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紫花苜蓿在干旱胁迫下的产量损失与抗旱性遗传研究进展

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摘要:紫花苜蓿是种植面积最广的多年生豆科饲草,由于其产量高、品质优良而被誉为“牧草之王”。干旱胁迫会对紫花苜蓿生长发育的各个阶段造成严重影响,导致产量损失。干旱胁迫对紫花苜蓿发芽率、分枝形成、茎伸长、叶片发育、根系发育等造成影响,可导致饲草产量减少70%以上。利用分子育种加速培育耐旱性苜蓿新品种是应对干旱胁迫的有效策略。然而紫花苜蓿抗旱性相关的遗传研究基础相对薄弱。前期研究主要集中于转基因和同源克隆。随着紫花苜蓿基因组的发布和测序技术的发展,全基因组关联分析和以转录组测序为代表的组学技术在紫花苜蓿抗旱相关基因的鉴定和抗旱遗传机制的解析中发挥了越来越重要的作用。本研究全面总结了干旱胁迫对紫花苜蓿产量的影响,并概述了近年来在紫花苜蓿抗旱性遗传研究领域取得的进展,旨在为紫花苜蓿抗旱育种提供参考依据。

关键词:紫花苜蓿;抗旱性;全基因组关联分析;产量

Research progress on yield loss under drought stress and drought resistance genetics of alfalfa (*Medicago sativa*)

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Abstract: Alfalfa (*Medicago sativa*) is the most widely cultivated perennial leguminous forage crop, acclaimed as the “king of forages” because of its high yield and superior quality. Drought stress has a significant impact on the growth and development of alfalfa, resulting in substantial yield reductions. It influences the germination rate, branch formation, stem elongation, leaf growth, and root development, potentially causing large decreases (>70%) in forage yield. Accelerating the breeding of drought-tolerant alfalfa varieties through molecular breeding is an effective strategy to mitigate the effects of drought stress on this forage crop. However, the genetic foundation of drought resistance in alfalfa remains largely unexplored. Previous research on alfalfa has mainly concentrated on transgenic methods and homologous cloning techniques. With the release of the alfalfa genome and advances in sequencing technology, genome-wide association studies and omics technologies based on transcriptome sequencing have played an increasingly important role in identifying drought-related genes and elucidating drought resistance mechanisms in alfalfa. This paper comprehensively summarizes the effects of drought stress on alfalfa yield, outlines recent

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advances in research on the genetic basis of drought resistance in alfalfa, and provides a reference for the breeding of drought-resistant alfalfa varieties.

Key words: alfalfa; drought resistance; genome-wide association studies; yield

干旱、盐碱、极端气候等环境胁迫影响了植物的整个生长周期[1],严重限制了作物的产量[2],是全球农业生产的主要制约因素。干旱胁迫是限制作物生产力的主要非生物因素,在过去几十年中对全球作物产量造成了极大损失[3-5],仅干旱造成的作物产量损失超过所有病原体的总和[6]。有研究表明,在干旱胁迫下,谷物减产幅度为25%~40%,而豆类则为20%~80%[2]。在过去的50年中,美国记录的约67%的作物损失是由干旱胁迫造成的[7]。预计到2050年,由于人口增长,农业用水需求可能是原来的2倍[6]。并且由于气候变暖、可利用淡水资源的减少和工业用水的增加,农业生产将面临更加严峻的干旱环境,全球主要作物减产幅度将超过50%[8-9]。

紫花苜蓿(*Medicago sativa*)是一种多年生豆科饲草,是全球分布最广、种植面积最大的饲草作物[10]。作为一种优质饲草,其产量高、品质优良,粗蛋白含量为18%~22%,同时富含维生素和矿物质,是畜牧业成本低廉且优质的蛋白质来源[11-12]。紫花苜蓿能够以干草、青贮、放牧等形式被家畜利用。同时,由于其具有共生固氮能力,可提高土壤氮含量,在农业生产中发挥着至关重要的作用[2]。除这些传统用途外,紫花苜蓿还具有作为生物燃料的潜力,同时也可作为生物反应器生产工业酶(如α-淀粉酶、纤维素酶和木质素过氧化物酶等)[10]。此外,紫花苜蓿还可作为蔬菜和保健品被人类利用[13]。畜牧业的发展以及苜蓿应用领域的多样化趋势增加了对紫花苜蓿的需求。

美国苜蓿种植面积位居世界第一,2022年收获面积达到603万hm^2,总产量达4350万t,干草产值超过100亿美元,是仅次于玉米(*Zea mays*)、大豆(*Glycine max*)和小麦(*Triticum aestivum*)的第四大作物(https://usda.library.cornell.edu/)。中国是苜蓿种植大国,同时也是苜蓿消费大国。中国苜蓿种植面积仅次于美国,位居世界第二,但国内苜蓿产量远远无法满足我国畜牧业发展需求,每年需大量进口苜蓿干草[14-15]。紫花苜蓿是以收获地上茎叶为主的饲草作物,其产量和生产稳定性受到干旱胁迫的严重影响[2,16]。而中国紫花苜蓿的种植区域主要分布于北方干旱和半干旱地区,干旱胁迫是制约我国苜蓿生产的主要因素之一。在有限的水土资源条件下获得更高的苜蓿产量是我国苜蓿育种工作的重点。因此,培育具有改良根系结构(root system architecture, RSA)、高水分利用效率的抗旱苜蓿品种是提高产量,保证苜蓿产业可持续发展的必要举措。传统育种方法依赖于周期性的表型选择,具有周期长、效率低且需要耗费大量人力等缺点。通过遗传改良提高作物的抗旱性是克服农业生产中干旱胁迫的有效策略。紫花苜蓿为同源四倍体(2n=4X=32)异花授粉植物,且有杂合度高、自交不亲和、近交抑制等特性[17],导致其分子育种进展缓慢。相比其他主要作物,人们对苜蓿耐旱性的遗传机制知之甚少。本研究就近年来国内紫花苜蓿抗旱方面的研究进展进行综述及展望。

1 干旱胁迫对苜蓿生产的影响

干旱胁迫对作物的影响主要取决于物种、基因型、发育阶段以及胁迫的强度及持续时间[2,8,18]。植物已经进化出复杂的调控网络使它们能够及时响应和适应环境变化。通常,干旱胁迫会诱导植物在形态、生理、生化和分子水平发生变化,以响应不利环境并生存[2,8,19-20]。这些适应性反应包括植物转录组、蛋白质组和代谢组的改变,可以通过修复胁迫损伤、平衡细胞稳态以及调节生长等方式,维持植物抗逆与生长的动态平衡[8,21]。在植物适应干旱胁迫期间,干旱会对多个生物过程产生破坏性影响,包括减慢代谢过程、破坏氧化还原和离子平衡、扰乱光合作用、降低养分同化速度等,这些生命活动的变化最终会降低植物的生长和产量[1,8]。总体而言,干旱胁迫会对植物高度、纤维素含量、节数、冠层、叶面积指数、茎叶干重和根系发育产生不利影响[8,22]。

1.1 紫花苜蓿根系结构改变以适应干旱胁迫

根系结构对植物的适应性和生产力起着至关重要的作用[23-25]。最近的研究强调了改变植物的RSA以提高植物抗逆性和总体产量的潜在好处[25-27]。通过优化RSA,可减少或避免在生长和非生物胁迫之间权衡所造成的

损失，从而提高产量和抗逆性。在干旱胁迫下，紫花苜蓿根系生长发育受阻、根系干重减少[28]，但根冠比和根茎长度比增加[19,29-30]。在干旱处理 9 d 后，强抗旱品种陇中苜蓿的根系平均直径、根体积、根系干重、维管束面积、木质部面积和次生木质部导管数目均显著高于中度抗旱品种陇东苜蓿和弱抗旱品种甘农 3 号[31]。对不同 RSA 种群的研究表明，较大根系的种群牧草产量更高，而根长更长的种群能更好地利用水资源，从而提高耐旱性[32]。在紫花苜蓿中过表达大豆 *WRKY20* 和 *ZFP1* 可促进根的长度，从而提高对干旱的耐受性[33-34]。同样，与胁迫条件下野生型植株相比，紫花苜蓿 *miR156* 过表达植株表现出更发达的根系，从而提高了抗旱性和总体生物量[35-37]。总之，这些研究表明调控苜蓿根系生长发育是提高产量和干旱耐受性的有效途径（表 1）。

1.2　干旱胁迫对紫花苜蓿产量的影响

有研究报道，在干旱胁迫下，紫花苜蓿的生物量减少幅度为 12%～73%[2]，在不同基因型材料间存在较大差异（表 1）。干旱影响苜蓿生长发育的各个阶段，包括发芽率、分枝形成、茎伸长、叶片发育、根系发育等，并最终导致减产。表 1 列出了各个生长阶段干旱胁迫对苜蓿形态及产量的影响。

干旱胁迫会降低紫花苜蓿种子发芽率并影响苗期形态建成。在温室中，施加不同浓度的聚乙二醇（polyethylene glycol，PEG）以模拟干旱胁迫[38]，结果显示随着 PEG 浓度升高，8 个基因型苜蓿材料的发芽率显著降低，胚芽、胚根长度和鲜重均显著减小。所测材料在所有性状上均存在显著差异，且在不同种质间表现出较大差异，其中材料 Yazdi 最耐旱，而 Ranger 最敏感。进一步选择 3 个基因型的材料（包括 Yazdi 和 Ranger）以评价其苗期抗旱性，结果表明在干旱胁迫下，苜蓿幼苗叶片数量和叶面积随 PEG 浓度升高而减少，茎、根长度缩短，茎和根干重显著降低，而根茎长度比则随着 PEG 浓度升高而增加。在田间条件下，对 10 个苜蓿品种施加 3 种不同程度的干旱处理，结果显示产量损失取决于干旱强度和基因型。在 3 种胁迫条件下，Baghdadi 品种产量下降幅度最小，而 Hamedani 品种下降幅度最大，在严重干旱条件下产量分别减少 22% 和 52%[39]。在温室中对 198 份苜蓿种质材料的抗旱性评价结果显示，相比对照组，干旱胁迫下群体平均鲜重和干重分别减少 61.9% 和 38.1%。具有高相对含水量的紫花苜蓿种质表现出相对较低的生物量损失[40]。利用相同的群体材料，在田间评估了干旱胁迫对紫花苜蓿产量的影响。一般而言，水分亏缺时生物量较对照显著降低。在一年的 3 次刈割中，第一次刈割对照组和干旱组均获得较高产量，干旱胁迫下群体平均产量损失为 37.4%；在第 3 次刈割时，产量损失高达 71.3%[41]。在田间条件下，通过 5 个苜蓿品种探究干旱胁迫对苜蓿形态特征的影响和形态的改变与苜蓿产量的关系[42]。设置了 3 个干旱胁迫水平：重度（灌溉时土壤水分为田间持水量的 25%）、中度（50%）和轻度（75%）。经 6 次连续扦插评估的结果显示，干旱胁迫下，鲜草产量、干草产量、株高、单位面积分枝数、叶面积指数、节间长度和节间数降低，而叶茎比增加。干草产量与株高、单位面积分枝数和叶面积指数呈正相关，而叶茎比与之则呈负相关。干旱胁迫导致苜蓿产量降低，主要是由株高降低、分枝数减少等表型形态的变化造成的。

1.3　紫花苜蓿抗旱育种研究进展

美国紫花苜蓿育种工作开展时间长且发展非常迅速，已经育成了一大批耐旱、高产、优质、持久性好等综合性状优良的苜蓿品种。仅 2023 年一年间就登记了 40 个苜蓿新品种（https://www.naaic.org/resource/stdtests.php）。美国和加拿大的紫花苜蓿耐旱育种一直依赖于黄花苜蓿（*Medicago falcata*），因为其具有极强的抗旱性。目前，已经培育出一些标志性的耐旱品种，包括：Grimm、Baltic、Cossack、Ladak 等[50]。Ladak 品种最初于 1914 年从印度北部的拉达克省引进，长期以来在旱地紫花苜蓿试验中被用作对照，是在抗旱性上表现最好的栽培品种之一。还为 Rambler、Travios、Roamer 和 Drylander 等耐旱品种的培育作出了巨大贡献[10]。中国与美国和加拿大相比，在紫花苜蓿育种方面还存在一定差距。中国苜蓿育种面临多个问题，包括：种质资源收集、评价、利用不充分；育种主要依托科研单位，育种工作人员不足、育种年限长、抗性品种培育进展缓慢；育成品种推广力度不足；育种技术相对落后等。中国现有的苜蓿品种主要具备高产、耐盐、抗寒、抗病虫、耐放牧、早熟和低纤维等特征，缺乏抗旱品种[51]。而我国苜蓿种植区域主要分布于北方干旱半干旱地区，培育抗旱、高产、优质的苜蓿新品种是适应我国苜蓿产业发展的必然选择，将是我国苜蓿育种工作的重要方向。我国苜蓿栽培历史超过 2000 年，有一大批适应地方干旱环境条件的优质紫花苜蓿种质材料，充分开发利用这些种质材料将有助于苜蓿抗旱育种[52]。

表 1 干旱胁迫对紫花苜蓿形态、产量的影响

Table 1 The impact of drought stress on alfalfa morphology and yield

材料数量 Material number	环境 Environment	发育阶段 Developmental stages	干旱相关表型 Drought-related phenotype	产量损失 Yield loss	参考文献 References
3份材料 3 materials	花盆,温室 Pots, greenhouse	苗期 Seedling stage	总干重和成活率下降,但根冠比增加。Total dry weight and survival rates decreased, but root-shoot ratio increased.	陇中苜蓿、陇东苜蓿和甘农3号生物量分别减少25.31%,40.35%和69.59%。Biomass of *M. sativa* cv. Longzhong, Longdong and Gannong No. 3 decreased by 25.31%, 40.35% and 69.59%, respectively.	[19]
8份材料 8 materials	花盆,温室 Pots, greenhouse	营养生长期 Vegetative stage	鲜重、干重减少,根茎干重比增加。Fresh weight, dry weight decreased, but root-shoot dry weight ratio increased.	产量损失为55%~75%。The yield decreased from 55% to 75%.	[29]
10份材料 10 materials	温室 Greenhouse	苗期 Seedling stage	茎和根的鲜重和干重、根和茎长减少,根茎长度比值增加。Stem and root fresh and dry weight, root and stem length decreased, but root-stem length ratio increased.	—	[30]
3份材料 3 materials	花盆,温室 Pots, greenhouse	苗期 Seedling stage	根系总长度、根系总表面积、根系平均直径、根体积和根尖数、根系干重、根直径降低。Total root length, total root surface area, average root diameter, root volume and number of root tips, root dry weight and root diameter decreased.	—	[31]
8份材料 8 materials	温室 Greenhouse	发芽期和苗期 Germination and seedling stages	发芽阶段:发芽率、胚芽和胚根的鲜重和干重降低;幼苗阶段:根长、茎长、叶面积、叶片数、根和茎干重降低,根茎长度比增加。Germination stage: germination rate, fresh weight and dry weight of plumules and radicles decreased; Seedling stage: root length, stem length, leaf area, number of leaves, root and stem dry weight decreased, but root-stem length ratio increased.	—	[38]
10份材料 10 materials	田间 Field	营养生长期 Vegetative stage	产量损失。Yield loss.	产量损失为22%~52%。The yield decreased from 22% to 52%.	[39]
198份材料 198 materials	花盆,温室 Pots, greenhouse	营养生长期 Vegetative stage	鲜重、干重减少。Fresh weight, dry weight decreased.	平均鲜重减少了61.9%,平均干重减少38.1%。The average fresh weight decreased by 61.9%, and the average dry weight decreased by 38.1%.	[40]
198份材料 198 materials	田间 Field	营养生长期 Vegetative stage	鲜重减少。Fresh weight decreased.	3次刈割的苜蓿产量损失分别为:37.4%、3.5%和71.3%。The alfalfa yield decreased by 37.4%, 3.5% and 71.3% in three successive harvest, respectively.	[41]
5份材料 5 materials	田间 Field	营养生长期 Vegetative stage	鲜重、干重、株高、单位面积分枝数、叶面积指数、节间长度和节间数减少,但叶/茎增加。Fresh forage yield, dry forage yield, plant height, stem number/unit area, leaf area index, internode length and internode number decreased, but leaf-stem ratio increased.	较轻度干旱胁迫,重度干旱胁迫下平均产量损失为37%。Compared with mild drought stress, the average yield loss under severe drought stress was 37%.	[42]
6份材料 6 materials	培养皿,温室 Petri dish, greenhouse	发芽期 Germination stage	发芽率、胚根、胚芽长度、种子活力指数降低。Germination rate, radicle and plumule length, and seed vitality index decreased.	—	[43]

续表 Continued Table

材料数量 Material number	环境 Environment	发育阶段 Developmental stages	干旱相关表型 Drought-related phenotype	产量损失 Yield loss	参考文献 References
11份材料 11 materials	温室 Greenhouse	苗期 Seedling stage	生物量降低、根茎比增加。Biomass decreased, but root to stem (R/S) ratio increased.	—	[44]
1份材料 1 material	花盆,温室 Pots, greenhouse	营养生长期 Vegetative stage	全株生物量、叶片数、茎伸长率和枝条相对生长速率、枝条/根减少。Whole plant biomass, leaf number, stem elongation rate and shoot relative growth rate, and shoot-root ratio decreased.	干重减少了 51%。The dry weight decreased by 51%.	[45]
5份材料 5 materials	温室 Greenhouse	营养生长期 Vegetative stage	干重、存活率、分枝数、根生物量减少。The shoot dry weight, survival rate, number of branches, and root biomass decreased.	第二次刈割时干重减少了 27.3%,第3次刈割时减少了 96.5%。The dry weight decreased by 27.3% in second harvest, and 96.5% in third harvest.	[46]
4份材料 4 materials	温室 Greenhouse	营养生长期 Vegetative stage	干重减少。Dry weight decreased.	干重减少 22.48%～34.45%。The dry weight decreased from 22.48% to 34.45%.	[47]
18份材料 18 materials	田间 Field	营养生长期 Vegetative stage	茎干重、总生物量减少。Shoot dry matter, total biomass decreased.	平均茎干重减少 28.5%,总干物质减少 36.5%。The average stem dry weight decreased by 28.5%, and the total dry matter decreased by 36.5%.	[48]
16份材料 16 materials	田间 Field	营养生长期 Vegetative stage	鲜重、干重减少、叶茎比降低。Fresh and dry weight, leaf-stem ratio decreased.	干重减少 13.8%～46.2%。The dry weight decreased by 13.8%－46.2%.	[49]

传统的紫花苜蓿育种策略依赖周期性表型选择,虽改良了耐盐、抗病虫等性状,但需要长期表型评价,选择效率低[53－54]。特别是对于多年生紫花苜蓿,表型评价更为费时。分子育种策略是从基因层面对育种材料进行定向选择/改变,避免了传统育种方法在表型评价上耗时费力的缺点,是未来紫花苜蓿育种的发展方向。在抗除草剂和低木质素方面,美国已推广转基因苜蓿品种。而中国仅有少量转基因品系,目前并没有转基因商业品种。兰州大学有3份耐旱紫花苜蓿转基因材料获批,进入大田中间试验。这些材料包括,转超旱生牧草“腾格里无芒隐子草(*Cleistogenes songorica*)”*CsLEA2*和*CsALDH12A1*基因的2份紫花苜蓿材料和1份转紫花苜蓿*MsNTF2*基因的紫花苜蓿材料。利用分子育种加速培育抗旱性苜蓿新品种是保障苜蓿产业发展的有效策略。数量性状基因座(quantitative trait locus, QTL)定位和全基因组关联分析(genome-wide association studies, GWAS)是开发紫花苜蓿抗旱分子标记的有效手段。目前已经通过这些方法鉴定到一些与苜蓿抗旱性相关的单核苷酸多态性(single nucleotide polymorphism, SNP)位点[41,55－57],然而这些分子标记还未被充分应用于紫花苜蓿抗旱分子标记育种中。紫花苜蓿抗旱分子标记辅助选择(marker assisted selection, MAS)育种还停留于理论阶段。

2　紫花苜蓿抗旱性相关基因挖掘与功能研究

与主要的作物相比,紫花苜蓿抗旱遗传机制解析及抗旱分子育种还存在较大差距。但前期已经通过转基因创制了耐旱种质材料,并通过同源克隆等方法在紫花苜蓿中鉴定到一些抗旱功能基因。并随着测序成本降低和组学技术发展,RNA-seq和GWAS在紫花苜蓿抗旱遗传基础解析中发挥积极作用,鉴定到一些抗旱功能基因(图1)。

2.1　紫花苜蓿抗旱性GWAS研究进展

由于紫花苜蓿是同源四倍体的异花授粉植物,其遗传背景相对复杂,导致对紫花苜蓿抗旱性状的遗传基础研究相对缓慢。定位紫花苜蓿抗旱性相关遗传位点、鉴定抗旱关键基因,解析其抗旱调控网络是紫花苜蓿抗旱分子育种面临的主要挑战。GWAS是鉴定复杂数量性状关联位点和候选基因的重要方法。该方法已在紫花苜蓿重

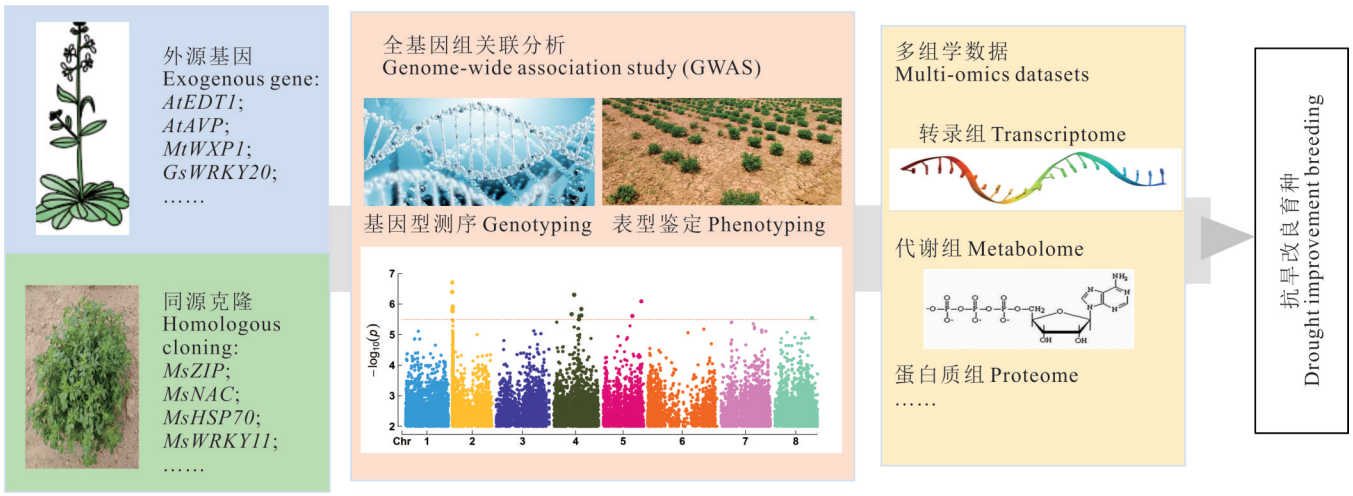

图1 紫花苜蓿抗旱相关基因研究进展

Fig. 1 Research progress on genes related to drought resistance of alfalfa

由于紫花苜蓿遗传背景复杂,前期的研究多通过在紫花苜蓿中导入外源基因或同源克隆抗旱相关基因以提高其抗旱性。随着测序技术的发展,全基因组关联分析和多组学数据,特别是转录组在紫花苜蓿抗旱相关基因的定位和功能研究中发挥着越来越重要的作用。Due to the complex genetic background of alfalfa, earlier research mainly focused on introducing exogenous genes or homologous cloning of drought-resistant genes to enhance its drought resistance. With the advancement of sequencing technology, genome-wide association analyses and multi-omics data, especially transcriptomics, are playing an increasingly important role in locating and studying the functions of drought-resistant genes in alfalfa.

要农艺性状遗传解析中广泛应用,鉴定到许多与产量、品质、抗旱、耐盐相关的关键位点[41,56,58]。在先前的研究中,由于其性价比高,简化基因组测序(genotyping by sequencing, GBS)在紫花苜蓿抗旱性GWAS研究中有广泛应用。在温室中对198份来自美国国家植物种质库的紫花苜蓿材料的抗旱性指数(drought resistance index, DRI)和叶片相对含水量(relative water content, RWC)两个抗旱重要指标进行统计,基于GBS-SNP的GWAS在DRI和RWC中分别鉴定到了19和15个显著位点。该研究中定位的抗旱相关SNP与之前报道的与苜蓿干旱条件下的生物量相关的QTL共定位[55]。在田间干旱条件下,对上述关联群体的紫花苜蓿在干旱胁迫下的产量和26个品质相关表型进行GWAS分析,定位到28个与产量相关的SNP位点和超过100个与品质相关的SNP位点。其中,15个SNP标记与产量和品质相关,这些位点可能同时参与对紫花苜蓿的产量和品质的调控。此外,仅有极少部分在干旱胁迫下定位到的SNP位点与水分充足条件下定位的SNP共点位,可能暗示了在干旱胁迫下紫花苜蓿的产量和品质的遗传调控网络与水分充足条件下不同[41,56]。在一项109份苜蓿基因型的GWAS研究中,使用RNA-seq数据进行SNP调用,总共获得了251577个SNP。使用株高、地上生物量和7个叶绿素荧光参数的抗旱系数进行GWAS研究,共定位到21个显著关联的位点。结合转录组数据,在8号染色体显著SNP标记(*MTR_42730294*)上下游2 kb范围发现了一个响应干旱胁迫的MYB-like转录因子(*MsMYBH*),并验证了该基因在苜蓿抗旱调控中的功能[57]。GWAS分析是定位紫花苜蓿抗旱性相关遗传位点和候选基因的可靠方法。然而,栽培紫花苜蓿是一种异花授粉的同源四倍体作物,具有丰富的遗传变异和复杂的遗传背景。在上述关联群体中进行的研究是基于GBS和RNA-seq获得的基因型数据,且参考基因组为紫花苜蓿近缘物种蒺藜苜蓿(*Medicago truncatula*),丢失了大量遗传信息,具有一定的局限性。随着紫花苜蓿染色体级别的高质量参考基因组的发布[59-61]及测序成本的下降,在后续的研究中开发高质量分子标记以定位紫花苜蓿抗旱关联位点及关键基因成为可能(表2)。

2.2 异源表达抗旱功能基因增强紫花苜蓿抗旱性

在拟南芥(*Arabidopsis thaliana*)、蒺藜苜蓿和其他植物中已经鉴定和表征了许多抗旱功能基因,是作物抗旱遗传改良的宝贵基因资源。转基因研究表明,异源表达*AgcodA*、*AtAVP1*、*AtEDT1*、*MtWXP1*、*GsZFP1*、*GsWRKY20*、*EsMcsu1*、*CsLEA*、*ZxABCG11*等基因成功增强了紫花苜蓿的耐旱性[33-34,62-69]。例如,将拟南芥

表 2　紫花苜蓿抗旱性全基因组关联分析研究汇总

Table 2　Summary of genome-wide association studies researches on drought resistance of alfalfa

群体大小 Population size (No.)	基因型测序 Genotyping	环境 Environment	表型 Phenotype	主要结果 Main results	参考文献 Reference
200	GBS	田间 Field	一年3次刈割的生物量Biomass of three harvests in one year	干旱条件下定位到28个与生物量相关的单核苷酸多态性(single nucleotide polymorphism, SNP)标记。28 SNP markers were associated with biomass under drought stress.	[41]
198	GBS	温室 Greenhouse	耐旱指数和叶片相对含水量 Drought resistance index (DRI) and leaf relative water content (RWC)	在DRI和RWC中分别鉴定了19和15个SNP。Nineteen and fifteen SNP associated with DRI and RWC, respectively.	[55]
198	GBS	田间 Field	26个品质相关性状26 forage quality traits	131个SNP与所有水分亏缺处理中的多个性状相关。131 SNP associated with multiple traits in all the water deficit treatments.	[56]
109	RNA-seq	温室 Greenhouse	株高、地上生物量和7个叶绿素荧光参数的抗旱系数Drought-resistance coefficients for plant height, above-ground biomass, and seven chlorophyll fluorescence parameters	在9个性状中共定位到21个显著SNP;验证了候选基因*MsMYBH*可增强苜蓿的抗旱性。A total of 21 significant SNPs were identified in nine traits; *MsMYBH* was confirmed to enhance alfalfa drought resistance.	[57]

Enhanced Drought Tolerance 1(*AtEDT1*)基因转入苜蓿中,转基因苜蓿植物的气孔尺寸增加,但气孔密度降低,这些气孔变化极大地减少了干旱胁迫下叶片中水分流失。同时,转基因苜蓿植物较野生型植株表现出更庞大的根系,有更大的根长、根重和根直径。在田间,干旱胁迫处理下的转基因植物有更高的生物量[64]。在紫花苜蓿中过表达蒺藜苜蓿*MtWXP1*基因,导致转基因苜蓿叶片角质层蜡质含量显著增加。同时,转基因株系在水分胁迫下和复水后表现出更高的光合作用速率、较高的相对含水量和叶片水势[65-66]。将来自旱生植物霸王(*Zygophyllum xanthoxylum*)的*ZxABCG11*转入紫花苜蓿,转基因苜蓿植株在田间干旱条件下干草产量比野生型高出50%。*ABCG11*编码三磷酸腺苷(adenosine triphosphate, ATP)结合盒转运蛋白,转基因苜蓿植株表现出更快的生长速度和更厚的叶片角质层,从而提高转基因苜蓿的保水能力和光合作用能力[63]。

2.3　紫花苜蓿抗旱基因鉴定及功能研究进展

通过同源克隆,在紫花苜蓿中鉴定到一些抗旱功能基因。前期由于紫花苜蓿遗传转化体系不成熟、效率低,在烟草(*Nicotiana tabacum*)和拟南芥中对*MsZIP*、*MsNAC*、*MsHSP17.7*、*MsZEP*、*MsHSP70*、*MsLEA4-4*、*MsCML46*、*MsVDAC*等进行功能验证,证明这些基因可参与调控紫花苜蓿抗旱性[70-77]。随着紫花苜蓿遗传转化体系的成熟,通过过表达和RNA干扰(RNA interference, RNAi)对其基因进行功能验证成为主要手段。过表达*MsWRKY11*的转基因植株表现出叶片的气孔密度降低和木质素含量增加的表型。通过体外和体内试验证明,*MsWRKY22*可以直接与*MsWRKY11*启动子中的W-box元件结合,并激活*MsWRKY11*的表达以调节苜蓿的耐旱性[78]。紫花苜蓿中,*MsDHN1*和*MsPIP2;1*分别编码脱水蛋白(保护剂)和水通道蛋白(控制水跨膜运输),在苜蓿生长和应对干旱胁迫的反应中发挥着至关重要的作用。缺水导致*MsPIP2;1*在Ser^{272}处磷酸化,导致mMYB的C端从质膜释放并易位至细胞核,其中*MsDHN1*的C端与mMYB△83相互作用,并促进mMYB响应缺水的转录活性。*mMYB*的过表达下调*MsCESA3*的表达,但通过与*MsCESA7*启动子直接结合上调其表达,并导致转基因毛状根具有高耐旱性[79]。此外,随着CRISPR技术在紫花苜蓿中的成功应用,利用该技术进行紫花苜蓿抗旱性研究也有一些报道。之前的研究发现在紫花苜蓿中,通过RNAi介导的*MsSPL8*下调植株表现对干旱和盐胁迫的耐受性[80]。利用CRISPR/Cas9诱导的*MsSPL8*基因突变进一步证实了*MsSPL8*在紫花苜蓿抗旱性中的功能[81]。

随着测序技术的发展,利用组学技术在紫花苜蓿中鉴定出一些抗旱的关键基因。通过转录组测序(RNA sequencing, RNA-seq)在紫花苜蓿中鉴定到2个功能未知的干旱诱导基因*MsDIUP1*(*DROUGHT-INDUCED*

UNKNOWN PROTEIN 1)和*MsNTF2L*(*NUCLEAR TRANSPORT FACTOR 2-LIKE*),并通过过表达和RNAi对其功能进行验证[82-83]。结果表明,*MsDIUP1*通过影响丙二醛和渗透保护剂(游离脯氨酸和可溶性糖)的含量调控紫花苜蓿的抗旱性[82]。而对*MsNTF2L*过表达和RNAi苜蓿植物的分析一致表明,*MsNTF2L*高度影响表皮蜡质沉积,并通过促进活性氧(reactive oxygen species, ROS)清除、气孔密度降低、脱落酸(abscisic acid, ABA)诱导的气孔关闭以及光合作用调节来赋予苜蓿干旱耐受性[83]。GWAS在紫花苜蓿抗旱功能基因的鉴定上也发挥了重要作用。对109份苜蓿种质材料的GWAS分析鉴定到一个增强苜蓿抗旱性的MYB样转录因子(*MsMYBH*)。*MsMYBH*过表达植物与野生型相比,其生物量和饲料品质得到提高。结合RNA-seq、蛋白质组学和染色质免疫沉淀分析表明,*MsMYBH*可以直接与*MsMCP1*、*MsMCP2*、*MsPRX1A*和*MsCARCAB*的启动子结合以提高其表达。这种相互作用使得过表达株系具有更好的水平衡、高光合作用效率以及清除过量的H_2O_2以适应干旱[57]。

2.4 MicroRNA调控紫花苜蓿对干旱胁迫的响应

MicroRNA(miRNA)是20~24个核苷酸长的内源性小RNA,通过不完全的序列互补性靶向mRNA,在转录后水平沉默或下调基因表达。越来越多的研究表明miRNA参与植物的各项生长发育活动[84],同时也参与植物对非生物胁迫的反应[85]。miRNA的表达受干旱胁迫诱导,并通过调节下游干旱响应基因以调节植物对干旱胁迫的耐受性。miRNA主要作用于下游的转录因子,已有研究表明miR159-MYB、miR169-NFYA、miR156-SPL、miR393-TIR1、miR160-ARF、miR167-ARF等模块参与植物的抗旱反应[85]。

在紫花苜蓿中,通过高通量测序在根中鉴定到18个干旱响应miRNA,在叶中则有12个干旱响应miRNA[86]。其中,miRNA156、miRNA159、miRNA160、miRNA166已经在其他植物中被证实参与干旱胁迫的响应和调控。miR156在紫花苜蓿中得到广泛关注,已有研究表明其调控苜蓿的生长发育[35],并且介导苜蓿的抗旱反应[36-37,87]。过表达*miR156*的苜蓿植株在干旱条件下表现出更高的存活率和较少的水分流失,增强了耐旱性[36]。进一步研究显示,低或者中等水平的*miR156*过表达可以通过诱导*WD40-1*表达量和沉默*SPL13*以增强花青素生物合成,从而增强紫花苜蓿的抗旱能力;而高过表达水平的*miR156*植株则表现出干旱敏感的表型[37]。对*miR156*的过表达植株进行转录组测序分析发现,除了*SPL13*外,还有6个*SPL*家族基因在转录水平上受到*miR156*调控[88]。其中,*MsSPL9*在过表达*miR156*的苜蓿植物中表达量下调。在干旱条件下,*MsSPL9*-RNAi紫花苜蓿植株与野生型相比表现出较少的叶片衰老,并增加了相对含水量,积累了更多的减轻胁迫的花青素[89]。这些结果表明,*miR156-SPL*(*MsSPL9*和*MsSPL13*)模块可通过调节花青素的生物合成参与调控紫花苜蓿的耐旱性(表3)。

3 总结与展望

干旱胁迫造成紫花苜蓿产量的严重损失,在田间干旱条件下,可使苜蓿减产70%以上[41]。然而,由于紫花苜蓿遗传背景复杂,对其抗旱性的遗传研究进展较为缓慢,特别是与水稻(*Oryza sativa*)、玉米等农作物相比研究基础还较为薄弱。尽管目前已经鉴定到一些与紫花苜蓿抗旱性相关的转录因子、编码保护性蛋白(胚胎发育晚期丰富蛋白、热休克蛋白、通道蛋白等)的基因和miRNA,但对这些基因参与紫花苜蓿抗旱调控的机制与调控网络的解析还有待进一步的深入研究。而随着测序技术的发展及成本的降低,多组学分析将在紫花苜蓿抗旱功能基因的定位及抗旱调控网络的解析中发挥越来越重要的作用。结合已有的研究成果,建议后续关于紫花苜蓿抗旱性的研究可从以下方面着手:

1)关注干旱胁迫对紫花苜蓿生殖生长阶段的影响。紫花苜蓿是一种饲草作物,多在现蕾期或初花期刈割,在进入初花期后,随着刈割时间推迟,产量上升但饲草品质急剧下降。因此,现有的与苜蓿抗旱性相关的研究多集中于种子萌发、幼苗及营养生长阶段,很少关注干旱胁迫对紫花苜蓿生殖生长阶段的影响,干旱胁迫对紫花苜蓿种子产量的影响鲜有报道。近年来,中国苜蓿种植面积不断增加,但国内苜蓿种子生产缺口很大,每年需进口大量苜蓿种子。因此,解析干旱胁迫对紫花苜蓿生殖生长阶段的影响,挖掘相关调控基因,对紫花苜蓿种子生产,保障我国草畜业健康发展具有重要意义。

表 3　紫花苜蓿耐旱性遗传研究进展

Table 3　Advances in genetic research on drought tolerance in alfalfa

基因 Genes	描述 Description	基因功能 Gene function	参考文献 Reference
GsZFP1	编码 Cys2/His2 型锌指蛋白 Encodes a Cys2/His2-type zinc-finger protein	过表达增强了紫花苜蓿的耐旱性。Overexpression enhanced drought resistance of alfalfa.	[33]
GsWRKY20	WRAKY 转录因子 WRKY-type transcription factor	更厚的角质层 Thicker cuticular layer	[34]
miR156/SPL13+*DFR/WD40-1*	miRNA，SPL 转录因子 miRNA，squamosa promoter binding protein-like（SPL）transcription factors	中等水平的 *miR156* 表达可抑制 *SPL13* 并增加 *WD40-1* 表达，以微调花青素生物合成的 *DFR* 表达，并调节紫花苜蓿的各种发育、生理和生化过程，从而提高抗旱能力。Moderate levels of *miR156* expression can inhibit *SPL13* and increase *WD40-1* expression，to fine-tune the expression of *DFR* involved in anthocyanin biosynthesis，and regulate various developmental，physiological，and biochemical processes in alfalfa，thereby enhancing drought resistance.	[36－37]
MsMYBH	MYB-like 转录因子 MYB-like transcription factor	保持水平衡、高光合作用效率以及清除过量的 H_2O_2。Maintaining water balance，high photosynthetic efficiency，and scavenging excess H_2O_2.	[57]
AtAVP1	液泡 H^+-焦磷酸酶（H^+-PPase）Vacuolar H^+-pyrophosphatase（H^+-PPase）	叶片和根系中积累更多的 Na^+、K^+ 和 Ca^{2+}。Accumulation of more Na^+，K^+ and Ca^{2+} in leaves and roots.	[62]
ZxABCG11	编码 ABC 转运蛋白 Encodes an ATP binding cassette（ABC）transporter	更高的蜡晶体密度和更厚的叶片角质层，从而提高转基因苜蓿的保水能力和光合作用能力。Higher wax crystal density and thicker leaf cuticular layer，thereby enhancing water retention and photosynthetic capacity of transgenic alfalfa.	[63]
AtEDT1	同源域亮氨酸拉链转录因子 Homodomain-leucine zipper transcription factor	降低气孔密度，增加根系发育，同时膜透性和丙二醛含量降低，但可溶性糖和脯氨酸含量较高。Reduced stomatal density，increased root development，while membrane permeability and malondialdehyde content decreased，but soluble sugar and proline content were higher.	[64]
MtWXP1	AP2 结构域的转录因子基因 AP2 domain-containing transcription factor gene	增加角质层蜡堆积，增强耐旱性。Increased accumulation of cuticular wax，enhancing drought resistance.	[65－66]
EsMcsu1	编码一种钼辅因子硫化酶 Encoding a molybdenum cofactor sulfurase	促进脱落酸生物合成，提高抗旱性。Promotes abscisic acid biosynthesis，enhancing drought resistance.	[67]
CsLEA	晚期胚胎发生丰富蛋白 Late embryogenesis abundant（LEA）proteins	较高的相对含水量和减少的膜损伤。Higher relative water content and reduced membrane damage.	[68]
AgcodA	来自土壤细菌 *Arthrobacter globiformis* 的 *codA* 基因，编码胆碱氧化酶 A *codA* gene from the soil bacterium（*Arthrobacter globiformis*），encoding choline oxidase	保持较高的相对含水量和增加甘氨酸甜菜碱和脯氨酸含量。Maintaining high relative water contents and increased levels of glycinebetaine and proline.	[69]
MsZIP	bZIP 转录因子 bZIP transcription factor	增加丙二醛含量、相对含水量、可溶性糖含量、可溶性蛋白含量和脯氨酸含量。Increased malondialdehyde content，relative water content，soluble sugar content，soluble protein content，and proline content.	[70]
MsNAC	NAC 转录因子 NAC transcription factor	—	[71]
MsHSP17.7	编码热休克蛋白 Encodes a small heat shock protein	增加根长。Increased root length.	[72]
MsZEP	编码玉米黄质环氧化酶 Encodes zeaxanthin epoxidase	影响各种生理途径、ABA 水平和胁迫响应基因表达。Affects various physiological pathways，ABA levels，and stress-responsive gene expression.	[73]
MsHSP70	编码热休克蛋白 Encodes heat shock proteins	相对含水量、脯氨酸含量、超氧化物歧化酶活性升高，丙二醛含量降低。Increased relative water content，proline content，and superoxide dismutase activity，while malondialdehyde content decreased.	[74]
MsLEA4-4	晚期胚胎发生丰富蛋白 Late embryogenesis abundant（LEA）proteins	更多的侧根和更高的叶绿素含量，可溶性糖水平和多种抗氧化酶活性升高，而脯氨酸和丙二醛水平显著降低。More lateral roots and higher chlorophyll content，increased soluble sugar levels and various antioxidant enzyme activities，while proline and malondialdehyde levels significantly decreased.	[75]

续表 Continued Table

基因 Genes	描述 Description	基因功能 Gene function	参考文献 Reference
MsCML46	编码钙调蛋白样蛋白 Encodes calmodulin-like protein	*MsCML46* 结合游离 Ca^{2+} 以促进信号转导并维持较高的 K^+/Na^+，保护细胞内稳态。*MsCML46* binds free Ca^{2+} to promote signal transduction and maintain a higher K^+/Na^+, protecting cellular homeostasis.	[76]
MsVDAC	编码电压依赖性阴离子选择性通道(VDAC)蛋白 Encodes voltage-dependent anion-selective channel (VDAC) protein	渗透稳态和胁迫响应基因表达。Osmotic homeostasis and stress-responsive gene expression.	[77]
MsWRKY11	WRKY转录因子 WRKY transcription factor	通过调控木质素生物合成和紫花苜蓿气孔开闭调控耐旱性。Enhances drought resistance by regulating lignin biosynthesis and alfalfa stomatal opening and closing.	[78]
MsDHN1-MsPIP2;1-MsmMYB	MsDHN1(脱水蛋白)、MsPIP2;1(水通道蛋白)，MsmMYB(一种膜锚定的 MYB 转录因子) MsDHN1 (dehydrin) and MsPIP2;1 (aquaporin), MsmMYB (a membrane-anchored MYB transcriptional factor MsmMYB)	缺水导致 MsPIP2;1 磷酸化，mMYB (mMYB△83)的 C 端易位并与 MsDHN1 相互作用，并促进 mMYB△83 响应缺水的转录活性。*mMYB* 和 *mMYBD83* 的过表达下调 *MsCESA3* 的表达，但通过直接结合其启动子上调 *MsCESA7* 的表达。Dehydration leads to phosphorylation of MsPIP2;1, C-terminal translocation of mMYB (mMYB△83) and interaction with MsDHN1, promoting the transcriptional activity of mMYB△83 in response to dehydration. Overexpression of *mMYB* and *mMYBD83* downregulates the expression of *MsCESA3*, but upregulates the expression of *MsCESA7* by directly binding to its promoter.	[79]
MsSPL8	SPL转录因子 Squamosa promoter binding protein-like (SPL) transcription factors	表达下调的植株延缓枯萎并快速恢复；*MsSPL8* 突变体在耐缺水能力方面有所提高。Down-regulation delayed wilting and recovered quickly; *MsSPL8* mutants displayed improvements in their ability to withstand water-deficit.	[80－81]
MsDIUP1	干旱诱导的未知蛋白 1(它缺乏任何可靠的保守结构域)Drought-induced unknown protein 1 (it lacked any confidently conserved domains)	参与胁迫信号传导、抗氧化防御和渗透调节的基因存在差异反应。Differential responses of genes involved in stress signal transduction, antioxidant defense, and osmotic regulation.	[82]
MsNTF2L	核转运因子 2-like Nuclear transport factor 2-like	通过调节叶片失水(通过调节气孔和蜡沉积)、抗氧化防御和光合作用调控苜蓿耐旱性。Regulates drought resistance by modulating leaf dehydration (through stomatal and wax deposition), antioxidant defense, and photosynthesis in alfalfa.	[83]
miR156/SPL9	miRNA	调节花青素的生物合成。Regulating anthocyanin biosynthesis.	[89]
TPS1-TPS2	酵母海藻糖-6-磷酸合酶 (TPS1) 和海藻糖-6-磷酸磷酸酶 (TPS2) 基因 Yeast trehalose-6-phosphate synthase (TPS1) and trehalose-6-phosphate phosphatase (TPS2) genes	海藻糖积累。Accumulation of trehalose.	[90]
MfLEA3	晚期胚胎发生丰富蛋白 Late embryogenesis abundant (LEA) proteins	转基因植物中积累 ROS 减少。Reduced accumulation of ROS in transgenic plants.	[91]
AtNDPK2	拟南芥核苷二磷酸激酶 2 Arabidopsis nucleoside diphosphate kinase 2	水分流失率降低，细胞膜损伤降低。Water loss rate and cell membrane damage were decreased.	[92]
AtABF3	脱落酸(ABA)响应元件结合因子 3 ABA-responsive element-binding factor 3	蒸腾速率降低，活性氧含量降低。A reduced transpiration rate and lower reactive oxygen species contents.	[93]
IbOr	甘薯橙基因 The sweetpotato orange gene	总类胡萝卜素水平更高，细胞膜损伤更少。Higher total carotenoid levels and lower cell membrane damage.	[94]
co-expression of bar+CsALDH genes	氧化应答和抗除草剂基因 An oxidative responsive gene (*CsALDH*) and herbicide resistance gene (*bar*)	Na^+ 含量降低，K^+ 含量升高；离子毒性降低，渗透调节维持；相对含水量升高，光系统变化减少，膜损伤减少。Lower Na^+ and higher K^+ content; Reduction of ion toxicity and maintenance of osmotic adjustment; Higher relative water content level, fewer changes in the photosystem, decreased membrane injury.	[95]

续表 Continued Table

基因 Genes	描述 Description	基因功能 Gene function	参考文献 Reference
MsTMT	编码γ-生育酚甲基转移酶 Encodes γ-tocopherol methyltransferase	减轻氧化损伤，积累更多渗透解离物质，提高水分利用效率。Alleviated oxidative damage, accumulation of more osmolytic substances and improved water use efficiency.	[96]

2)完善紫花苜蓿基因组。尽管已经有染色体级别的紫花苜蓿基因组发表[59-61]，但是由于紫花苜蓿基因组具有高杂合、高重复度、高倍性等特征，现有的紫花苜蓿参考基因组并不完善。*T2T*基因组指的是具有端粒到端粒的高质量、高准确性和高连续性的完整基因组。已相继完成了拟南芥[97]、水稻[98]、玉米[99]等多个植物的*T2T*基因组的组装。此外，单个紫花苜蓿的基因组仅能代表该基因型的遗传信息，无法代表紫花苜蓿物种的遗传多样性。泛基因组(pangenome)是指某一物种多个个体基因组信息的集合，有助于揭示物种内丰富的遗传变异，挖掘新的功能基因，解析品种形成的分子基础，深化对物种遗传多样性的认识[100]。基因组测序技术的发展、测序成本的降低以及基因组组装算法的进步，有助于完善紫花苜蓿基因组信息，并指导抗旱功能基因及其他重要农艺性状相关基因的挖掘。

3)利用 Indel、SV 等标记进行紫花苜蓿抗旱性状的关联分析。SNP、插入/缺失变异(insertion-deletion，Indel)、结构变异(structural variations，SV)和表观遗传变异共同导致了物种内和物种间观察到的可遗传表型多样性[101-102]。基于SNP的GWAS分析在紫花苜蓿抗旱性状解析中发挥了关键作用，鉴定到一大批与紫花苜蓿抗旱性紧密关联的SNP变异[55-57]。然而，随着研究的深入，研究人员发现SNP变异并不能完全解释植物基因组的变异，Indel、SV等标记对植物表型变异的贡献对于植物育种者协助培育改良品种非常重要。随着基因组技术的快速发展，以更高的分辨率和准确性鉴定Indel、SV等标记成为可能。Indel、SV等标记的开发将加深对紫花苜蓿进化、驯化和育种过程中的基因组变化的认知。同时将这些变异与表型结合进行关联分析，将有助于鉴定关键的候选基因，为后续优异基因资源的发掘提供重要参考。

4)结合多组学数据解析紫花苜蓿抗旱性遗传基础。植物具有复杂的调控网络，单一的组学研究不够全面，往往不能很好地解释具体的生物学现象。组学技术和生物信息学的发展为植物研究人员提供了前所未有的机会，可通过对多个组学数据集的联合分析来解析复杂的生物学现象。通过多种组学研究方法，如表型组、基因组、转录组、代谢组、蛋白组、表观组等的结合，可以从不同层面揭示性状的遗传基础和调控机制，更全面反映植物遗传变异和表型变异的关联，鉴定重要农艺性状的标记位点和基因，从而提高育种效率[103-105]。在紫花苜蓿中，基因组、转录组、代谢组等数据已有报道，但很少有研究结合多个组学数据集解析苜蓿的抗旱性遗传机制。随着测序技术的进一步发展与成本的降低，多组学研究将在紫花苜蓿的遗传机制研究中发挥不可替代的作用，将会成为苜蓿抗旱育种的强大工具。

5)利用CRISPR/Cas基因编辑技术创制抗旱高产紫花苜蓿种质材料。CRISPR/Cas基因编辑系统已成为生命科学领域的重要技术，广泛用于各种植物的基因功能验证和种质材料创制。然而，相较于其他作物，紫花苜蓿的同源四倍体特性使得CRISPR/Cas 9系统的应用面临诸多挑战。早期研究表明，苜蓿的基因组编辑效率为1.7%～8.4%[60,106]，而现阶段已经研发出多种不同类型的紫花苜蓿基因编辑载体，显著提高了基因编辑的效率，已达50%以上[107]。结合全基因组关联研究(GWAS)和组学技术的迅猛发展，越来越多的抗旱候选基因得以识别，CRISPR技术有望成为后续基因功能验证及抗旱紫花苜蓿材料创制中最有效的手段。并且随着CRISPR技术的发展，通过对产量、品质、抗旱性及其他抗逆相关基因进行多基因编辑以改善紫花苜蓿的综合性能成为可能。

参考文献 References：

[1] Nadeem M, Li J J, Yahya M, *et al*. Research progress and perspective on drought stress in legumes: A review. International Journal of Molecular Sciences, 2019, 20(10): 2541.

[2] Inès S, Talbi O, Nasreddine Y, *et al*. Drought tolerance traits in *Medicago* species: A review. Arid Land Research Management, 2022, 36(1): 67-83.

[3] Bartels D, Sunkar R. Drought and salt tolerance in plants. Critical Reviews in Plant Sciences, 2005, 24(1): 23-58.

[4] Kim W, Iizumi T, Nishimori M. Global patterns of crop production losses associated with droughts from 1983 to 2009. Journal of Applied Meteorology Climatology, 2019, 58(6): 1233-1244.

[5] Matiu M, Ankerst D P, Menzel A. Interactions between temperature and drought in global and regional crop yield variability during 1961-2014. PLoS One, 2017, 12(5): e0178339.

[6] Gupta A, Rico-Medina A, Caño-Delgado A I. The physiology of plant responses to drought. Science, 2020, 368(6488): 266-269.

[7] Comas L H, Becker S R, Cruz V M V, *et al*. Root traits contributing to plant productivity under drought. Frontiers in Plant Science, 2013, 4: 442.

[8] Yadav B, Jogawat A, Gnanasekaran P, *et al*. An overview of recent advancement in phytohormones-mediated stress management and drought tolerance in crop plants. Plant Gene, 2021, 25: 100264.

[9] Li Y P, Ye W, Wang M, *et al*. Climate change and drought: a risk assessment of crop-yield impacts. Climate Research, 2009, 39(1): 31-46.

[10] Yu L X, Kole C. The alfalfa genome. Cham, Switzerland: Springer, 2021.

[11] Radović J, Sokolović D, Marković J. Alfalfa-most important perennial forage legume in animal husbandry. Biotechnology in Animal Husbandry, 2009, 25(5/6): 465-475.

[12] Bora K S, Sharma A. Phytochemical and pharmacological potential of *Medicago sativa*: A review. Pharmaceutical Biology, 2011, 49(2): 211-220.

[13] Li A, Liu A, Du X, *et al*. A chromosome-scale genome assembly of a diploid alfalfa, the progenitor of autotetraploid alfalfa. Horticulture Research, 2020, 7: 194.

[14] Bai Z, Ma W, Ma L, *et al*. China's livestock transition: Driving forces, impacts, and consequences. Science Advances, 2018, 4(7): eaar8534.

[15] Wang Q B, Zou Y. China's alfalfa market and imports: Development, trends, and potential impacts of the US-China trade dispute and retaliations. Journal of Integrative Agriculture, 2020, 19(4): 1149-1158.

[16] Ashrafi M, Azimi-Moqadam M R, Moradi P, *et al*. Effect of drought stress on metabolite adjustments in drought tolerant and sensitive thyme. Plant Physiology and Biochemistry, 2018, 132: 391-399.

[17] Kumar S. Biotechnological advancements in alfalfa improvement. Journal of Applied Genetics, 2011, 52(2): 111-124.

[18] Gall H L, Philippe F, Domon J M, *et al*. Cell wall metabolism in response to abiotic stress. Plants, 2015, 4(1): 112-166.

[19] Zhang C, Shi S, Liu Z, *et al*. Drought tolerance in alfalfa (*Medicago sativa* L.) varieties is associated with enhanced antioxidative protection and declined lipid peroxidation. Journal of Plant Physiology, 2019, 232: 226-240.

[20] Kapoor D, Bhardwaj S, Landi M, *et al*. The impact of drought in plant metabolism: How to exploit tolerance mechanisms to increase crop production. Applied Sciences, 2020, 10(16): 5692.

[21] Zhang H, Zhu J, Gong Z, *et al*. Abiotic stress responses in plants. Nature Reviews Genetics, 2022, 23(2): 104-119.

[22] Davis R, Earl H, Timper P. Effect of simultaneous water deficit stress and *Meloidogyne incognita* infection on cotton yield and fiber quality. Journal of Nematology, 2014, 46(2): 108.

[23] Ranjan A, Sinha R, Singla-Pareek S L, *et al*. Shaping the root system architecture in plants for adaptation to drought stress. Physiologia Plantarum, 2022, 174(2): e13651.

[24] Maqbool S, Hassan M A, Xia X, *et al*. Root system architecture in cereals: progress, challenges and perspective. The Plant Journal, 2022, 110(1): 23-42.

[25] Rogers E D, Benfey P N. Regulation of plant root system architecture: implications for crop advancement. Current Opinion in Biotechnology, 2015, 32: 93-98.

[26] Seo D H, Seomun S, Choi Y D, *et al*. Root development and stress tolerance in rice: the key to improving stress tolerance without yield penalties. International Journal of Molecular Sciences, 2020, 21(5): 1807.

[27] Jeong J S, Kim Y S, Redillas M C, *et al*. *OsNAC5* overexpression enlarges root diameter in rice plants leading to enhanced drought tolerance and increased grain yield in the field. Plant Biotechnology Journal, 2013, 11(1): 101-114.

[28] Castroluna A, Ruiz O, Quiroga A, *et al*. Effects of salinity and drought stress on germination, biomass and growth in three varieties of *Medicago sativa* L. Avances en Investigación Agropecuaria, 2014, 18(1): 39-50.

[29] Slama I, Tayachi S, Jdey A, *et al*. Differential response to water deficit stress in alfalfa (*Medicago sativa*) cultivars: Growth, water relations, osmolyte accumulation and lipid peroxidation. African Journal of Biotechnology, 2011, 10(72): 16250－16259.

[30] Riasat M, Saed-Mouchehsi A, Jafari A A. Effect of drought stress levels on seedling morpho-physiological traits of alfalfa (*Medicago sativa*) populations grown in glasshouse. Journal of Rangeland Science, 2020, 10(1): 86－97.

[31] Zhang C M, Shi S L, Liu Z, *et al*. Effects of drought stress on the root morphology and anatomical structure of alfalfa (*Medicago sativa*) varieties with differing drought-tolerance. Acta Prataculturae Sinica, 2019, 28(5): 79－89.
张翠梅, 师尚礼, 刘珍, 等. 干旱胁迫对不同抗旱性苜蓿品种根系形态及解剖结构的影响. 草业学报, 2019, 28(5): 79－89.

[32] Lamb J, Barnes D, Henjum K J C S. Gain from two cycles of divergent selection for root morphology in alfalfa. Crop Science, 1999, 39(4): 1026－1035.

[33] Tang L, Cai H, Ji W, *et al*. Overexpression of *GsZFP1* enhances salt and drought tolerance in transgenic alfalfa (*Medicago sativa* L.). Plant Physiology and Biochemistry, 2013, 71: 22－30.

[34] Tang L, Cai H, Zhai H, *et al*. Overexpression of *Glycine soja WRKY20* enhances both drought and salt tolerance in transgenic alfalfa (*Medicago sativa* L.). Plant Cell, Tissue and Organ Culture, 2014, 118: 77－86.

[35] Aung B, Gruber M Y, Amyot L, *et al*. MicroRNA156 as a promising tool for alfalfa improvement. Plant Biotechnology Journal, 2015, 13(6): 779－790.

[36] Arshad M, Feyissa B A, Amyot L, *et al*. MicroRNA156 improves drought stress tolerance in alfalfa (*Medicago sativa*) by silencing SPL13. Plant Science, 2017, 258: 122－136.

[37] Feyissa B A, Arshad M, Gruber M Y, *et al*. The interplay between miR156/SPL13 and DFR/WD40-1 regulate drought tolerance in alfalfa. BMC Plant Biology, 2019, 19(1): 1－19.

[38] Safarnejad A J P J B. Morphological and biochemical response to osmotic stress in alfalfa (*Medicago sativa* L.). Pakistan Journal of Botany, 2008, 40(2): 735－746.

[39] Maghsoodi M, Razmjoo J. Identify physiological markers for drought tolerance in alfalfa. Agronomy Journal, 2015, 107(1): 149－157.

[40] Zhang T, Kesoju S, Greene S L, *et al*. Genetic diversity and phenotypic variation for drought resistance in alfalfa (*Medicago sativa* L.) germplasm collected for drought tolerance. Genetic Resources and Crop Evolution, 2018, 65: 471－484.

[41] Yu L X. Identification of single-nucleotide polymorphic loci associated with biomass yield under water deficit in alfalfa (*Medicago sativa* L.) using genome-wide sequencing and association mapping. Frontiers in Plant Science, 2017, 8: 1152.

[42] Afsharmanesh G. Study of some morphological traits and selection of drought-resistant alfalfa cultivars (*Medicago sativa* L.) in Jiroft, Iran. Plant Ecophysiology (Jiroft Branch), 2009, 1(3): 109－118.

[43] Hamidi H, Safarnejad A. Effect of drought stress on alfalfa cultivars (*Medicago sativa* L.) in germination stage. American-Eurasian Journal of Agricultural and Environmental Sciences, 2010, 8(6): 705－709.

[44] Hanson A, Xu L, Johnson P S, *et al*. Identification and characterization of drought-tolerant alfalfa (*Medicago sativa* subsp. *falcata*) germplasm. Proceedings of the South Dakota Academy of Science, 2015, 94: 263－272.

[45] Gorai M, Hachef A, Neffati M. Differential responses in growth and water relationship of *Medicago sativa* (L.) cv. Gabès and *Astragalus gombiformis* (Pom.) under water-limited conditions. Emirates Journal of Food and Agriculture, 2010, 7(1): 1－12.

[46] Annicchiarico P, Pecetti L, Tava A. Physiological and morphological traits associated with adaptation of lucerne (*Medicago sativa*) to severely drought-stressed and to irrigated environments. Annals of Applied Biology, 2013, 162(1): 27－40.

[47] Farissi M, Bouizgaren A, Faghire M, *et al*. Agrophysiological and biochemical properties associated with adaptation of *Medicago sativa* populations to water deficit. Turkish Journal of Botany, 2013, 37(6): 1166－1175.

[48] Moghaddam A, Vollmann J, Wanek W, *et al*. Suitability of drought tolerance indices for selecting alfalfa (*Medicago sativa* L.) genotypes under organic farming in Austria. Crop Breeding Journal, 2012, 2(2): 79－89.

[49] Benabderrahim M, Hamza H, Haddad M, *et al*. Assessing the drought tolerance variability in Mediterranean alfalfa (*Medicago sativa* L.) genotypes under arid conditions. Plant Biosystems, 2013, 149(2): 395－403.

[50] Boe A, Kephart K D, Berdahl J D, *et al*. Breeding alfalfa for semiarid regions in the northern Great Plains: History and additional genetic evaluations of novel germplasm. Agronomy, 2020, 10(11): 1686.

[51] Shi S, Nan L, Smith K F. The current status, problems, and prospects of alfalfa (*Medicago sativa* L.) breeding in China. Agronomy, 2017, 7(1): 1.

[52] Yang Q C. Guide for alfalfa planting zone and cultivar. Beijing: China Agricultural University Press, 2012.

杨青川. 苜蓿种植区划及品种指南. 北京：中国农业大学出版社，2012.

[53] Li X, Brummer E C. Applied genetics and genomics in alfalfa breeding. Agronomy, 2012, 2(1): 40－61.

[54] Volenec J, Cunningham S, Haagenson D, *et al*. Physiological genetics of alfalfa improvement: past failures, future prospects. Field Crops Research, 2002, 75(2/3): 97－110.

[55] Zhang T, Yu L X, Zheng P, *et al*. Identification of loci associated with drought resistance traits in heterozygous autotetraploid alfalfa (*Medicago sativa* L.) using genome-wide association studies with genotyping by sequencing. PLoS One, 2015, 10(9): e0138931.

[56] Lin S, Medina C A, Boge B, *et al*. Identification of genetic loci associated with forage quality in response to water deficit in autotetraploid alfalfa (*Medicago sativa* L.). BMC Plant Biology, 2020, 20(1): 1－18.

[57] Shi K, Liu J, Liang H, *et al*. An alfalfa MYB-like transcriptional factor MsMYBH positively regulates alfalfa seedling drought resistance and undergoes MsWAV3-mediated degradation. Journal of Integrative Plant Biology, 2024, 66(4): 683－699.

[58] Liu X P, Hawkins C, Peel M D, *et al*. Genetic loci associated with salt tolerance in advanced breeding populations of tetraploid alfalfa using genome-wide association studies. The Plant Genome, 2019, 12(1): 180026.

[59] Long R C, Zhang F, Zhang Z W, *et al*. Genome assembly of alfalfa cultivar Zhongmu-4 and identification of SNPs associated with agronomic traits. Genomics, Proteomics and Bioinformatics, 2022, 20(1): 14－28.

[60] Chen H, Zeng Y, Yang Y, *et al*. Allele-aware chromosome-level genome assembly and efficient transgene-free genome editing for the autotetraploid cultivated alfalfa. Nature Communications, 2020, 11(1): 2494.

[61] Shen C, Du H, Chen Z, *et al*. The chromosome-level genome sequence of the autotetraploid alfalfa and resequencing of core germplasms provide genomic resources for alfalfa research. Molecular Plant, 2020, 13(9): 1250－1261.

[62] Bao A K, Wang S M, Wu G Q, *et al*. Overexpression of the Arabidopsis H^+-PPase enhanced resistance to salt and drought stress in transgenic alfalfa (*Medicago sativa* L.). Plant Science, 2009, 176(2): 232－240.

[63] Liu L B, Bao A K, Li H J, *et al*. Overexpression of *ZxABCG11* from *Zygophyllum xanthoxylum* enhances tolerance to drought and heat in alfalfa by increasing cuticular wax deposition. The Crop Journal, 2023, 11(4): 1140－1151.

[64] Zheng G, Fan C, Di S, *et al*. Over-expression of *Arabidopsis EDT1* gene confers drought tolerance in alfalfa (*Medicago sativa* L.). Frontiers in Plant Science, 2017, 8: 316380.

[65] Zhang J Y, Broeckling C D, Blancaflor E B, *et al*. Overexpression of *WXP1*, a putative *Medicago truncatula* AP2 domain-containing transcription factor gene, increases cuticular wax accumulation and enhances drought tolerance in transgenic alfalfa (*Medicago sativa*). The Plant Journal, 2005, 42(5): 689－707.

[66] Jiang Q, Zhang J Y, Guo X, *et al*. Physiological characterization of transgenic alfalfa (*Medicago sativa*) plants for improved drought tolerance. International Journal of Plant Sciences, 2009, 170(8): 969－978.

[67] Zhou C, Ma Z, Zhu L, *et al*. Overexpression of *EsMcsu1* from the halophytic plant *Eutrema salsugineum* promotes abscisic acid biosynthesis and increases drought resistance in alfalfa (*Medicago sativa* L.). Genetics and Molecular Research, 2015, 14: 17204－17218.

[68] Zhang J, Duan Z, Zhang D, *et al*. Co-transforming *bar* and *CsLEA* enhanced tolerance to drought and salt stress in transgenic alfalfa (*Medicago sativa* L.). Biochemical and Biophysical Research Communications, 2016, 472(1): 75－82.

[69] Li H, Wang Z, Ke Q, *et al*. Overexpression of *codA* gene confers enhanced tolerance to abiotic stresses in alfalfa. Plant Physiology and Biochemistry, 2014, 85: 31－40.

[70] Li Y, Sun Y, Yang Q, *et al*. Isolation and characterization of a gene from *Medicago sativa* L., encoding a bZIP transcription factor. Molecular Biology Reports, 2013, 40(2): 1227－1239.

[71] Wang Y X. Characterization of a novel *Medicago sativa NAC* transcription factor gene involved in response to drought stress. Molecular Biology Reports, 2013, 40(11): 6451－6458.

[72] Li Z Y, Long R C, Zhang T J, *et al*. Molecular cloning and characterization of the *MsHSP17.7* gene from *Medicago sativa* L. Molecular Biology Reports, 2016, 43(8): 815－826.

[73] Zhang Z, Wang Y, Chang L, *et al*. *MsZEP*, a novel zeaxanthin epoxidase gene from alfalfa (*Medicago sativa*), confers drought and salt tolerance in transgenic tobacco. Plant Cell Reports, 2016, 35(2): 439－453.

[74] Li Z, Long R, Zhang T, *et al*. Molecular cloning and functional analysis of the drought tolerance gene *MsHSP70* from alfalfa (*Medicago sativa* L.). Journal of Plant Research, 2017, 130(2): 387－396.

[75] Jia H, Wang X, Shi Y, *et al*. Overexpression of *Medicago sativa LEA 4-4* can improve the salt, drought, and oxidation resistance of transgenic *Arabidopsis*. PLoS One, 2020, 15(6): e0234085.

[76] Du B, Chen N, Song L, *et al*. Alfalfa (*Medicago sativa* L.) *MsCML46* gene encoding calmodulin-like protein confers tolerance to abiotic stress in tobacco. Plant Cell Reports, 2021, 40(10): 1907－1922.

[77] Yang M, Duan X, Wang Z, *et al*. Overexpression of a *voltage-dependent anion-selective channel* (*VDAC*) protein-encoding gene, *MsVDAC*, from *Medicago sativa* confers cold and drought tolerance to transgenic tobacco. Genes, 2021, 12(11): 1706.

[78] Wen W, Wang R, Su L, *et al*. *MsWRKY11*, activated by *MsWRKY22*, functions in drought tolerance and modulates lignin biosynthesis in alfalfa (*Medicago sativa* L.). Environmental and Experimental Botany, 2021, 184: 104373.

[79] Lv A, Su L, Fan N, *et al*. The *MsDHN-MsPIP2;1-MsmMYB* module orchestrates the trade-off between growth and survival of alfalfa in response to drought stress. Plant Biotechnology Journal, 2023, 22(5): 1132－1145.

[80] Gou J, Debnath S, Sun L, *et al*. From model to crop: functional characterization of *SPL8* in *M. truncatula* led to genetic improvement of biomass yield and abiotic stress tolerance in alfalfa. Plant Biotechnology Journal, 2018, 16(4): 951－962.

[81] Singer S D, Burton Hughes K, Subedi U, *et al*. The CRISPR/Cas9-mediated modulation of squamosa promoter-binding protein-like 8 in alfalfa leads to distinct phenotypic outcomes. Frontiers in Plant Science, 2021, 12: 774146.

[82] Luo D, Zhang X, Liu J, *et al*. Drought-induced unknown protein 1 positively modulates drought tolerance in cultivated alfalfa (*Medicago sativa* L.). The Crop Journal, 2023, 11(1): 57－70.

[83] Luo D, Liu J, Wu Y, *et al*. NUCLEAR TRANSPORT FACTOR 2-LIKE improves drought tolerance by modulating leaf water loss in alfalfa (*Medicago sativa* L.). The Plant Journal, 2022, 112(2): 429－450.

[84] Waheed S, Zeng L. The critical role of miRNAs in regulation of flowering time and flower development. Genes, 2020, 11(3): 319.

[85] Singroha G, Sharma P, Sunkur R. Current status of microRNA-mediated regulation of drought stress responses in cereals. Physiologia Plantarum, 2021, 172(3): 1808－1821.

[86] Li Y, Wan L, Bi S, *et al*. Identification of drought-responsive microRNAs from roots and leaves of alfalfa by high-throughput sequencing. Genes, 2017, 8(4): 119.

[87] Arshad M, Gruber M Y, Hannoufa A. Transcriptome analysis of microRNA156 overexpression alfalfa roots under drought stress. Scientific Reports, 2018, 8(1): 9363.

[88] Gao R, Austin R S, Amyot L, *et al*. Comparative transcriptome investigation of global gene expression changes caused by miR156 overexpression in *Medicago sativa*. BMC Genomics, 2016, 17(1): 658.

[89] Hanly A, Karagiannis J, Lu Q S M, *et al*. Characterization of the role of SPL9 in drought stress tolerance in *Medicago sativa*. International Journal of Molecular Sciences, 2020, 21(17): 6003.

[90] Suárez R, Calderón C, Iturriaga G. Enhanced tolerance to multiple abiotic stresses in transgenic alfalfa accumulating trehalose. Crop Science, 2009, 49(5): 1791－1799.

[91] Shi H, He X, Zhao Y, *et al*. Constitutive expression of a group 3 LEA protein from *Medicago falcata* (MfLEA3) increases cold and drought tolerance in transgenic tobacco. Plant Cell Reports, 2020, 39(7): 851－860.

[92] Wang Z, Li H, Ke Q, *et al*. Transgenic alfalfa plants expressing *AtNDPK2* exhibit increased growth and tolerance to abiotic stresses. Plant Physiology and Biochemistry, 2014, 84: 67－77.

[93] Wang Z, Su G, Li M, *et al*. Overexpressing *Arabidopsis ABF3* increases tolerance to multiple abiotic stresses and reduces leaf size in alfalfa. Plant Physiology and Biochemistry, 2016, 109: 199－208.

[94] Wang Z, Ke Q, Kim M D, *et al*. Transgenic alfalfa plants expressing the sweetpotato orange gene exhibit enhanced abiotic stress tolerance. PLoS One, 2015, 10(5): e0126050.

[95] Duan Z, Zhang D, Zhang J, *et al*. Co-transforming *bar* and *CsALDH* genes enhanced resistance to herbicide and drought and salt stress in transgenic alfalfa (*Medicago sativa* L.). Frontiers in Plant Science, 2015, 6: 1115.

[96] Ma J, Qiu D, Gao H, *et al*. Over-expression of a γ-tocopherol methyltransferase gene in vitamin E pathway confers PEG-simulated drought tolerance in alfalfa. BMC Plant Biology, 2020, 20(1): 226.

[97] Naish M, Alonge M, Wlodzimierz P, *et al*. The genetic and epigenetic landscape of the *Arabidopsis* centromeres. Science, 2021, 374(6569): eabi7489.

[98] Song J M, Xie W Z, Wang S, *et al*. Two gap-free reference genomes and a global view of the centromere architecture in rice. Molecular Plant, 2021, 14(10): 1757－1767.

[99] Chen J, Wang Z, Tan K, *et al*. A complete telomere-to-telomere assembly of the maize genome. Nature Genetics, 2023, 55(7): 1221－1231.

[100] Schreiber M, Jayakodi M, Stein N, *et al*. Plant pangenomes for crop improvement, biodiversity and evolution. Nature Reviews Genetics, 2024, 25(8): 563－577.

[101] Zhou Y, Zhang Z, Bao Z, *et al*. Graph pangenome captures missing heritability and empowers tomato breeding. Nature, 2022, 606(7914): 527－534.

[102] Guo J, Cao K, Deng C, *et al*. An integrated peach genome structural variation map uncovers genes associated with fruit traits. Genome Biology, 2020, 21(1): 258.

[103] Yang L, Yang Y, Huang L, *et al*. From single- to multi-omics: future research trends in medicinal plants. Briefings in Bioinformatics, 2023, 24(1): bbac485.

[104] Wang M, Wang Y, Li X, *et al*. Integration of metabolomics and transcriptomics reveals the regulation mechanism of the phenylpropanoid biosynthesis pathway in insect resistance traits in *Solanum habrochaites*. Horticulture Research, 2024, 11(2): uhad277.

[105] Kuang L, Yan T, Gao F, *et al*. Multi-omics analysis reveals differential molecular responses to cadmium toxicity in rice root tip and mature zone. Journal of Hazardous Materials, 2024, 462: 132758.

[106] Gao R, Feyissa B A, Croft M, *et al*. Gene editing by CRISPR/Cas9 in the obligatory outcrossing *Medicago sativa*. Planta, 2018, 247(4): 1043－1050.

[107] Zhao H, Zhao S, Cao Y, *et al*. Development of a single transcript CRISPR/Cas9 toolkit for efficient genome editing in autotetraploid alfalfa. The Crop Journal, 2024, 12(3): 788－795.