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综 述 Reviews

异黄酮的生物合成及代谢工程研究进展

李笑佳, 包爱科*
兰州大学农业农村部草牧业创新重点实验室, 兰州大学草地农业科技学院, 兰州730000
*通信作者(baoaik@lzu.edu.cn)

摘要: 植物异黄酮是一类广泛存在于豆科植物中的次级代谢物, 能够促进根瘤菌结瘤和固氮, 并在植物与环境的相互作用中起着关键的作用。异黄酮因其具有雌激素活性对人体健康有诸多益处, 如降低患骨质疏松症、心血管疾病和癌症的风险以及缓解更年期症状。此外, 异黄酮还能够促进畜禽的生长发育以及提高肉、蛋、奶等畜禽产品的品质。近年来, 植物中异黄酮的生物合成及其分子机制的研究受到关注并取得了重要进展, 在此基础上, 众多研究者通过基因工程手段成功提高了植物中的异黄酮含量。本文重点对近年来有关植物异黄酮的结构和功能、其生物合成机制及其代谢工程的最新研究进展进行综述, 并对未来相关研究进行了展望, 旨在为相关研究提供有益的参考。

关键词: 异黄酮; 豆科植物; 苯丙烷代谢; 生物合成; 遗传改良

Advances in biosynthesis and metabolic engineering of isoflavones

LI Xiaojia, BAO Aike*
Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou 730000, China
*Corresponding author (baoaik@lzu.edu.cn)

Abstract: Plant isoflavones are a class of secondary metabolites widely present in legumes, which can promote nodulation and nitrogen fixation of rhizobium, and play a key role in the plant-environment interaction. Because of their estrogenic activity, isoflavones show many health benefits, such as reducing the risk of osteoporosis, cardiovascular disease and cancer, as well as relieving menopausal symptoms. In addition, isoflavones can also promote the growth and development of livestock and improve the quality of meat, eggs, milk and other livestock and poultry products. In recent years, the biosynthesis and molecular mechanism of isoflavones in plants have attracted great attention and made important progress. On this basis, many researchers have successfully improved isoflavone content in plants through genetic engineering. In this paper, we review the recent research progress on the structure and function, biosynthesis mechanism as well as metabolic engineering of isoflavones in plants, and prospects for future studies, aiming to provide useful references for related studies.
Key words: isoflavones; legumes; phenylpropane metabolism; biosynthesis; genetic improvement

异黄酮是一类具有雌激素活性的植物化学物质, 其不仅能够诱导慢生根瘤菌的结瘤, 影响植物根际微生物群落的多样性, 还在植物应对各种非

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生物和生物胁迫中发挥作用(Subramanian等2006; Dastmalchi等2017)。异黄酮还具有重要的药用价值, 能够预防冠心病、痴呆症和癌症, 对于改善更年期综合征、肥胖症、炎症和衰老等症状也有一定的作用(Strong等2014; Zhang等2014a), 同时还具有抗皱、美白和皮肤保湿的功效(Lim等2014)。此外, 在饲料中添加异黄酮对反刍动物及家禽的生长、产蛋和产奶量、繁殖以及抗病等方面均具有积极的影响, 且能提升肉、蛋、奶的品质(Grgic等2021)。

目前发现异黄酮含量最丰富的植物主要是大豆(*Glycine max*)和红三叶(*Trifolium pratense*), 除此之外, 紫花苜蓿(*Medicago sativa*)、百脉根(*Lotus corniculatus*)、鹰嘴豆(*Cicer arietinum*)、葛根(*Pueraria lobata*)、羽扇豆(*Lupinus micranthus*)等其他豆科植物中也含有少量异黄酮(Kaufman等1997)。近年来, 因异黄酮的诸多益处, 市场上对富含异黄酮的保健品及食品、甚至药品的需求量不断增加, 使异黄酮的结构与功能、其在植物中的生物合成及分子机制成为众多研究者关注的热点问题, 这将有助于通过代谢工程及基因工程等技术提高植物中异黄酮的含量, 为大量生产异黄酮及其功能性食品和药物奠定重要基础。

1 异黄酮的结构与作用

1.1 异黄酮的结构

异黄酮类化合物是一类特殊的代谢物, 在自然界通常以游离型苷元和结合型糖苷2种形式存在(赵慧颖等2022), 但只有游离型苷元才具有代谢活性, 而结合型糖苷可在葡糖苷酶的作用下水解为游离的异黄酮苷元(Rekha和Vijayalakshmi 2011)。截止目前, 已经从300多种植物中鉴定到2 400多种异黄酮(Sajid等2021)。异黄酮类化合物是由两个苯环连接到一个杂环上的多酚类化合物, 在苯环上有不同的取代基, 它们的结构因甲基化、羟基化和糖基化的程度不同而具有一定的差异(Deng等2019; 图1)。异黄酮包含12种不同的异构体, 分为4种化学形式, 即苷配基(aglycones)、7-*O*-葡萄糖苷(7-*O*-glucosides)、6″-*O*-乙酰基-7-*O*-葡萄糖苷(6″-*O*-acetyl-7-*O*-glucosides)和6″-*O*-丙二酰基-7-*O*-葡萄糖苷(6″-*O*-malonyl-7-*O*-glucosides) (Deng等2019; Bernatoniene等2021; 表1)。

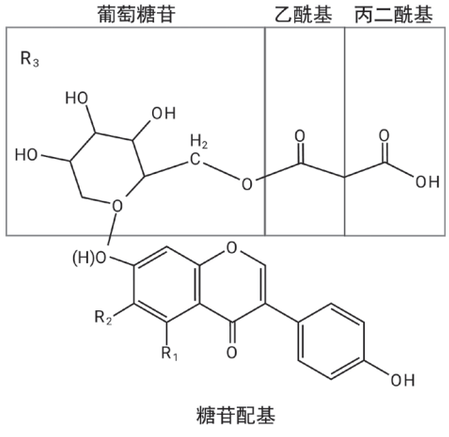

图1 异黄酮类化合物的化学结构式

Fig. 1 The chemical structure formula of isoflavones

参考Deng等(2019)和Bernatoniene等(2021)文献绘制。

异黄酮类化合物的主要单体包括大豆黄素(7, 4’-二羟基异黄酮, daidzein)、染料木素(7, 4’-二羟基-6-甲氧基异黄酮, genistein)、芒柄花素(7-羟基-4’-甲氧基异黄酮, formononetin)、鹰嘴豆芽素A (5, 7-二羟基-4’-甲氧基异黄酮, biochanin A)和黄豆黄素(7, 4’-二羟基-6-甲氧基异黄酮, glycitein) (Spagnuolo等2014), 它们与天然雌激素(17β-雌二醇, 17β-estradiol)的结构具有很高的相似性(图2), 这使异黄酮能够与雌激素受体结合并表现出类似于天然雌激素的生物活性(Rafii 2015)。

1.2 异黄酮的作用

1.2.1 异黄酮在植物中的功能

异黄酮类化合物在植物-环境相互作用中发挥着重要的作用, 能够促进豆科植物共生结瘤、抑制病原菌感染, 并丰富植物根际微生物群落的多样性(White等2015; Dastmalchi等2017)。Sugiyama等(2016)研究发现大豆在营养生长阶段通过异黄酮偶联物水解β-葡萄糖苷酶水解葡糖苷来分泌大量的异黄酮以吸引根瘤菌。Subramanian等(2006)证明异黄酮可通过诱导慢生根瘤菌结瘤基因的表达而促进大豆根瘤菌结瘤。此外, 异黄酮还在植物防御各种生物和非生物胁迫中起着关键的作用(Kubes等2019)。已有研究表明, 异黄酮类化合物如芒柄

表1 异黄酮类化合物的主要异黄酮苷元及其异构体

Table 1 The main isoflavones aglycones and their isomers

结构形式	R1	R2	R3	异黄酮
苷配基(aglycone)	H	H	H	大豆黄素(daidzein)
	OH	H	H	染料木素(genistein)
	H	OCH_3	H	黄豆黄素(glycitein)
葡萄糖苷(glucoside)	H	H	$C_6O_5H_{11}$	大豆苷(daidzin)
	OH	H	$C_6O_5H_{11}$	染料木苷(genistin)
	H	OCH_3	$C_6O_5H_{11}$	黄豆黄苷(glycitin)
6″-*O*-乙酰基葡萄糖苷	H	H	$C_6O_5H_{11}$+OCH_3	6″-O-乙酰基大豆苷(6″-*O*-acetyldaidzin)
(6″-*O*-Acetylglucosides)	OH	H	$C_6O_5H_{11}$+OCH_3	6″-O-乙酰基染料木苷(6″-*O*-acetylgenistin)
	H	OCH_3	$C_6O_5H_{11}$+OCH_3	6″-O-乙酰基黄豆黄苷(6″-*O*-acetylglycitin)
6″-*O*-丙二酰基葡萄糖苷	H	H	$C_6O_5H_{11}$+$COCH_2COOH$	6″-O-丙二酰基大豆苷(6″-*O*-malonyldaidzin)
(6″-*O*-malonylglucosides)	OH	H	$C_6O_5H_{11}$+$COCH_2COOH$	6″-O-丙二酰基染料木苷(6″-*O*-malonylgenistin)
	H	OCH_3	$C_6O_5H_{11}$+$COCH_2COOH$	6″-O-丙二酰基黄豆黄苷(6″-*O*-malonylglycitin)

数据来自Deng等(2019)和Bernatoniene等(2021)文献。

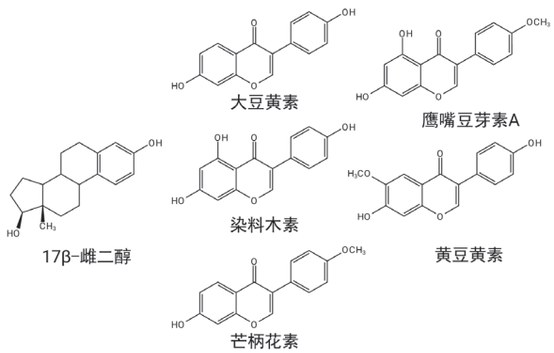

图2 17β-雌二醇和主要异黄酮单体的结构式

Fig. 2 Structural formulas of 17 β-estradiol and main isoflavone monomers

花素可以保护白三叶(*Trifolium repens*)免受虫害, 如象鼻虫啃食(Franzmayr等2012)。染料木素可以增强大豆对大豆斑疹病菌(*Xanthomonas axonopodis* pv. *glycines*, *Xag*)的抗性, 增强大豆的抗氧化能力, 促进大豆叶片中酚类物质的积累(Zhang等2022)。同时大豆种子含有较高的异黄酮而对白粉病具有一定的抗性(Deng等2019)。植物应对非生物胁迫方面, 研究发现异黄酮类化合物的积累还可保护植物免受冷害、冻害和盐胁迫的影响(Khan等2012)。在高温胁迫下, 大豆可将豆荚中的异黄酮或关键代谢前体物质转移到种子中以抵御高温对大豆种子的伤害(Chennupati等2012)。而外源异黄酮浸泡过的种子可减轻干旱胁迫幼苗的细胞损伤, 增强其抗氧化活性和光合作用, 提高栽培大豆的耐旱性(Tian等2014)。

1.2.2 异黄酮对人类的益处

作为一种天然的植物雌激素, 异黄酮不仅是潜在的化学预防剂, 而且是非常重要的治疗性天然药物, 同时还具有抗氧化特性。这些特性使得异黄酮能够预防子宫内膜癌、乳腺癌和前列腺癌等癌症以及心血管和神经性疾病, 还可改善更年期综合征、肥胖症、炎症和衰老等疾病引发的症状(Liang等2014; Zhang等2014b; Yu等2016; Zhong等2018; Pang等2020; Kim 2022)。研究表明, 大豆中的异黄酮能够抑制蛋白酪氨酸激酶的活性, 从而起到预防冠心病和认知障碍/痴呆症的作用(Ferguson等2014; Sekikawa等2019)。异黄酮还可通过直接影响β细胞的增殖和葡萄糖刺激的胰岛素分泌, 有效缓解糖尿病症状(Vinayagam和Xu 2015)。此外, 异黄酮可以减少肠源性内毒素, 加速酒精代谢, 促进肝毒性化学物质的解毒, 同时减缓肝细胞凋亡、恢复自噬活性, 对肝脏起到很好的保护作用(Alipour和Karimi-Sales 2020)。由于其多效性, 异黄酮类化合物被认为是治疗绝经期雌激素减少等相关疾病的一种天然替代品(Bernatoniene等2021), 特

别是红三叶中提取的鹰嘴豆芽素A和芒柄花素在缓解妇女更年期不适方面的作用已在临床试验中得到了证实, 并且相较于其他植物中提取的异黄酮化合物副作用最小(Tucak等2018)。

1.2.3 异黄酮在家畜生产中的作用

研究发现, 饲喂一定浓度的异黄酮, 可对动物的生长、产蛋和产奶等产生积极的影响(Payne等2001; Grgic等2021; Tsugami等2022)。研究发现, 在饲料中添加红三叶干草可以抑制牛和反刍动物胃中的高产氨瘤胃细菌(hyper ammonia-producing bacteria, HAB), 促进瘤胃中纤维素分解细菌的增殖, 提高反刍动物的平均日增重(Harlow等2020)。用三叶草混合饲料喂养奶牛后, 牛奶中异黄酮浓度显著升高(Andersen等2009)。在饲料中添加高浓度的异黄酮能显著增加鸡蛋蛋黄中染料木素和大豆苷元的含量, 还能改善母鸡产蛋后期的产蛋量和鸡蛋的品质, 以及产蛋母鸡的骨骼状况(Lv等2019)。另外, 饲料中添加适量的异黄酮能提高草鱼肌肉中蛋白质和总多不饱和脂肪酸(polyunsaturated fatty acid, PUFA)的含量, 提升鱼肉的综合品质(Yang等2019)。其次, 异黄酮对猪的生长、免疫系统、繁殖以及抗病等方面产生一定的影响(Li等2020)。作为一种免疫调节饲料添加剂, 异黄酮还能减弱呼吸综合征病毒(porcine reproductive and respiratory syndrome virus, PRRSV)对猪造成的不利影响, 降低猪感染PRRSV的比率(Smith等2020)。除此之外, 异黄酮还能通过降解高羊茅种子纤维而缓解动物因高羊茅中毒造成的血管收缩、体重和肌肉发育不良以及采食量降低等生理症状, 其中红三叶的治疗效果最佳(Ault-Seay等2020; Harlow等2022)。但也有报道称, 由于绵羊子宫内的雌激素受体是奶牛的2~4倍, 异黄酮在绵羊体内的代谢速度比在奶牛体内更快, 饲喂大量红三叶干草会对绵羊的生殖产生不利的影响(Hloucalová等2016; Grgic等2021)。绵羊长期摄入植物雌激素的安全范围应不超过0.5%~1% (干重), 其中芒柄花素的含量不应超过0.3% (干重) (Pace等2006, 2011; Nichols等2013)。

1.3 异黄酮在植物中的分布及影响其含量的因素

目前, 针对大豆异黄酮的研究相对较多, 然而红三叶中异黄酮的浓度更高, 达到10~25 $mg \cdot g^{-1}$, 是大豆的3~25倍(Grgic等2021)。此外, 众多其他豆科植物也能够大量合成异黄酮, 如鹰嘴豆、葛根、豌豆 (*Pisum sativum*)、百脉根、小冠花 (*Coronilla varia*)和苜蓿等。豆科植物中的异黄酮类化合物主要有染料木素、大豆黄素、鹰嘴豆芽素A和芒柄花素, 它们的含量在不同物种间具有一定的差异, 例如: 在红三叶中的主要为芒柄花素和鹰嘴豆芽素A, 在紫花苜蓿中主要是美迪紫檀素(medicarpin), 豌豆中主要是豌豆素和美迪紫檀素, 而在大豆中则主要是大豆黄素、染料木素和黄豆黄素(Du等2010)。

虽然异黄酮广泛存在于豆科植物中, 但影响植物组织中异黄酮含量的因素也有很多, 包括生长环境、植物种类、组织类型, 发育阶段以及储存方式等(Mustonen等2018; Tucak等2019; Wyse等2021)。研究发现, 低温会增加大豆中丙二酰异黄酮的含量, 高温则使其总异黄酮含量降低(Wang等2022)。Song等(2018)发现大豆异黄酮的活性成分总体上呈现从北(高纬度)到南(低纬度)的下降趋势。还有研究发现降雨量和海拔对异黄酮的影响比温度更大, 且异黄酮含量越高, 植物适应多变且复杂的天气条件的能力可能更强(Mustonen等2018; Enkhbat等2021)。在植物种类方面, Butkutė等(2018)发现红三叶、中间车轴草(*Trifolium medium*)、紫花苜蓿、天蓝苜蓿(*Medicago lupulina*)、红豆草(*Onobrychis viciifolia*)、甜叶黄芪(*Astragalus glycyphyllos*)和鹰嘴紫云英(*Astragalus cicer*)的异黄酮含量存在显著差异。陈寒青等(2004)对红三叶不同部位(花、茎和叶)中4种主要异黄酮(鹰嘴豆芽素A、芒柄花素、染料木素和大豆黄素)的含量进行了测定, 发现叶中异黄酮含量最高(0.856%), 茎中次之(0.403%), 花中最低(0.258%)。并且, 采收期与三叶草异黄酮含量之间呈现直接相关性, 特别是对芒柄花素的含量影响最大(Visnevschi-Necrasov等2013; Mustonen等2018)。此外, Wyse等(2021)发现, 紫花苜蓿鲜样比干样中含有更高浓度的香豆素和其他种类异黄酮。

2 植物中异黄酮生物合成及其关键酶基因

异黄酮的合成过程来源于苯丙烷代谢途径的一个分支, 而苯丙烷代谢途径是高等植物中主要

的次级代谢途径之一, 主要分为3个部分(图3)。第一部分是苯丙氨酸生物合成途径, 苯丙氨酸生物合成的起始是苯丙氨酸经苯丙氨酸裂解酶(phenylalanine ammonia lyase, PAL)脱氢生成肉桂酸, 而PAL是初级代谢途径中第一个关键酶(Weise等2017)。随后, 肉桂酸在肉桂酸4-羟化酶(cinnamic acid 4-hydroxylase, C4H)和4-香豆酸辅酶连接酶(4-coumarate-CoA ligase, 4CL)共同催化下生成*p*-香豆酰辅酶A, 这是苯丙氨酸生物合成的最后一步, 其中C4H是苯丙烷代谢途径的第一种细胞色素P450单加氧酶(Dinkins等2021)。第二部分是中间产物的生成, *p*-香豆酰辅酶A被查尔酮合酶(chalcone synthase, CHS)和查尔酮还原酶(chalcone reductase, CHR)催化生成异甘草素和柚皮素查尔酮, 然后经查尔酮异构酶(chalcone isomerase, CHI)催化合成黄烷酮(甘草素和柚皮素), 而甘草素和柚皮素是合成其他代谢物的重要前提物质, 其中甘草素是大豆黄素生物合成所必需的底物(Zhang等2014c)。第三部分是异黄酮的生物合成, 异黄酮合酶(isoflavone synthase, IFS)是异黄酮合成途径的第一个关

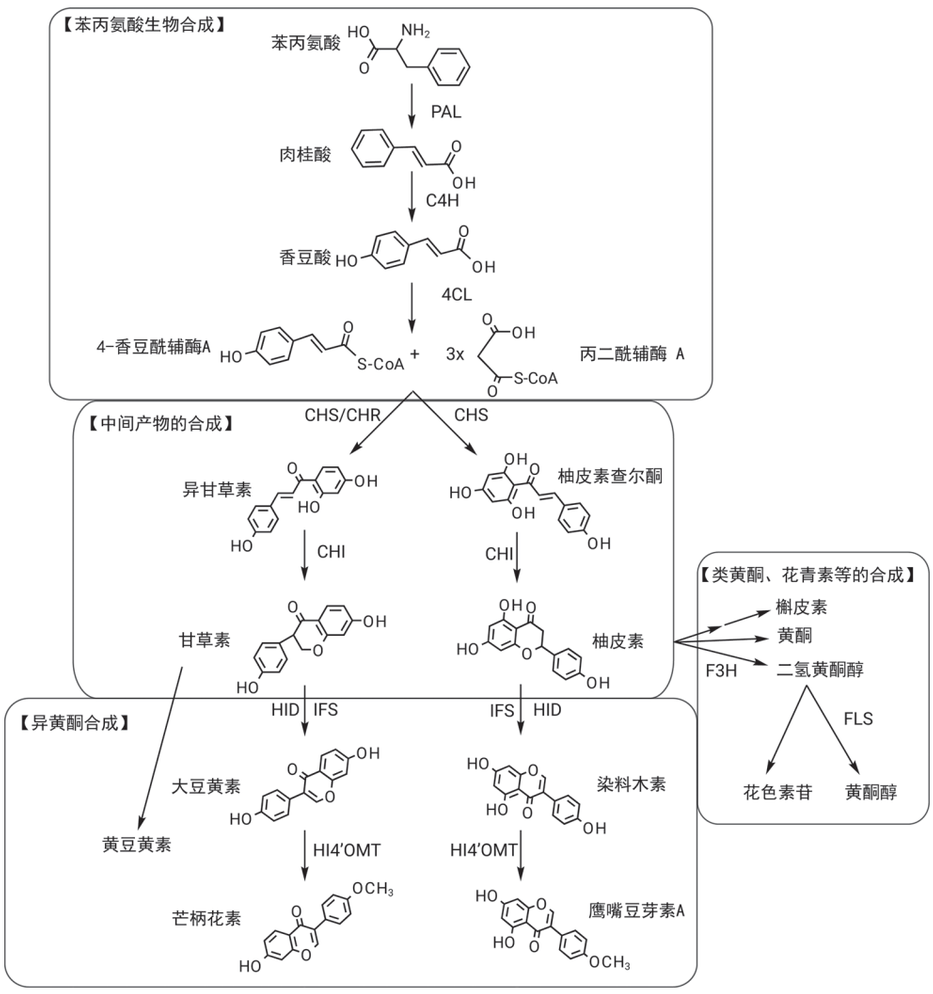

图3 异黄酮的生物合成途径

Fig. 3 Biosynthesis pathway of isoflavones

参考Dinkins等(2021)和Kim (2022)文献绘制。

键酶, 也是丙烷代谢途径中第二种细胞色素P450单加氧酶, 经羟化反应将黄烷酮转化成2-羟基异黄酮, 2-羟基异黄酮可以直接脱水并经2-羟基异黄酮类脱水酶(2-hydroxyisoflavanone dehydratase, HID)催化生成4′-羟基异黄酮(大豆黄素和染料木素), 也可以经2-羟基异黄酮4′-O-甲基转移酶(isoflavone 4′-O-methyltransferase, HI4'OMT)甲基化后脱水生成4′-甲氧基异黄酮(芒柄花素和鹰嘴豆芽素A) (Shimamura等2007)。此外还有类黄酮和花青素等其他途径也和异黄酮生物合成共用同一底物, 在黄烷酮3-羟化酶(flavonoid hydroxylase, F3H)和黄酮醇合成酶(flavonol synthase, FLS)的催化下, 由柚皮素生成黄酮醇和花色素苷, 柚皮素还可被催化生成黄酮、槲皮素等其他次级代谢物(Yu和McGonigle 2005)。异黄酮生物合成过程中的关键酶已经通过各种分子生物学和遗传学方法进行了大量的研究, 这些关键酶的研究推进了我们对异黄酮生物合成和催化机理的了解, 为后续进行代谢工程提供了强有力的指导。下文将逐一介绍异黄酮生物合成过程中的关键酶基因。

PAL是催化苯丙烷途径的第一个限速酶, 引导碳流从初级代谢进入次级代谢, 控制碳通量到各种生物活性小分子芳香族化合物, 以及木质素、细胞壁的结构成分(Zhang等2013; Kong 2015)。PAL通常以同源四聚体的形式存在, 在单子叶植物和一些双子叶植物中催化L-苯丙氨酸的脱氨(PAL活性) (Kong 2015)。PAL催化特性在植物的生长发育和逆境防御、工业以及生物医学领域发挥着重要作用(Dyshlyuk等2021; Feng等2022)。PAL具有不同的异构体, 由多基因编码, 并且几乎在所有真核生物中都是保守的(Feng等2022)。其最早从大麦(*Hordeum vulgare*)中分离出来, 随后在细菌(Lovelock和Turner 2014)、真菌(Kawatra等2020)、藻类(Arafa等2020)和各种植物中被发现(Shang等2012; Jun等2018)。

CHS属于III型聚酮合酶(type III polyketide synthases, PKS), 在结构上是最简单的PKS, 是植物中特有的, CHS由单拷贝基因编码, 在不同植物中序列高度保守(Wang等2018; Saslowsky等2000)。CHS可催化一系列脱羧、缩合和环化反应(Tropf等1995)。如催化一分子*p*-香豆酰辅酶A和三分子丙二酰辅酶A的缩合反应得到查尔酮衍生物柚皮素查尔酮, 是合成类黄酮、异黄酮和花青素等次级代谢物第一个限速酶(Yu等2018)。CHS在多种高等植物中均得到广泛研究(Durbin等2000; Deng等2014)。水稻(*Oryza sativa*)基因组包含31个*CHS*基因, 其中只有*OsCHS8*和*OsCHS24*编码具有活性的CHS, 具有催化形成柚皮素查尔酮的活性(Park等2020)。此外, CHS的活性受植物发育和组织分化中内源机制的调节, 并在紫外线防护、抗病和抗虫等方面发挥着重要的作用(Park等2020; Geng等2022)。

CHR是5-脱氧异黄酮类生物合成的关键酶, 它通过与CHS共同作用, 催化丙二酰基辅酶A与*p*-香豆酸辅酶A来产生异甘草素。CHR以多基因的形式存在于豆科植物中, 大豆基因组有11个*CHR*基因, 亚细胞定位分析表明GmCHR5存在于细胞质和细胞核中。蛋白质-蛋白质相互作用分析表明, GmCHR5可以与IFS同工酶相互作用, 共同催化异黄酮的生物合成(Mameda等2018)。同时, 不同的*CHR*基因在植物中发挥着不同的作用, 大豆*GmCHR5*在根和种子中参与5-脱氧异黄酮类化合物的积累, 而*GmCHR1*和*GmCHR6*在对病原菌的防御机制中起关键作用(Nakayama等2019)。

CHI是一种非常稳定的酶, 它能催化分子内环化反应, 将双环查尔酮转化为三环(2S)-黄烷酮(Du等2010; Yin等2019)。尽管柚皮素查尔酮在水溶液中可以快速自发地环化, 但CHI的存在可以将反应速度提高2.4×10^6倍(Dare等2020)。到目前为止, 已经在许多植物中发现了4种类型的CHI并进行了功能鉴定, 其中I型和II型CHI被认为是真正的CHI, 具有典型的CHI酶活性(Chao等2021)。I型CHI广泛存在于苔类、苔藓、蕨类植物和大多数维管束植物中, II型CHI是豆科植物特有的(Yin等2019; Chao等2021)。I型CHI只异构化2',4',4',6-四羟基查尔酮, 存在于所有植物中, 然而, II型CHI不仅能异构化2',4',4',6-四羟基查尔酮, 还能异构化2',4',4-四羟基查尔酮, 后一种化合物与异黄酮类化合物的形成有关(Nan等2022)。而III型和IV型CHI是功能不同的非催化成员(Park等2021)。由于其重要作用, CHI在拟南芥(*Arabidopsis thaliana*)、水稻、苹果

(*Malus* spp.)、梨(*Pyrus* spp.)、牡丹(*Paeonia suffruticosa*)等植物以及微生物中被研究(Yin等2019; Dare等2020; Park等2021)。此外, CHI在植物对各种病原物的反应中起着至关重要的作用, 其通过抑制病原体生长和发育来减轻植物因病原体攻击而造成的伤害(Zhou等2018)。

IFS是进入异黄酮生物合成途径中第一个关键酶, 将苯丙烷中心代谢通路引向异黄酮类代谢通路分支, 催化二氢黄酮醇生成异黄酮苷元(Cheng等2013)。异黄酮苷元在UDP-糖基转移酶(UDP-glycosyltransferase, UGT)和丙二酰基糖基转移酶(MT)的作用下可以转化为结合的、活性较低的糖苷形式, 促进异黄酮类化合物在植物不同部位之间的转移和储存(Franzmayr等2012; Deng等2019)。IFS属于细胞色素P450超家族的CYP93C亚家族, 又名2-羟基异黄酮(2-hydroxyisoflavanone synthase, IFS; Shimamura等2007)。作为一个多基因家族, 编码功能蛋白的2个基因(*IFS1*和*IFS2*)已从大豆、豌豆、甜菜(*Beta vulgaris*)、红三叶、白三叶、小扁豆(*Lens culinaris*)中被鉴定(Visnevschi-Necrasov等2013)。研究发现, *IFS1*和*IFS2*在表达上存在组织特异性, *IFS1*主要在根和种皮中表达, 而*IFS2*主要在种子中表达(Dhaubhadel等2003)。在此之后, 研究人员将*IFS*基因克隆并在烟草(*Nicotiana tabacum*)、拟南芥和水稻等非豆科植物中表达, 为非豆科植物产生异黄酮类化合物奠定基础(Sreevidya等2006; Tan等2012; Yin等2017)。

HID将IFS催化形成的产物脱水产生大豆黄素或染料木素(Shimamura等2007)。在异黄酮生物合成途径中, 前三种酶CHS、CHI和IFS在酶和基因水平上得到了广泛的研究, 而对HID催化2-羟基异黄酮脱水以产生异黄酮的性质尚未清楚, 主要是因为其底物2-羟基异黄酮的不稳定性(Du等2010)。不同植物中的HID对2-羟基异黄酮有不同的底物特异性, 这种底物特异性反应了不同植物中的异黄酮类化合物具有不同的结构(Akashi等2005)。此外, 与酶催化反应相比, 底物的自发脱水速度较慢, 因此植物中2-羟基异黄酮类化合物的脱水主要依赖于HID酶的催化(Akashi等2005)。还有研究发现, 大豆*HID*过表达确实积累了大豆苷元和染料木素, 这表明HID是提高异黄酮生产率的关键决定因素(Shimamura等2007)。

随着研究的深入, 其他一些与异黄酮合成有关的酶也被不断鉴定出来, 例如: 从葛根中鉴定到的一种异黄酮7-*O*-葡萄糖基转移酶(isoflavone 7-*O*-glucosyltransferase PlUGT1), 其能够将大豆苷、染料木苷和芒柄花苷分别转化为大豆黄素、染料木素和芒柄花素(Li等2014); 大豆异黄酮7-*O*-葡萄糖基转移酶(isoflavone 7-*O*-glucosyltransferase or UDP-glucose, GmIF7GT或GmUGT1)参与异黄酮偶联物的生物合成(Köster和Barz 1981; Funaki等2015); 而异黄酮还原酶(isoflavone reductase, IFR)是植物界独有的, 被认为对于植物在各种生物和非生物环境胁迫的反应中发挥着至关重要的作用, 也有学者认为IFR是植物体内异黄酮类植物保卫素生物合成途径中的一种酶(Cheng等2015)。

3 异黄酮的代谢工程

3.1 豆科植物中异黄酮合成的基因工程

随着人们对异黄酮生物合成机制的深入了解, 通过调控植物异黄酮的生物合成对畜牧业、制药行业以及临床等方面具有非常重要的现实意义。目前已有众多研究证明可以通过基因工程手段有效提高豆科植物的异黄酮含量。例如超表达大豆*GmCHI*、*GmCHR*和*GmUGT*等异黄酮合成途径中关键酶编码基因提高了大豆异黄酮含量(Wu等2017; Yin等2019; Nguyen等2020); 超表达大豆异黄酮葡萄糖苷丙二酰基转移酶(isoflavone malonyltransferases)基因*GmIMaT3*增加了转基因大豆中大豆黄素和染料木素的含量(Ahmad等2017); 将油棕(*Elaeis guineensis*)中与IFS相似的P450基因(*EgP450*)导入泰国野葛根(*Pueraria mirifica*)中, 发现转基因植株中大豆黄素和染料木素的含量均得到显著提升(Xu等2017)。

近年来, 在豆科牧草中也有通过基因工程改变异黄酮含量的相关研究。Deavours和Dixon (2005)将蒺藜苜蓿(*Medicago truncatula*)异黄酮合酶基因*MtIFS1*转入紫花苜蓿, 转基因植株中染料木素和鹰嘴豆芽素A的含量都有所增加。还有研究利用CRISPR/Cas9技术对红三叶异黄酮生物合成关键

酶*IFS1*进行功能敲除, 发现基因编辑植株中的芒柄花素、鹰嘴豆芽素A和染料木素的含量显著降低(Dinkins等2021)。异黄酮的生物合成网络极其复杂, 并且异黄酮的积累依赖于通路中的多个酶基因的表达(Chu等2017)。因此, 不仅单一酶基因表达的改变可以增加或减少异黄酮的积累, 多基因的协同表达对异黄酮的合成效率也具有促进的作用。大豆*IFS1*、*CHS*和*CHI*以及*F3H*的协同作用, 可以显著促进紫花苜蓿异黄酮和原花青素的生物合成(Gou等2016)。

除了苯丙烷途径中的关键酶基因, 也可通过调节其上游的转录因子的表达来增加或减少植物异黄酮含量。目前已发现MYB和bHLH转录因子参与调控植物异黄酮的生物合成。例如: 大豆*GmMYBJ3*可以通过调控大豆中的*CHS8*和*CHI1A*基因的表达参与异黄酮的生物合成(Zhao等2017); 超表达大豆*GmMYB133*促进了两个关键异黄酮生物合成基因(*GmCHS8*和*GmIFS2*)的表达, 从而增加了大豆毛状根中总异黄酮含量(Bian等2018); 大豆*GmMYB29*可以激活*IFS2*和*CHS8*基因的启动子, 其在大豆毛状根中的超表达和RNA干扰(RNAi)分别导致异黄酮含量增加和减少(Chu等2017); Sarkar等(2019)筛选了能够激活大豆异黄酮生物合成途径中关键酶基因*GmCHS8*、*GmIFS1*和*GmIFS2*的候选MYB转录因子, 最终确定了3个基因(*GmMYB102*、*GmMYB280*和*GmMYB502*)作为异黄酮类生物合成的候选调控基因, 它们的超表达均增加了大豆毛状根中总异黄酮的积累。此外, 大豆bZIP转录因子GmbZIP5与R1MYB转录因子GmMYB176可以相互作用, 共同调节大豆异黄酮类化合物的生物合成(Vadivel等2021)。

研究发现, 除了改变参与其生物合成关键酶基因的表达直接调控异黄酮的含量, 还可通过抑制苯丙烷途径的其他分支来减少其对共同底物的竞争, 从而间接影响异黄酮的合成(Dastmalchi等2017)。例如: Jiang等(2014)通过RNAi将大豆*GmF3H*和黄酮合酶II (*GmFNSII*)基因沉默, 显著提高了干扰株系中异黄酮的含量; 由于F3H、F3H和FNSII与异黄酮合成关键酶IFS有共同的底物, Zhang等(2020)利用CRISPR/Cas9介导的多重基因编辑技术同时敲除了大豆中*GmF3H1*、*GmF3H2*和*GmFNSII-1*基因, 结果发现与野生型相比, T_3代纯合三重突变体的叶片异黄酮含量约为野生型的2倍。

3.2 异黄酮在非豆科植物中的代谢工程

近年来, 因异黄酮的诸多益处, 通过代谢工程对非豆科植物的次生代谢途径进行基因改造以获得异黄酮类化合物越来越受到研究者们的关注。已有研究证明, 通过转入异黄酮合成相关基因, 可以在拟南芥(Yin等2017)、水稻(Sreevidya等2006)和烟草(Pandey等2014b)等非豆科植物中合成异黄酮类化合物。Pičmanová等(2013)将豌豆*PsIFS*基因在拟南芥中超表达后检测到染料木素和鹰嘴豆芽素A的积累。Pokhrel等(2021)将大豆异黄酮合酶*GmIFS1*基因转入水稻中, 在转基因水稻中检测到了染料木素, 同时发现转基因水稻对稻瘟病具有一定的抗性。Sohn等(2014)将韩国的大豆品种‘Sinpaldalkong II’的*SpdIFS1*和*SpdIFS2*基因转入水稻中, 转基因水稻种子中的染料木素的含量达到100 μg·g^{-1}。Zhang等(2014c)将*GmCHS2*基因转入烟草中, 在转基因植株中检测到了异甘草素(异黄酮合成前体物质)。还有研究将拟南芥*AtMYB12*和大豆*GmIFS1*基因共同转入烟草中, 发现其叶片中黄酮醇的含量升高, 并且有大量染料木素糖缀合物的积累(Pandey等2014a)。Suntichaikamolkul等(2019)将泰国野葛根*PmIFS*基因和拟南芥R2R3 MYB12转录因子基因*AtMYB12*在烟草中表达后产生了染料木素的积累。Shi等(2021)在烟草中超表达红三叶转录因子基因*TpMYB30*或*TpRSM1-2*, 转基因植株异黄酮含量较野生型均显著增加。Tian和Dixon (2006)通过将蒺藜苜蓿异黄酮合酶和查尔酮异构酶基因*IFS*和*CHI*同时导入烟草, 转基因植株的花瓣和幼叶产生的染料木素和染料木苷占总黄酮量的比例高于单独转化*IFS*基因的植株。类似地, 分别导入大豆*GmIFS*基因后, 在转基因生菜(*Lactuca sativa*)叶片、矮牵牛(*Petunia hybrid*)叶片和花瓣、番茄(*Solanum lycopersicum*)叶片和果皮以及洋葱(*Allium cepa*)愈伤组织中均发现了染料木素的积累(Liu等2007; Shih等2008; Malla等2021)。

3.3 增加植物中异黄酮含量的其他途径

据报道, 异黄酮等一些酚类化合物能够吸收

紫外线B (ultraviolet radiation B, UVB)辐射, 对植物起到很好的保护作用(Schreiner等2012)。同时, UVB辐射已被证明可以促进各种植物中异黄酮的产生, 如大豆(Ma等2018)、百脉根(Kaducová等2022), 小麦(*Triticum aestivum*; Tian等2022)和黄芪(*Astragalus membranaceus*; Liu等2020)。UVB辐射能诱导大豆芽中参与异黄酮生物合成基因的表达, 增加了异黄酮的积累(Jiao等2016)。Kaducová等(2022)研究发现UVB辐射能够诱导百脉根中含量最丰富的异黄酮类化合物vestitol的生物合成。此外, 增强UVB辐射会增加红三叶中芒柄花素和鹰嘴豆芽素A的含量(Swinny和Ryan 2005)。γ-氨基丁酸(γ-aminobutyric acid, GABA)是一种信号分子, 参与次级代谢物的积累以及植物发育和新陈代谢的调节, 研究表明GABA可以调节UVB照射下大豆芽中异黄酮的积累(Gu等2022)。

除了紫外线辐射外, 还有一些因子可以诱导异黄酮等次级代谢物的合成。例如乙烯处理可以激活参与异黄酮生物合成的结构基因, 从而导致大豆植物中异黄酮含量的增加(Yuk等2016)。黄芪毛状根培养物(hairy root cultures)被证明可以高效生产异黄酮类化合物(Jiao等2014)。还有研究发现, 80 mmol·L^{-1} NaCl能够诱导大豆中2-羟基异黄酮合酶基因(*GmIFS1*)的表达, 显著提高其叶片和种子中的异黄酮含量(Jia等2017)。

4 总结与展望

作为一种植物雌激素, 异黄酮对于植物自身生长和抵御病虫害, 预防和治疗人类疾病及畜牧增产均具有十分重要的作用。因此植物异黄酮的生物合成以及代谢工程研究成为当下研究的热点, 亟待从以下4个方面取得突破: 首先, 随着研究的不断深入, 参与异黄酮代谢合成过程中的关键酶以及相关转录因子相继被发现, 但它们在异黄酮生物合成过程中的相互作用关系尚待厘清, 且其编码基因的功能还需要进一步验证; 其次, 有关miRNA在异黄酮生物合成过程中的作用机制仍未被深入研究(Gupta等2019), 发掘并鉴定调控异黄酮生物合成的重要miRNA, 将有助于高含量异黄酮植物的创制; 第三, 植物苯丙烷代谢途径各分支间的关系及其调控机制有待深入探究, 这将有助于通过控制苯丙烷代谢途径的其他分支而间接提高异黄酮的合成效率; 最后, 目前对于异黄酮的研究多集中于大豆以及中草药植物葛根, 而对于牧草及非豆科植物涉及异黄酮含量遗传改良方面的报道相对较少, 尚无育成品种应用推广, 因此通过多种生物育种手段培育高异黄酮含量牧草及作物新品种应是下一步需要重点关注的研究方向。

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