

玉米含硫氨基酸代谢机制研究进展

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摘要: 玉米是重要的粮食和饲料作物, 为畜禽提供必需的代谢能和营养物质, 饲料的营养价值与蛋白质及氨基酸组成比例密切相关。玉米籽粒蛋白质中氨基酸组成不平衡, 添加豆粕可以补充玉米中缺乏的赖氨酸和色氨酸。然而, 含硫氨基酸(半胱氨酸和蛋氨酸)也是大豆中的限制性氨基酸, 其中蛋氨酸直接影响畜禽的机体蛋白质合成速率, 进而影响肉蛋奶的产量。因此, 提高玉米含硫氨基酸特别是蛋氨酸含量对畜牧业发展意义重大。我国玉米种质资源中缺乏高蛋白种质, 且育种进程缓慢。近年来, 随着人们对植物硫元素的吸收和转运机制的深入研究, 初步构建了含硫氨基酸代谢调控网络。本研究概括了近年来关于提高作物含硫氨基酸代谢机制的研究结果, 提出将群体遗传学、比较基因组学及分子生物学相结合挖掘候选基因的新策略, 并利用现代生物育种技术提高玉米籽粒蛋氨酸含量, 为优质蛋白玉米遗传改良提供参考。

关键词: 玉米; 硫元素; 蛋氨酸; 籽粒蛋白质

Research Progress on Sulfur-rich Amino Acid Metabolism in Maize

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Abstract: Maize is an important food crop that is primarily used as feed to provide necessary metabolizable energy and nutrients for livestock and poultry. The nutritional value of feed is closely associated with the proportion of protein and amino acid composition. However, maize protein exhibits an imbalanced amino acid content, adding soybean meal supplements the lysine and tryptophan lacking in maize. However, sulphur-containing amino acids (cysteine and methionine) are also limiting amino acids in soybean. Methionine has a direct effect on the rate of protein synthesis in the bodies of livestock and poultry, which in turn affects the production of meat, eggs, and milk. Consequently, it is of great importance that there be a significant enhancement of the sulphur-containing amino acid content, particularly that of methionine, in maize, with a view to facilitating the development of animal husbandry. One limitation of China's maize germplasm resources is the lack of high methionine varieties. Additionally, the breeding process is relatively slow. In recent years, extensive research has been conducted on the absorption and transport mechanism of sulfur elements in plants, leading to the initial construction of a metabolic regulatory network for sulfur-containing amino acids. In this study, we summarized the research results of improving the metabolic mechanism of sulfur-containing amino acids in crops, proposed a new strategy of combining population genetics, comparative genomics and molecular biology to mine candidate genes, and used modern biological breeding to improve the methionine content of maize, so as

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to provide a reference for genetic improvement of high-quality protein maize.

Key words: maize; sulfur element; methionine; grain protein

玉米(*Zea mays* L.)是畜禽最佳的能量饲料,它的营养价值和品质对饲料品质具有重要影响,在饲料中添加60%左右的玉米,可为畜禽提供65%的代谢能和20%的蛋白^[1]。然而,普通玉米蛋白质中含硫氨基酸(蛋氨酸、半胱氨酸)、赖氨酸、色氨酸等必需氨基酸缺乏。有研究表明,蛋氨酸含量为0.42%时畜禽的产蛋量和产蛋质量达到较高水平^[2], Wu等^[3]发现饲料中的蛋氨酸对北京鸭的蛋白质和脂质代谢也起着关键作用,适量的蛋氨酸可以改善生长抑制和过量脂肪沉积。此外,蛋氨酸的代谢产物S-腺苷甲硫氨酸在调控机体的甲基化过程中发挥作用,从而对细胞的炎症反应产生影响^[4],蛋氨酸还可转化为半胱氨酸后参与谷胱甘肽的合成,增强生物体的抗氧化防御机制^[5]。然而蛋氨酸在玉米中平均含量仅为0.17%,无法满足动物生长所需,需要在饲料中添加豆粕来维持氨基酸平衡。

随着畜牧业的迅速发展,我国豆粕需求量逐年递增且严重依赖进口,2022年我国进口大豆9108万吨,其中饲料用量8145万吨,饲料中89.4%的蛋氨酸为禽类玉米-豆粕型日粮的第一限制性氨基酸^[6],影响畜禽对蛋白质的利用效率,在饲料中添加人工合成氨基酸又会大幅度增加饲料成本,并且在某些情况下可能降低畜禽的生长速度^[7]。因此,提高玉米中蛋氨酸含量十分必要。

玉米主要通过根部吸收土壤中的无机硫酸盐,在叶绿体中经过多种酶的同化及基因调控积累含硫氨基酸。国内外通过常规育种、改造植物内源蛋白基因、改变植物贮藏蛋白组分等多种方式以提高玉米含硫氨基酸含量,并进行了调控机理解析。本文综述了植物含硫氨基酸代谢机制,并提出常规育种与分子育种、信息育种相结合的策略,为含硫氨基酸在优质高蛋白玉米育种的应用研究提供理论参考。

1 含硫氨基酸的结构特点及同化途径

1.1 含硫氨基酸的结构特点

半胱氨酸(Cys, cysteine),分子式 $C_3H_7NO_2S$,分子量121.158。半胱氨酸分为左旋半胱氨酸(L型)和右旋半胱氨酸(D型)(图1A),在动植物体内主要为L型半胱氨酸,它在体内具有生理活动性,可以通过同一条或不同肽链上的半胱氨酸脱水缩合形成

二硫键,维持蛋白质稳定的空间结构。半胱氨酸合成路径广泛,主要通过饲料摄入、内源蛋白质的周转和前体物质的转化合成^[8],胱氨酸、半胱氨酸和蛋氨酸三者之间可以相互转化。

蛋氨酸(Met, methionine),又名甲硫氨酸,分子式 $C_5H_{11}NO_2S$,分子量149.205。根据旋光性不同,蛋氨酸也分为L型和D型(图1B)。大多数植物、真菌、细菌可以利用环境中的糖、有机氮源、无机氮源和硫源合成蛋氨酸,而畜禽体内不能自主合成蛋氨酸,必须从饲料中获取,因此蛋氨酸是饲料中必不可少的营养成分,在饲料中添加适量L-蛋氨酸可增加肉蛋产量^[9]。目前化学合成法主要生产DL-蛋氨酸,动物摄入后,L-蛋氨酸直接参与蛋白质合成,而D-蛋氨酸需要通过2步耗能反应转化为L-蛋氨酸后才能参与蛋白合成^[10]。因此,在畜禽的生长性能和饲料利用效率方面,L-蛋氨酸优于D-蛋氨酸和DL-蛋氨酸。

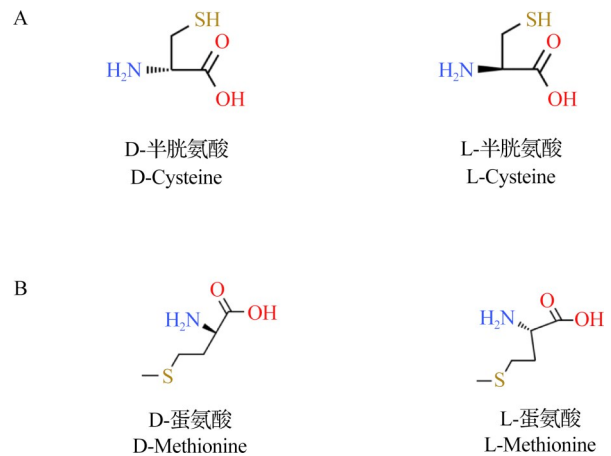


图1 含硫氨基酸结构式

Fig.1 Sulfur-containing amino acid structure formula

1.2 硫元素的吸收、转运与同化

硫元素是植物生长发育中所必需的营养元素,是含硫氨基酸半胱氨酸和蛋氨酸生物合成的重要原料。玉米对硫的积累随着生育期呈现“V”型曲线,在玉米营养生长期主要分布在叶片、茎秆、叶鞘中,籽粒形成后,籽粒中硫的分配量随着灌浆而增多,表明含硫氨基酸的运输是一个从源到库的过程,始于硫元素的吸收与同化^[6]。植物主要通过两种方式积累硫元素,一种通过根系细胞吸收无机硫酸盐,另一种是通过叶片光合作用累积有机硫化

物^[11]。硫元素通常以SO₄²⁻的形式存在于土壤和水中^[12],在硫元素的吸收与转运过程中有多种硫酸盐转运蛋白的参与,植物可以通过高亲和力与低亲和力转运蛋白从土壤中吸收不同浓度的硫酸盐^[13]。高等植物进化出硫酸根转运蛋白家族SULTRs,它介导从土壤中吸收SO₄²⁻并将其运输到整个生物体^[14-15]。在植物叶片细胞中,硫酸盐在进入叶绿体后,会被还原为硫化物,然后通过一系列耗能的过程将还原态硫转化为有机物,从而作为合成植物激素乙烯和多胺的来源。因此,提高细胞内硫含量有利于增强植物光合作用进而促进有机物的合成和积累^[16]。

刘烁然^[17]研究发现施硫肥可以提高玉米籽粒中半胱氨酸含量,半胱氨酸含量的增加促进了蛋白质中二硫键的形成及必需氨基酸的交联,可以同步提高籽粒蛋白质和蛋氨酸、半胱氨酸、缬氨酸等必需氨基酸含量。刘开昌等^[18]对高油1号、掖单13和长单26各施用硫肥90 kg/hm²后发现不同品种间硫的吸收量存在显著差异,籽粒蛋白质含量较对照分别提高了8.54%、6.32%、5.79%。因此,玉米品质及养分利用效率与不同的遗传背景以及硫肥的施用有关。

在模式植物拟南芥中硫酸盐整个同化过程需要3个关键酶:ATP 硫酸化酶(ATPS,ATP sulfurylase)、5'-腺苷酰硫酸还原酶(APR, adenosine 5'-phosphosulfate

reductase)和亚硫酸还原酶(SiR, sulfite reductase),硫酸盐在ATP 硫酸化酶的激活下转化为5'-腺苷酰硫酸(APS, adenosine-5'-phosphosulfate),随后,5'-腺苷酰硫酸在5'-腺苷酰硫酸还原酶作用下生成亚硫酸盐,亚硫酸盐经过亚硫酸还原酶还原成硫化物进入含硫氨基酸代谢通路。

1.3 含硫氨基酸代谢通路

游离氨基酸的合成存在紧密的调控网络,研究表明,调节某种氨基酸的代谢会影响其他氨基酸的水平^[19]。在含硫氨基酸代谢通路中,丝氨酸乙酰转移酶(SAT, serine acetyltransferases)是整个植物硫稳态的关键调节因子之一,丝氨酸乙酰转移酶催化丝氨酸合成O-乙酰丝氨酸(OAS, O-acetyl serine)(图2)^[20]。随后O-乙酰丝氨酸(硫醇)裂解酶(OAS-TL, O-acetyl serine(thiol)lyase)催化O-乙酰丝氨酸中已激活的乙酰基团置换成硫化物,从而释放出半胱氨酸。半胱氨酸作为唯一具有活性巯基的氨基酸^[21],是合成许多具有重要生物学功能的含硫代谢产物的前体,如谷胱甘肽(GSH, glutathione)和蛋氨酸。胱硫醚γ-合成酶(CGS, cystathionine γ-synthase)是催化蛋氨酸合成的第一个关键酶,是蛋氨酸合成途径中的限速酶,催化半胱氨酸和天冬氨酸(Asp, aspartate)缩合反应生成胱硫醚。蛋氨酸合成的第二步反应由胱硫醚β-裂解酶(CBL, cystathionine β-lyase)催化,生成同型半胱氨酸(Hcy,

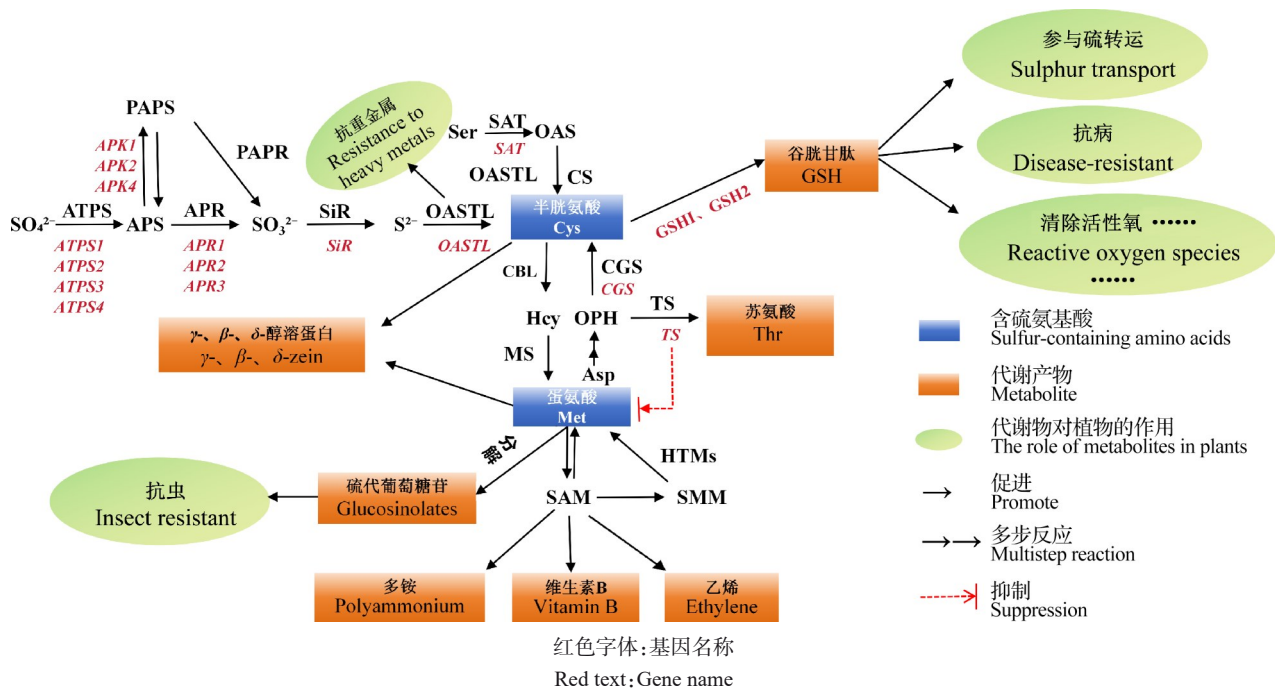


图2 植物中含硫氨基酸合成和代谢途径

Fig.2 Synthesis and metabolic pathways of sulfur-containing amino acids in plants

homocysteine),随后被运出叶绿体,然后同型半胱氨酸在蛋氨酸合酶(MS, methionine synthase)的催化作用下其巯基基团发生甲基化合成蛋氨酸。在植物体中大约有80%的蛋氨酸在S-腺苷甲硫氨酸合酶(SAMS, S-adenosylmethionine synthase)的作用下进一步转化成S-腺苷甲硫氨酸,部分蛋氨酸经过甲基转移酶的作用转化为S-甲基甲硫氨酸(SMM, S-methyl methionine)^[22]。S-甲基甲硫氨酸通过同型半胱氨酸S-甲基转移酶(HMTs, homocysteine methyltransferase)的活性重新转化为蛋氨酸,这可能是另外一条合成蛋氨酸的途径^[23-25]。另外,苏氨酸合成酶(TS, threonine synthetase)位于苏氨酸(Thr, threonine)和蛋氨酸生物合成的分支点,与胱硫醚 γ -合成酶竞争共同底物O-磷酸高丝氨酸(OPH, O-acetyl-serine), Bartlem等^[26]报道了在拟南芥中由于苏氨酸合酶基因上1个单碱基的突变导致氨基酸翻译提前终止,苏氨酸合成酶基因表达量下降,蛋氨酸含量显著提高。

1.4 半胱氨酸与其他代谢产物的联系

半胱氨酸作为硫酸盐同化途径产生的第一个碳/氮还原硫产物,不仅是蛋白质的组成成分,还是蛋氨酸生物合成的来源,许多其他含硫代谢产物如谷胱甘肽也参与植物生长、信号传导、胁迫反应和抗真菌发育^[27]。在玉米组织培养过程中添加半胱氨酸,可以诱发胚的氧化还原途径,进一步通过提高农杆菌侵染效率来提高玉米的转化效率^[28]。此外,半胱氨酸的合成是碳、氮和硫同化的3个主要途径的结合点^[29]。半胱氨酸的前体O-乙酰丝氨酸(OAS, O-acetyl serine)来源于碳氮同化途径,因此硫同化途径的最后一个酶半胱氨酸合成酶(CS, cysteine synthase)的活性也可能是改变碳氮通量的关键。目前研究已经建立了植物中同化硫酸盐和硝酸盐还原之间的相互作用网络^[30-35],由L-谷氨酸、L-半胱氨酸和甘氨酸组成的谷胱甘肽是一种非蛋白硫醇化合物,具有参与硫元素转运、基因表达调控及清除细胞内活性氧的功能^[36],也是非生物和生物胁迫下细胞内氧化还原环境的关键调节因子^[37],在维持细胞氧化还原状态方面具有特殊作用^[38]。

1.5 蛋氨酸与其他代谢产物的联系

氨基酸代谢与能量和碳水化合物代谢、碳氮预算以及蛋白质合成和次级代谢的需求紧密关联。作为在植物细胞生长和发育中起主要作用的蛋氨酸,对植物的初级和次级代谢至关重要,是谷胱甘肽、维生素、辅因子和硫化化合物的硫供体^[39]。S-腺

苷甲硫氨酸是蛋氨酸重要的次级代谢物质,它控制着多种基本代谢物的水平,包括乙烯、多胺、生物素和植物铁载体^[40]。S-腺苷甲硫氨酸作为一种初级烷基供体,它不仅调控叶绿素的合成,还参与细胞壁形成和许多次生代谢物的合成等关键过程^[41]。此外,蛋氨酸被分解生成硫代葡萄糖苷(Glucosinolates),这是一类植物次生代谢物,可积累植物器官中近30%的总硫含量,并对食草昆虫和病原体具有驱避活性^[42]。

2 含硫氨基酸代谢与植物生长发育的关系

2.1 含硫氨基酸代谢基因与非生物胁迫的关系

植物经过长期进化形成了抵御低温、干旱、重金属等非生物胁迫的机制,植物可通过富含半胱氨酸肽、植物螯合素的螯合作用耐受重金属胁迫。蛋氨酸合成途径中S-腺苷甲硫氨酸是植物体内重要的生物甲基供体、胺和乙烯合成的前体,它可调控细胞过程。樊金萍等^[43]从大豆中克隆SAM基因并将该基因导入烟草中,提高了转基因烟草对低温胁迫、干旱及盐胁迫的抗性。Harada等^[44]将水稻胞质半胱氨酸合成酶基因RCSI转化烟草(红花烟草)植株,并检测它们对镉的敏感性,研究结果表明转基因植株的半胱氨酸合成酶活性是野生型植株的3倍,而且镉胁迫后表现出明显的耐受性。O-乙酰丝氨酸(硫醇)裂解酶(OAS-TL, O-acetylserine (thiol) lyase)在植物抗金属胁迫中也起重要作用,Wang等^[45]通过转座子插入方式敲除水稻OsOASTL-A1发现,水稻根中半胱氨酸、谷胱甘肽和植物螯合素的水平显著降低,对砷酸盐胁迫的敏感性增强。

2.2 含硫氨基酸含量与贮藏蛋白积累的关系

目前优质蛋白玉米(QPM, quality protein maize)主要是利用基因o2(*opaque2*)提高籽粒赖氨酸和色氨酸水平,玉米胚乳修饰基因可以将o2的粉质胚乳转变为不同程度的硬质胚乳且赖氨酸含量几乎不降低^[46]。Li等^[47]通过PacBio三代基因组测序、极端个体混池测序(BSA, bulk segregation analysis)和转录组测序(RNA-seq)联合分析,解析了优质蛋白玉米基因组结构变异,挖掘硬质胚乳潜在的修饰因子,推进了高赖氨酸玉米育种进程。在植物中,半胱氨酸的增加有助于蛋白质中二硫键的形成和必需氨基酸的交联,从而提高籽粒蛋白质的含量。Wang等^[48]在玉米中鉴定到了1个新的氨基酸转运体ZmAAP6,过表达ZmAAP6株系中蛋氨酸、半胱

氨酸等多种氨基酸含量显著增加,增加了玉米籽粒醇溶蛋白和总蛋白的积累,是玉米种子氨基酸和蛋白质含量的正调节因子。植物中约90%的硫存在于含硫氨基酸中,调节植物硫同化途径也可改变蛋氨酸含量。来源于细菌的腺苷5'-磷酸硫酸还原酶和3'-磷酸腺苷-5'-磷酸硫酸还原酶(PAPR, 3'-phosphoadenosine-5'-phosphosulfate reductase)能够促进植物体内硫酸盐的还原,在玉米中增强5'腺苷酰硫酸还原酶和3'-磷酸腺苷-5'-磷酸硫酸还原酶的酶活性不仅提高10 kDa δ -玉米醇溶蛋白基因的表达,种子总蛋白含量也提高了2%以上^[49]。这些结果说明硫和蛋氨酸含量的变化可以影响植物种子中储存蛋白的组成,从而在一定程度上提高种子的营养品质。

3 作物含硫氨基酸改良研究进展

玉米作为重要的畜禽饲料来源,培育高含硫氨基酸含量的作物品种具有重要意义。随着基因工程的发展和基因编辑技术的成熟,玉米育种逐渐从传统育种转向了通过遗传工程的手段来缩短育种周期,使玉米不仅具有较高的谷物蛋氨酸含量,还含有农艺性状和谷物品质性状的有用变异,为含硫氨基酸玉米育种提供有效途径。

3.1 利用常规育种技术创制高蛋白种质

高蛋白玉米育种工作因缺乏可用的基因资源而面临较大困难,导致高蛋白种质资源匮乏。研究人员从衣阿华坚秆综合种(BSSS)中选育出BSSS-53自交系,该自交系中蛋氨酸含量比其他玉米自交系高30%左右,是由编码10 kDa δ -醇溶蛋白的结构基因*DZS10*自然突变导致的,该基因受反式调节基因*DZRI*的转录后调控^[50-51]。该自交系被用作回交转育中的供体以培育高蛋白自交系^[52],但*DZRI*基因存在亲本印记很难作为供体转入到其他自交系中。研究人员还利用回交转育方法开发具有较高谷物蛋氨酸含量的基础广泛的育种群体^[53],经过8代回交筛选后,高蛋白群体的蛋氨酸含量平均为0.26%,而低蛋氨酸群体的蛋氨酸水平平均为0.20%,但群体的农艺表现较差。基因工程育种和回交育种可能依赖于不同的遗传机制,因此可以将两种育种方法结合来增加籽粒蛋氨酸含量。

3.2 改造植物内源基因

一是调控硫同化途径基因的表达。在植物生长过程中,硫的吸收和还原是氨基酸生物合成的瓶

颈。通过调节编码硫酸盐同化过程所需的3个关键酶基因的表达可以促进半胱氨酸的合成。拟南芥中有4个ATP硫酸化酶编码基因,其中定位于质体的*APS1*、*APS3*、*APS4*参与硫同化过程^[54]。5'-腺苷酰硫酸还原酶受到*APR1*、*APR2*、*APR3*基因的调控作用。Wang等^[55]在拟南芥中克隆了编码亚硫酸还原酶基因*SiR*,该基因在硫稳态和半胱氨酸代谢等多种代谢过程中发挥着重要作用。*GSH1*和*GSH2*编码 γ -谷氨酰半胱氨酸合成酶,其催化谷胱甘肽生物合成途径中的第一步和限速步骤,即谷氨酸和半胱氨酸的结合,在低硫环境下,*SiR*基因表达量显著提高,而参与谷胱甘肽转化的基因表达量下降。

二是通过调节蛋氨酸代谢途径中的关键基因表达,能够在一定程度上增加植物体内的游离蛋氨酸含量。Avraham等^[56]在拟南芥中利用转基因的方法过表达内源*AtCGS*基因,经过测定,在转基因植株中S-甲基蛋氨酸和蛋氨酸的含量分别较野生型增加了19倍和2.2倍。在苜蓿^[57]、烟草^[58]和马铃薯^[59]中过表达*AtCGS*基因,转基因植株中游离蛋氨酸含量分别提高了32倍、20倍和6倍。在水稻^[60]和玉米^[61]中分别过表达*EcSAT*和*AtSAT1*基因也可显著提高蛋氨酸含量,同时发现在过表达*AtSAT1*的玉米植株叶片中硫同化增加,并且籽粒中富含蛋氨酸残基的10 kDa δ -醇溶蛋白亚基含量较野生型显著提高^[62-63],成熟籽粒中的蛋白质也积累到更高的水平。Kastoori等^[64]通过转基因方法将大肠杆菌3'-磷酸腺苷-5'-磷酸硫酸还原酶基因*EcPAPR*插入玉米高蛋白自交系B101基因组中,促进了玉米植株叶片部位积累蛋氨酸,最终玉米籽粒中的蛋氨酸含量较其受体材料增加了57.6%。

3.3 改变植物储藏蛋白组分

玉米籽粒中氨基酸组成不平衡,缺乏含硫氨基酸等必需氨基酸,蛋氨酸作为蛋白质合成启动所需的甲硫基tRNA的组分,对蛋白质含量有重要影响。玉米中醇溶蛋白分为4大类: α 类(19 kDa和22 kDa)、 β 类(15 kDa)、 γ 类(50 kDa、27 kDa和16 kDa)和 δ 类(18 kDa和10 kDa)。其中22 kDa α 和19 kDa α -玉米醇溶蛋白是最主要的贮藏蛋白,但几乎不含赖氨酸和蛋氨酸,营养品质较差。Wang等^[65]发现半胱氨酸与蛋氨酸含量呈高度正相关,含硫氨基酸含量与蛋白质含量呈显著正相关。 γ 类醇溶蛋白含有半胱氨酸残基数最高达21%,10 kDa δ -醇溶蛋白和18 kDa δ -醇溶蛋白是蛋氨酸残基数占比较高的蛋白,10 kDa δ -醇溶蛋白中蛋氨酸残基数占20%,

而 18 kDa δ -醇溶蛋白的蛋氨酸残基数占比可达 23% 以上^[66]。10 kDa δ -醇溶蛋白和 18 kDa δ -醇溶蛋白分别由无内含子的 *DZS10* 和 *DZS18* 基因编码,然而,许多玉米种质中编码 18 kDa δ -醇溶蛋白的结构基因大多存在突变,导致活性丧失。 δ -醇溶蛋白的遗传存在遗传印迹^[67],常规育种途径很难转育到其他玉米背景中,限制了其在玉米育种中的应用。

Dinkins 等^[68]将编码 15 kDa 的玉米醇溶蛋白基因在大豆种子中过表达,与对照相比,这些品系的蛋氨酸含量增加了 12%~20%,半胱氨酸含量增加

了 15%~35%。将玉米中特异型表达的 γ -醇溶蛋白编码基因导入大豆种子,氨基酸分析结果表明, γ -醇溶蛋白转基因种子的半胱氨酸和蛋氨酸含量分别提高了 29.33% 和 18.57%^[69]。在此基础上,同时增加蛋氨酸代谢“源”和“库”的新策略,可以进一步提高玉米种子中总蛋氨酸含量^[70]。如表 1 所示,通过敲除大豆中富含蛋氨酸的玉米 11 kDa δ 醇溶蛋白基因,并在富硫环境下种植,发现能够显著提高外源蛋白的含量^[71]。这表明硫的可利用性对储藏蛋白的积累具有重要影响。

表 1 植物含硫氨基酸代谢相关基因

Table 1 Genes related to metabolism of sulfur-containing amino acids in plants

基因名称 Gene name	受体植株 Acceptor plant	表达模式 Expression pattern	蛋氨酸含量 Methionine content changes	参考文献 Reference
<i>AtCGS</i>	拟南芥	过表达	提高 19 倍	[56]
<i>AtCGS</i>	苜蓿	过表达	提高 32 倍	[57]
<i>AtCGS</i>	马铃薯	过表达	提高 6 倍	[59]
<i>EcSAT</i>	水稻	过表达	提高 4.8 倍	[60]
<i>AtSAT1</i>	玉米	过表达	提高 1.4 倍	[61]
<i>EcPAPR</i>	玉米	插入	提高 57.6%	[64]
<i>TSl</i>	拟南芥	提前终止突变	提高 22 倍	[26]
玉米 β 醇溶蛋白基因 β -zein	大豆	过表达	提高 12%~20%	[68]
玉米 γ -醇溶蛋白基因 γ -zein	大豆	过表达	提高 15.5%~18.6%	[69]

醇溶蛋白基因的启动子中有一些保守的顺势作用元件受相应的转录因子调控,*O2*、*PBF* 可以调控 22 kDa α -醇溶蛋白^[72],*PBF*、*OHPI*、*OPH2*、*ZmbZIP22* 可以调控 27 kDa γ -玉米醇溶蛋白基因的表达^[73-74],*ZmNAC128* 和 *ZmNAC130* 可以特异性地激活 16 kDa γ -玉米醇溶蛋白基因的表达^[75]; *ZmMADS47* 可以调控 50 kD γ 类醇溶蛋白^[76]; *ZmABI19* 与 *ZmbZIP29* 蛋白互作,能够协同增强 *O2* 的表达,在胚乳灌浆期过表达可以增加籽粒蛋白含量^[77]。

4 多组学联合挖掘关键调控基因

全基因组关联分析 (GWAS, genome-wide association study) 是研究基因型与表型关联的主要分析方法,表达量性状位点 (eQTL, expression quantitative trait loci) 研究将基因结构变异和表达性状联系起来,在解析植物性状的关键基因和调控网络方面发挥了重要作用,已在多个物种中得以应用,如水稻^[78-81]、油菜^[82]、小麦^[83]、玉米^[84-87]、番茄^[88]等。Yang 等^[89]组装了热带小粒玉米基因组,通过与 B73、Mo17

基因组比较,并对 368 个自交系授粉后 15 d 的 25008 个基因的转录组数据进行联合 eQTL 分析,鉴定了 207 个具有主导结构变异 (SV, structural variations) 关联的 eQTL 和 17632 个具有主导 SNP 关联的 eQTL,首次克隆了影响粒重的关键基因。

多组学联合分析能更好地理解生物学现象及机体内物质的代谢途径,从而全面地解析生物分子功能和调控机制。Niu 等^[90]通过多组学分析筛选到了藜麦萌发过程中参与赖氨酸积累的候选基因,并探讨了影响赖氨酸生物合成的因素;Xing 等^[91]整合了来自全基因组关联分析的 QTL,数量性状核苷酸 (QTNs, quantitative trait nucleotides) 的大量数据,以及包括转录组、蛋白质组和代谢组在内的多组学数据,并进一步分析它们之间的相互作用以预测一些与氮素利用效率相关的候选基因。综合基因组学、系统生物学和现代育种技术,为深入研究玉米等作物高氮素利用效率分子机理和选育氮高效品种提供重要基因资源。Li 等^[92]综合利用基于代谢物的全基因组关联分析 (mGWAS, metabolome genome-wide association study)、甲基化数量性状基因座

(mQTL, methylation quantitative trait loci)、转录组测序和植物稳定转化等多种手段在水稻6号染色体上定位并鉴定了1个控制水稻多种营养代谢物积累以及食味品质的数量性状位点,为水稻营养育种提供了新资源。

5 展望

玉米作为畜禽饲料的主要成分,改善玉米籽粒蛋白质中氨基酸的平衡并进一步提高含硫氨基酸含量对于促进畜牧业发展意义重大。氨基酸代谢与植物营养品质表型密切相关,在植物体中存在复杂的代谢调控网络,解析玉米含硫氨基酸代谢机理并通过转基因技术与传统回交育种相结合,在适宜的遗传背景下可以提高玉米叶片中硫酸盐的同化能力,生产出蛋氨酸含量较高的玉米,对畜禽具有显著的营养价值。玉米中富含硫的醇溶蛋白在饲喂雏鸡的日粮中具有生物可利用性,可以取代人工

合成的蛋氨酸甲酯补充剂。

目前,组学方法已被广泛应用于氨基酸代谢及其关联的研究^[93-96]。将组学方法与杂交育种相结合的现代生物育种技术可以为创制高蛋白新种质提供技术支撑(图3)。长期以来,完整的端粒到端粒(T2T, telomere-to-telomere)基因组一直是基因组研究的目标,2023年6月,Chen等^[97]完成了首个玉米基因组无间隙组装,揭示了大量存在于不同着丝粒间和不同亚端粒区的基因组序列和结构变异。本研究提出通过基因-蛋白质-代谢物联合分析含硫氨基酸调控网络,并利用比较基因组学方法分析基因结构变异与表型间的联系,甚至可以挖掘到调控玉米含硫氨基酸表型的新基因。此外,CRISPR/Cas持续优化的基因编辑技术已经允许一种或几种关键调节酶的表达或活性被改变^[98],这些分析方法可以丰富高蛋白玉米种质资源,加快高蛋白高蛋白玉米种质的创制进程。

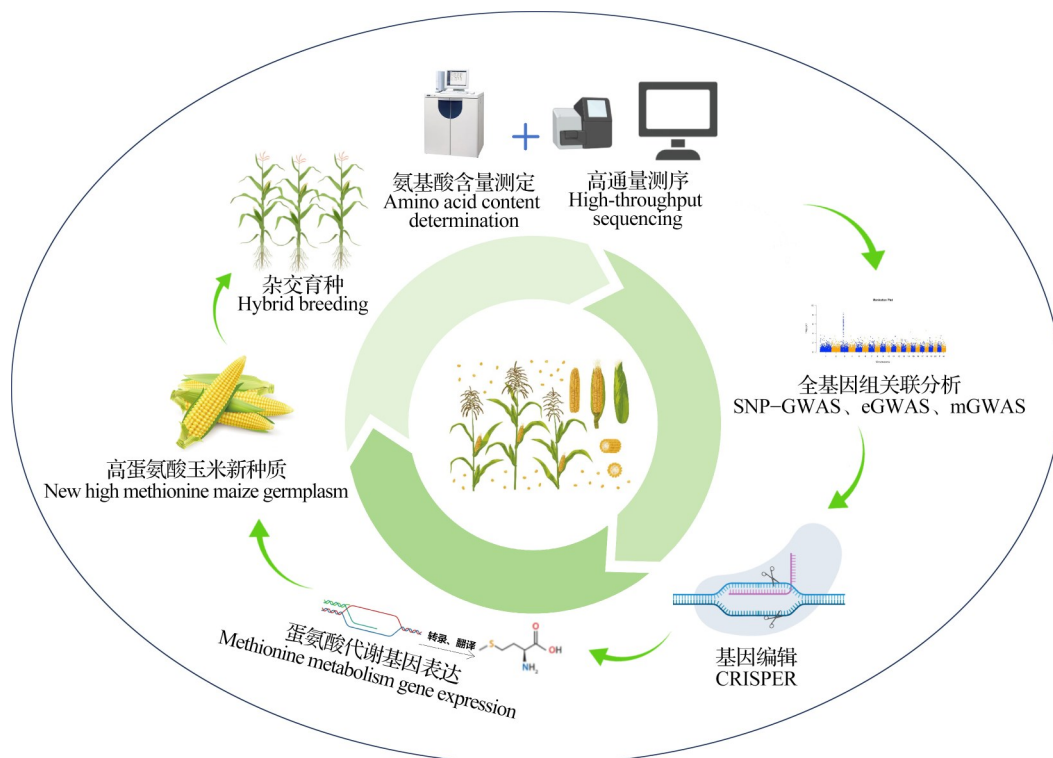


图3 高蛋白氨基酸玉米育种流程图

Fig.3 Flow chart of high methionine maize breeding

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