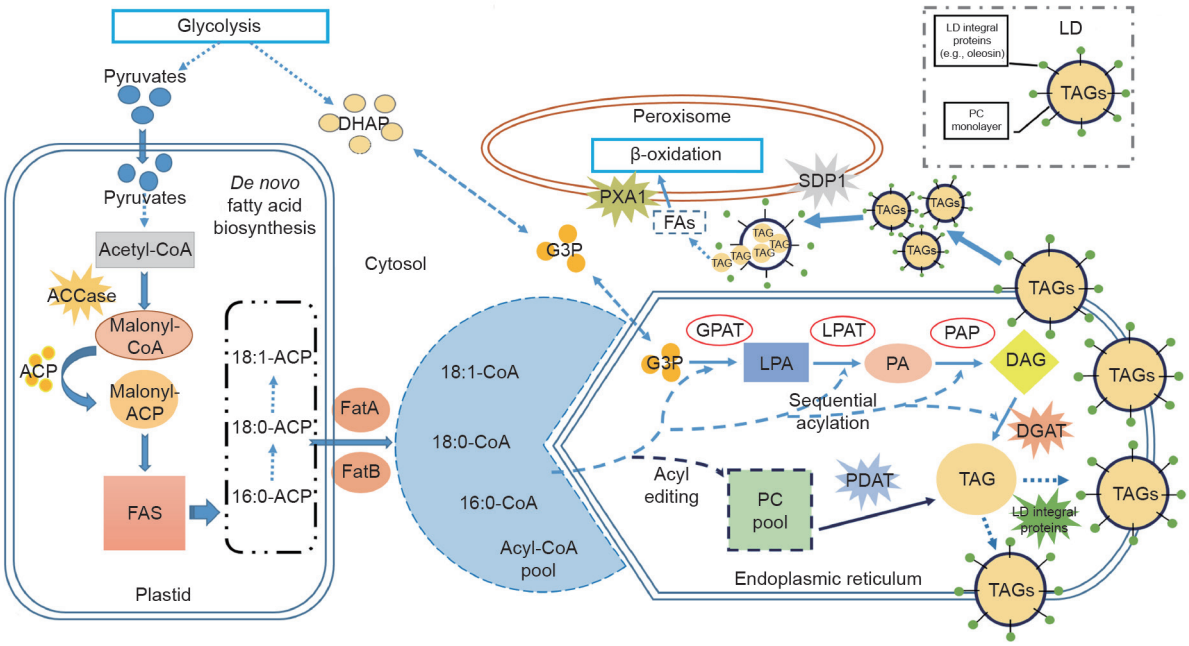

图2. 植物TAG的生物合成和转换。植物细胞质体（即叶绿体）是重新进行脂肪酸“从头”合成的主要场所，在其中具有不同链长度的脂肪酸如棕榈酸（16:0-ACP）和硬脂酸（18:0-ACP）的产生可以由特定的FAS系统启动。在细胞溶质中产生的“酰基CoA库”是ER中用于随后的TAG合成的酰基的主要供体，其中Kennedy途径和酰基CoA独立途径协作以支持TAG组装。ER中的“PC池”提供了酰基CoA独立途径的直接底物。DHAP：磷酸二羟丙酮；CoA：辅酶A；ACP：酰基载体蛋白；FAS：脂肪酸合成酶；ACCase：乙酰辅酶A羧化酶；FatA/FatB：酰基-ACP硫酯酶A/B；G3P：*sn*-甘油-3-磷酸盐；GPAT：*sn*-甘油-3-磷酸酰基转移酶；LPA：溶血磷脂酸；LPAAT：溶血磷脂酸酰基转移酶；PA：磷脂酸；PAP：磷脂酸磷酸酶；DAG：二酰基甘油；DGAT：二酰甘油酰基转移酶；TAG：三酰甘油；PC：磷脂酰胆碱；PDAT：磷脂：二酰甘油酰基转移酶；LD：脂滴；SDP1：糖依赖型1；PXA1：过氧化物酶体ABC转运蛋白1；FA：脂肪酸。

可以有效控制LD的大小、移动和融合[77]。另外，LD的功能似乎不仅限于储存能量。据报道，拟南芥叶片中的LD还能够作为亚细胞工厂来合成植物抗毒素以控制衰老和真菌感染，而且相关的油体固醇蛋白也具有将雌二醇转化为生物活性酮化合物的能力[78]。

在种子以外的油脂组织如鳄梨（*Persea americana*）的中果皮内，LD整合蛋白未被大量发现。相反，一组被称为LD相关蛋白（LDAP）的小蛋白则被发现为中果皮LD蛋白组中的主要蛋白[79]。LDAP与小橡胶颗粒蛋白（SRPP）具有同源性，SRPP因橡胶树（*Hevea brasiliensis*）和俄罗斯蒲公英（*Taraxacum brevicornic-ulatum*）这类橡胶生产植物的乳铁蛋白细胞中存储橡胶颗粒（*cis*-1,4-polyisoprene）的相关性而得名[80]。与油质蛋白不同，LDAP可能不被整合到LD中，而是以等向性的方式与颗粒表面相联合[81]。据报道，LDAP蛋白可能具有两个主要功能：一是增强油体的稳定性以防止LD的融合或分散；二是通过形成酶复合物促进新脂质的合成[80,82–84]。

3.2. 单基因工程策略增加 TAG 积累

3.2.1. 加强上游调控

满足油脂生物合成所需的脂肪酸来源广泛，但主要依赖于淀粉的生物降解、糖酵解和直接光合碳固定。相比之下，TAG组装和LD形成以及随后的脂质转换却是被严格调控的生物化学过程，其中的几种限速酶发挥着主导作用。过去，扩大脂肪酸来源（push）、增进TAG合成（pull）、巩固LD的形成和稳定（package）及最小化脂质降解（project）的遗传调控策略主要集中在这几类限速酶基因上[18,68]。然而，由于植物中的碳利用不仅仅只限于分解代谢，更参与到一系列生理调控之中，所以单独操纵特定基因的表达对实现整体TAG生物合成的全局变化效果有限（表1）[20,85–100]。

WRINKLED1（WRI1）转录因子最初在拟南芥突变体中发现，与野生型[101]相比，其种子表皮皱缩并且可溶性糖含量较高[101]。研究表明，对该转录因子的单基因操作可以作为脂肪酸生物合成上游调控的代表性策略。WRI1通过调控一系列下游基因的表达对脂肪酸的生物合成起着关键的作用[102]。体外（in vitro）实验已经证明WRI1直接结合参与脂肪酸合成的多种基因的启动子，包括ACCase的生物素羧基载体蛋白亚基、ACP、烯酰-ACP还原酶、β-酮脂酰-ACP还原酶、质体丙酮酸激酶、丙酮酸脱氢酶的BCCP亚基和FAD2等[103–105]。在玉米[106]和油菜[107]中异位过表达*WRI1*的研究都发现种子中的油含量显著提高。当在拟南芥营

表1 脂代谢主要基因遗传操作的代表性研究

Target plant	Target organ	Promoter and target gene	Beneficial effects	Other effects	References
Solanum tuberosum	Tuber	StGBSS::*AtWRI1*	TAG increased to 1% of DW	Increased sugar and membrane lipids	[20]
Brassica napus	Leaf	CaMV35S::*BnWRI*1	63% increase of TAG	Facilitated flowering	[85]
Brachypodium distachyon	Leaf	ZmUBI1::*Bd*WRI1	32.5 fold increase of TAG	Cell death in leaf	[86]
Arabidopsis thaliana	Leaf	CaMV35S::*Rc*LEC2	2.7 fold increase of TAG	Morphological variations	[87]
Arabidopsis thaliana	Leaf	AtNAP::*At*LEC2	3 fold increase of TAG	Reduced MGDG and PG	[88]
Solanum tuberosum	Tuber	CaMV35S::*AtACCa*se	5 fold increase of TAG	Improved fatty acids biosynthesis	[89]
Arabidopsis thaliana	Seed	CaMV35S::*SiDG*AT	45% increase of oil content	Increased seed size and weight	[90]
Arabidopsis thaliana	Leaf	CaMV35S::*AtPDAT*	28 fold increase of TAG	Altered membrane lipids	[91]
Arabidopsis thaliana	Seed	CaMV35S::*AtPDAT*	10% increase of TAG	Increased seed germinating rate	[92]
Arabidopsis thaliana	Seed	CaMV35S::*RcPDA*T	27% increase of hydroxyl fatty acid in TAG	Altered PC fatty acid profile	[93]
Brassica napus	Seed	VfUSP::*BnSDP1*	8% increase of oil content	Slightly decreased seed vigor	[94]
Jatropha curcas	Seed	CaMV35S::*JcSDP1*	30% increase of TAG	Decreased protein and FFAs	[95]
Arabidopsis thaliana	Leaf	CaMV35S::*AtABHD5*	Increased phospholipids	Altered galactolipids composition	[96]
Arabidopsis thaliana	Leaf	CaMV35S::*AtCGI-58*	7 fold increase in LD abundance	Altered leaf TAG composition	[97]
Arabidopsis thaliana	Leaf	CaMV35S::*AtPXA1*	4 fold increase of TAG	Membrane damage	[98]
Arabidopsis thaliana	Leaf	CaMV35S::*AtPXA1*	10–20 fold increase of TAG	Leaf necrosis and wilting	[99]
Arabidopsis thaliana	Leaf	CaMV35S::*AtSEPIN*	Increased LD number and size	10% increase of seed oil	[100]

Bn: *Brassica napus*; *Bd*: *Brachypodium distachyon*; St: *Solanum tuberosum*; *At*: *Arabidopsis thaliana*; *Rc*: *Ricinus communis*; *Si*: *Sesamum indicum*; *Jc*: *Jatropha curcas*; VfUSP: *Vicia faba* unknown seed protein; CaMV: cauliflower mosaic virus; GBSS: granule-bound starch synthase; *WRI1*: WRINKLED1; *LEC2*: leafy cotyledon 2; *ACCase*: acetyl-CoA carboxylase; *DGAT*: diacylglycerol acyltransferase; *PDAT*: phospholipid:diacylglycerol acyltransferase; *SDP1*: sugar-dependent 1; *ABHD5*: AB-hydrolase domain-containing gene 5; *CGI-58*: comparative gene identification-58; *PXA1*: peroxisomal ABC-transporter 1; MGDG: mono-galactosyldiacylglycerol; PG: phosphatidylglycerol.

养组织中异位表达时，WRI1也诱导产油量增加了5.8倍[108]。同样，在块茎特异性启动子的转录控制下表达来自拟南芥的*WRI1*后，转基因马铃薯块茎内的TAG积累显著增加，高达干重的1% [20]，表明了WRI1不仅可以在种子中而且还能够在光合和非光合营养组织中增强脂质生物合成。油棕和枣椰子（*Phoenix dactylifera*）的比较研究则表明，WRI1同源基因在这些不同的棕榈种类中的功能不同，或促成储油或促进糖的积累[109,110]。

此外，一个称为14-3-3的高度保守磷酸肽结合蛋白通过与AP2结构域的结合而与WRI1相互作用，进而调节TAG的合成代谢[111]。*14-3-3*和*AtWRI1*的共表达导致本生烟草（*Nicotiana benthamiana*）和稳定获得的转基因植物中均发现TAG的大量积累，表明14-3-3能够增强WRI1的转录活性[112]。在马铃薯块茎中过表达*14-3-3*基因的早期研究则表明，随着叶片中可溶性糖和儿茶酚胺含量的增加，总脂质含量也增加了69%[113]。另一方面，位于WRI1调控网络上游的转录因子LEC2[114]，也在植物碳分配控制中发挥着重要作用[115]。据报道，在拟南芥中*LEC2*基因的衰老诱导表达导致转基因叶片中的TAG增加了3倍[88]。然而，在表达*LEC2*的植物中亦观察到包括单/二-半乳糖基二酰基甘油（MGDG/DGDG）和磷脂酰甘油（PG）在内的重要膜脂的急剧减少，这反映了膜脂质体内平衡的破坏。

除转录因子外，ACCase的过表达也可促进植物总脂质的增加[89]。ACCase在麻风树中的表达被发现与种子发育期间油的积累相关[116]。为了将更多的碳转移到脂肪酸的生物“从头”合成中，在拟南芥、马铃薯和玉米中作为催化淀粉生物合成的主要步骤的限速酶ADP-葡萄糖焦磷酸化酶（AGPase）经RNAi下调以扩大脂肪酸合成所需的碳来源，但是效果却较为有限[89,108,117]。相比之下，包括FatA1、FatA2和FatB在内的硫酯酶家族参与了脂肪酸从脂质脂肪酸“从头”合成到细胞质的运输，在烟草营养组织中被过量表达后，TAG的生物合成得以进一步提高[118]。

3.2.2. TAG 高效组装

在叶片组织中，TAG通常作为淀粉生产的副产物合成，其功能却不仅仅局限于能量供体[16,119]。据报道，TAG还可以通过在叶片衰老过程中转化膜酰脂，将残余的酰基CoA结合到中性脂质中以进一步支持库器官的成熟[120]。目前这方面的基因调控策略主要集中在直接增强限速酰基转移酶的表达，如DGAT和PDAT[74,121]。在大多数植物物种中，DGAT家族由3个不同的成员组成，包括*DGAT1*、*DGAT2*和*DGAT3* [122]。已

知*DGAT1*是在Kennedy途径中组装TAG的主效基因，而DGAT2则部分参与到不常见脂肪酸如羟基脂肪酸的生物合成中[123–125]。最近，DGAT3被假定为衰老叶片中由质体半乳糖脂释放的游离酰基CoA的清除剂[126]。*DGAT1*和*DGAT2*的异源表达都能够增强植物中TAG的积累[127,128]。在酵母突变体中过表达来自麻风树的DGAT表明，DGAT1可以诱导TAG增加16.6%，DGAT2诱导TAG增加14.3%，表明它们在TAG积累中起关键作用[68]。

PDAT的遗传操作也已经被用来增强酰基CoA的非依赖途径[92,129]。同时，*PDAT*还被认为是一种涉及不常见脂肪酸代谢的多功能基因[130]。最近关于拟南芥中脂质体变化的研究表明，在热胁迫下，由于PDAT的上调，TAG和极性脂质的水平可以同时改变，这表明PDAT可能参与到比DGAT更复杂的脂质代谢网络中[131]。此外，在酰基编辑程序中，为了调节植物细胞内稳态，PDAT与胆碱磷酸转移酶（CPT）可能协同工作从而将PC转化为DAG以调控极性脂和中性脂的动态平衡[75,132]。由PDAT介导的TAG积累途径的独特特征，值得在植物源脂质代谢学中进一步探索。

3.2.3. TAG 包装

植物油质蛋白和哺乳动物的脂滴包被蛋白在酵母细胞中的异源表达实验表明，这些LD整合蛋白能够促进TAG的包装隔离并加速随后的LD聚合[133]。因此植物油质蛋白和其他油体相关蛋白成为脂质代谢工程的目标，用于增强植物油种子及营养组织中的TAG生产[77,134,135]。

在拟南芥中已知至少有17个差异表达的*Oleosin*基因，表明这些基因受到高度调控[136]。拟南芥油质蛋白的敲除或插入突变均可导致少量LD的增加[137]。大豆油质蛋白在转基因水稻中的过表达可产生更多但更小的LD，与非转基因对照相比，含油量增加37%~46%，而TAG的总体脂肪酸成分能得以保持不变[138]。当半胱氨酸-油质蛋白在拟南芥中与*DGAT1*共表达时，不同营养组织中总脂肪酸含量都得到了不同程度的提高[139]。

再者，非整合蛋白（如LDAP）显然亦扮演着重要角色，例如稳定LD的表面以防止LD的融合或分散，并通过形成酶复合物促进新脂质的合成[77,140]。与LD相关的其他主要非整合蛋白包括来自玉米的油体相关蛋白1（OBAP1）[141,142]及来自拟南芥的SEIPIN [100]也被发现在TAG的包装和积累中发挥着潜在作用。目前已在拟南芥中鉴定出3个LDAP基因，所有这些基因都是特异性结合在LD表面的，并且在热和冷温度胁迫期间*LDAP1*和*LDAP3*能够特异性地诱导LD的形成[77]。过表达LDAP的转基因拟南芥植株表现出较高的营养和生殖生长速率，抗旱性也显著提高[143]。

3.2.4. 预防 LD 降解

LD的分解、TAG降解和脂肪酸的β-氧化是脂肪代谢的常规循环。因此，LD的维持和脂解的抑制是减少植物营养组织中油分丢失的目标之一[144]。糖依赖性1（SDP1）和过氧化物酶体ABC-转运蛋白1（PXA1）是目前公认的脂解酶。SDP1最初在拟南芥种子中被发现，是分解LD的主要酶[94]。脂解酶的详细机制一直不甚清楚，直到最近的研究表明SDP1可通过过氧化物酶体延伸和脂蛋白在过氧化物酶体与LD的相互作用中接触LD。这种过程通常在植物幼苗发育的早期阶段被激活，并受LD数量的高度调控，暗示SDP1的表达活性在一定程度上与TAG的含量有关[145,146]。早期关于植物SDP1的研究主要集中在突变体上，证明了TAG含量可随着*SDP1*的缺失而显著增加[147,148]。利用转录组学分析的进一步研究表明，SDP1可以在几乎所有的植物组织中表达，意味着油脂降解并不是组织特异性的，因此通过抑制脂肪分解以增加植物营养组织中LD的储存是可行的[149]。对油籽中*SDP1*表达的RNAi抑制虽然影响到了种子活力，但最终使油产量增高了8% [94]。另一种脂解酶PXA1在功能上可能负责将脂肪酸运输至过氧化物酶体中[150]。*PXA1*缺失的拟南芥突变体能够在脂质水解过程中基本上保留FFA[151]，但主要是胞浆中的α-亚麻酸[98]。据推测，PXA1可能无法独立工作，主要通过与称为比较基因识别-58（*CGI-58*）的基因相互作用，该基因最初在哺乳动物脂肪的自噬中被发现，并在植物脂解中起关键作用[152,153]。而干扰*CGI-58*在拟南芥中的表达不但使脂质生物合成上调，而且还显现出与PXA1表达活性的正相关性[154]。这在随后的研究中得以证实，*CGI-58*作为表达调节因子可以正向调节大多数非种子植物组织中的PXA1活性[155]。

除了直接操纵植物营养组织中的TAG生物合成外，胚乳特异性玉米同系物淀粉分支酶（SBE）的内源性替代能够通过提高种子产量达3倍来增加拟南芥的总含油量[156]。虽然TAG的增加主要归因于单株植物角果的高度发育，但这一研究仍然带来了新的见解，即碳分配

过程中涉及的其他间接碳水化合物途径亦可能提高植物的产油率。

3.3. 多基因工程策略

最近的技术进步显著增强了通过同时表达多个转基因来操纵复杂的脂质代谢网络的能力。植物中TAG的生物合成是一个被高度调控的过程，与从最初的碳分配到处于动态平衡间的脂质合成有关的多种生物化学途径紧密相关[16]。参与植物脂质代谢的生化途径相应地受多种限速酶的调控[157]。作为植物营养组织中短暂存在的副产物，由于这些关键基因的复杂转录调控，TAG通常不能被连续合成或大量积累[68]。即便如此，大多数限速酶的单基因操作在增加油脂积累方面已经表现出有效的作用，而同时调控不同基因以获得进一步增强的TAG生产手段则将更加有效。在这种综合策略下，扩大作为“源”的脂肪酸生物“从头”合成并降低以LD为“库”的TAG消耗依然是提高植物含油量的主要方法[26,158]。因此，包括上游转录因子、TAG装配的决定性酰基转移酶、LD整合蛋白和下游脂解酶等主要因素被同时视为协同调控脂质代谢网络的主要目标。本生烟草已经被用于基因瞬时表达的分析，能够快速评估复杂的TAG生物合成途径中不同基因组合的表达效果[18,159]。目前已开发出一套能够对脂质生产的多基因工程及酶活性优化的有效评估系统，这对于成功开发新的油料作物范例至关重要。

表2[17,19,21,91,108,160–162]总结了近期通过组合多种转基因以增强植物营养组织中TAG产生的代表性研究。*WRI1*、*DGAT1*和*OLEOSIN1*基因的同时过表达能够将烟草叶中的TAG含量提高到干重的15%，远远超过了单独表达这些基因所能达到的水平[18]。这3个基因在分别指导光合碳分配到脂肪酸生物合成、TAG装配和LD的形成中各自起到了关键的作用，其协同功能不但进一步增加了TAG的含量，也使膜脂的水平有小幅度增长。而当通过RNAi干涉*SDP1*的表达或导入拟南芥LEC2至高含油量的转基因烟草之后，TAG在烟草叶片干重中的含量空前地增加至30%~33%，已达到油料种子的标准[17]。同样，转基因甘蔗中也表现出了类似的结果，当在表达*WRI1-DGAT1-OLEOSIN1*的甘蔗中*AGPase*和*PXA1*的表达被抑制后，地上营养组织中TAG被提高了95倍，而单基因工程仅能使每个单基因转基因品系内的TAG增加1.5~9.5倍[19]。在地下马铃薯块茎中也证实了多转基因调控对TAG积累的协同效应[17]。马铃薯块茎是重要的淀粉来源，鲜重含有16%~20%的淀粉、2%~2.5%的马铃薯糖蛋白和72%~75%的水，而脂质仅占约0.1%~0.15%[163]。当通过同时过量表达*WRI1*、*DGAT1*和*OLEOSIN1*基因，转基因马铃薯品系在块茎干重中可积累至3%的TAG，与野生型相比含油量增加了近100倍[37]。这与单独表达WRI1转录因子的转基因马

表2 植物中脂质的多基因工程的代表性研究

Target plant	Target organ	Promoter and target gene	Beneficial effect	Other effect	Reference
Nicotiana tabacum	Leaf	enTCUP2::*NbSDP1* AtSAG12::*AtLEC2*	30%–33% accumulation of TAG	Transitory starch reduction	[17]
Saccharum officinarum	Leaf	OsUbi3::*AtWRI1* ZmUbi3::*ZmDGAT1-2* CaMV35S::*ZmPXA1,SoAGPase,AtOLE1*	95 fold increase of TAG	43 fold increase of TAG in stem	[19]
Solanum tuberosum	Tuber	StB33::*AtWRI1,AtOLE1* CaMV35S::*AtDGAT1*	100 fold increase of TAG	Reduced starch, increased soluble sugar content	[21]
Arabidopsis thaliana	Leaf	CaMV35S::*AtPDAT1,AtSDP1,AtPXA1*	3 fold increase of total lipids	Delayed growth and development	[91]
Arabidopsis thaliana	Leaf	CaMV35S::*AtWRI1,AtAGPase*	5.8 fold increase of oil content	Decreased starch and chlorophyll	[108]
Crambe abyssinica	Seed	Napin::*BnFAE1,LdLPAAT,CaFAD2*	28% increase of erucic acid	Decreased C18 unsaturated fatty acids	[160]
Arabidopsis thaliana	Leaf	CaMV35S::*VfFAD2,VfDGAT2*	21% increase in total lipids	Increased polyunsaturated fatty acids	[161]
Arabidopsis thaliana	Seed	AtSUS2::*AtWRI1,AtDGAT1* VfUSP::*AtSDP1*	1.2 fold increase of TAG	Increased seed mass, decreased seed number per plant	[162]

Nb: *Nicotiana benthamiana*; *Zm*: *Zea mays*; *Ld*: *Limnanthes douglasii*; *Vf*: *Vernicia fordii*; enTCUP: tobacco constitutive expression cryptic promoter; OsUbi3: *Oryza sativa* ubiquitin promoter; CaMV: cauliflower mosaic virus; B33: potato tuber-specific patatin promoter B33; SUS: sucrose synthase; VfUSP: *Vicia faba* unknown seed protein; *SAG*: senescence associated gene; Napin: *Brassica napus* seed specific promoter; *AGPase*: ADP-glucose pyrophosphorylase; *OLE*: oleosin; *FAE1*: fatty acid elongase1; *LPAAT*: lysophosphatidic acid acyltransferase; *FAD2*: microsomal oleate desaturase.

铃薯形成了鲜明的对比，该转录因子在块茎中获得了1% TAG干重的积累[20]。尽管人们对植物地下组织内TAG合成的详细信息至今仍知之甚少，但可以明确的是，在植物营养组织中，针对总体脂质代谢网络的多基因操纵策略在重新分配碳以合成目前最稳定且能量最高的三酰甘油化合物中是十分有效的。

4. 转基因对 TAG 积累的转录控制

与有细胞毒性的FFA不同，TAG在植物细胞质中的大量积累基本上是无害的，因为它通常是一种具有良好亲水性和非细胞毒性的稳定化合物[164]。然而，在高油转基因品系中的生物量生产和TAG积累之间却存在折衷[37,91]。将碳从常见的代谢过程引导至TAG的生物合成通路可能会对本身不积累TAG的营养组织产生代谢冲突。这种代谢负担通常反映为植株生长缓慢和生物量减少。因为高生物量的积累是作物具有经济价值的直接体现，所以这种结果是不理想的。广泛用于驱动目标基因过表达以增强TAG生物合成的CaMV35S启动子被认为会引起转基因植物生化网络平衡的紊乱[165–167]。因此，TAG在植物营养组织中的持续性生产或许不是具有普遍应用性的生产模式[68]。在时间和空间两个维度上精确调控目标基因的表达因此显得至关重要[168,169]。

为了避免这些不良影响，如表1和表2所示，通过启动子介导的诱导系统进行调节TAG产生的程序化途径可成为一种新型手段。叶绿体特异性RuBisCO小亚基（SSU）启动子用于转基因烟草叶片中*WRI1*和*OLEOSIN1*的表达调控，以控制转基因在绿色组织中的转录活性[18]。这种尝试成功地将叶片油含量提高到了工业生产标准，而不会对其他重要的生理和生物化学性状产生明显的不良影响。随着整个植物生物量的早期建成，衰老阶段被认为可能是植物高水平产油的时期之一[170]。尽管有报道称人们担心在植物细胞器的衰老期间叶绿体的自然降解会使碳固定能力会被削弱，但衰老诱导型启动子却依旧能够在植物营养组织中进一步增加TAG的含量[170–174]。

5. 提高非种子植物组织中 TAG 积累的其他关键问题

虽然在基因发现方面有重大进展，但许多基因在脂肪酸生物合成和脂质代谢中的作用仍然难以确定。即使在基因组信息已相对明确的拟南芥等模式植物中，最近的全基因组关联分析研究也揭示了大量先前未知的与脂质代谢密切相关的基因[175]。对于许多没有进行基因组测序的油料植物，比较转录组学可用于生成大数据库，正如油棕与枣椰之间的比较研究所示，这些数据集则可以被用于发掘主要的脂质调控候选基因[109,176]。

在开展脂质代谢工程之前，有必要确认给定的目标植物物种在理论上是否具有商业可行性。对于基于植物油原料来源的生物柴油生产来说尤其如此。例如，高产量和高生物量作物如马铃薯块茎已被认为是潜在的可储油作物。最近在马铃薯块茎中已获得适度提高的油脂积累值得用来进一步研究TAG代谢，以及典型淀粉积累贮藏器官中油与淀粉之间错综复杂的关系[20,177]。因为在解剖学上相似的黄色莎草块茎中TAG的含量可达到其干重的30%左右，表明至少在理论上可进一步提高转基因马铃薯块茎中的TAG积累而同时对植物生长和块茎产量不引发严重影响[65]。

对于一个特定目标油料作物基因供体的选择，受体和供体物种之间的进化距离也非常重要。尽管关于从进化上较远的供体向受体中转入基因的研究偶有报道，但成功率相当有限[178]。例如，特定转录因子（如WRI1）的异位表达需要考虑它们与其同源结合位点的相容性。最近已有研究发现*WRI1*的高表达具有致死性，尤其是当基因供体和受体植物之间的同源性非常高时更是如此[86]。这样的结果说明需要在分子和生化水平上更详细地研究目标作物和基因供体之间的代谢网络是否差异显著。

同时表达多个外源基因的传统方法涉及转基因在转化载体中的叠加。线性载体内多个转基因的物理排列对于减少产生反义RNA兼触发转基因沉默的可能性至关重要[179]。进一步的考虑是可通过包含几个不同的启动子来协调基因表达，因为重复使用相同的启动子可能导致同源DNA序列的不稳定。实际上这种转基因叠加的方法仅适用于少量基因，因为插入的基因片段太大会有增加重组率的风险。而且，通过单个载体导入与TAG生物合成途径有关的多个转基因的尝试往往也会受到将转基因克隆到大载体中的技术限制。这种限制可以通过两种或更多种转化载体以共转化的形式加以克服，或者通过连续转化步骤和随后通过有性杂交来解决。此外，随着基因合成成本的迅速下降以及合成和组装大型DNA插入片段的稳步进展，可以设想在不久的将来将更复杂和更高水平的TAG生物合成途径导入目标作物基因组。

一些研究指出，当可用的FFA被激活参与TAG生物合成途径时，可以改变重要的膜脂质如磷脂和半乳糖脂

的合成[180,181]。植物细胞的质膜介导与干旱和极端温度相关的渗透调节，亦可作为对细菌、真菌和昆虫等生物胁迫对抗的主要屏障。另外，膜脂还是用于定位或对环境波动进行远端响应的信号分子的脂质存储库。例如，在内源性信号转导和植物应激反应系统中起关键作用的中间化学物质如磷酸（PA）水平的变化经研究发现与植物的生长和生理调控有关[182,183]。此外，涉及脂质信号传导的基因才刚刚开始被揭示，并且很有可能许多未被深入研究的脂质相关基因也发挥着信号传导或细胞膜重塑的功能（如PDAT）。在组学上十分需要对营养组织中引入TAG生物合成途径的转基因油料作物进行系统评价[184–187]。新的富油植物的环境适应性、实际生产力和市场可行性以及多点田间测试也因此非常重要[20]。由于全球生态恶化，在不利条件下作物的生存也应加以考虑。最近发现有TAG降解缺陷的植物能够承受长时间的黑暗和氧化损伤，表明植物组织中高度积累的TAG可能会更有效地抵抗非生物胁迫[188]。

6. 总结与展望

植物油在土地使用、粮食安全、生物燃料生产和许多非食品应用方面具有重要意义。传统的植物育种对提高油脂产量至关重要，但由于缺乏可用于获得复杂的油脂积累途径的遗传多样性而受到限制。通过酶过量表达或引入对负反馈调节不敏感的酶变体，特别是在多个转基因的协同表达的情况下，重新调控TAG生物合成中的限速步骤已经取得了很大的进展。

转基因来源、诱导型启动子和目标植物物种的适当选择和匹配对于建立生产上可行且不会严重影响转基因植物正常发育的高生物量植物油生产平台具有重要意义。高生物量植物油生产的进一步改进需要系统生物学的方法来获得对植物源TAG代谢网络的更多了解，而且还需要对生物合成途径进行复杂的多级调控，包括碳分配和协调脂质代谢以及转换的转录因子。实现这一目标需要系统生物学和代谢网络重建方面的有机结合和更深入的认知。最后，组学技术和先进的基因组编辑能力的结合将极大地扩展在植物营养组织内增强TAG积累的能力。

合成生物学在当前的脂质代谢工程中已能够提供快速组装多种基因的可行性，这体现在可互换和模块化组装的转基因载体试剂盒的开发和运用上。可以设想，未来的高油性状还可以与具有极大健康效益或工业价值的其他产量性状相结合，如ω-3长链多不饱和脂肪酸。这种“双重目的”作物有可能被直接用作新型健康食品、动物饲料以及重要的油脂化学原料。再者，目前流行的基因组编辑工具（如CRISPR/Cas9）可用于消除或最小化因TAG大量积累而引起的代谢竞争，同时有效地引导代谢流向植物油的生物合成，甚至是修饰脂质生物合成酶中的特定氨基酸位点以改善酶活性或改变其底物的特异性。

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Compliance with ethics guidelines

Xiao-Yu Xu, Hong-Kun Yang, Surinder P. Singh, Peter J. Sharp, and Qing Liu declare that they have no conflict of interest or financial conflicts to disclose.

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