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浮萍的研究及应用

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摘要 水生漂浮植物浮萍隶属于天南星科, 共5个属, 36个种, 其中的芜萍属是世界上最小的开花植物. 浮萍个体微小(大小约0.5 mm~1.5 cm)、结构简单(仅由叶状体和假根组成)、无性繁殖快(约24 h翻一倍), 富含淀粉和蛋白, 对环境适应能力强, 尤其对氮磷、重金属和有机污染物具有较强的富集能力, 还广泛分布于世界各类淡水生境, 如池塘、湖泊、稻田等. 上述优势特征使浮萍在生物学、生物能源开发以及污染修复等方面的研究和应用取得了令人瞩目的进展. 目前, 近10个浮萍物种已经建立了高效的遗传转化体系, 且常用浮萍植物, 包括紫萍(*Spirodela polyrhzia*)、青萍(*Lemna minor*)、青萍(*Lemna gibba*)和芜萍(*Wolffia australiana*)已经完成全基因组测序, 为利用分子生物学和生物信息学技术手段开发和挖掘浮萍中有重要应用前景的功能基因以进行浮萍品质改良奠定了基础. 本文介绍了浮萍的起源、分类和进化, 及其在形态解剖学、生理学、遗传转化和组学方面的研究, 并详细阐述了浮萍在食品、饲料和生物能源开发, 以及污染修复中的应用进展, 最后对浮萍未来的研究方向和应用前景进行了展望, 为开展浮萍基础生物学研究和资源利用提供参考.

关键词 浮萍, 进化, 生理, 组学, 生物能源, 污染修复

水生漂浮植物浮萍(duckweeds)隶属于天南星科浮萍亚科(Lemnoideae), 包含5个属: 紫萍属(*Spirodela*)、少根紫萍属(*Landoltia*)、青萍属(*Lemna*)、扁无根萍属(*Wolffiella*)、芜萍属(*Wolffia*), 共36个种, 其中的芜萍属(*Wolffia*)是世界最小的开花植物[1](图1(a)). 浮萍广泛分布于世界各类淡水生境, 如湖面、池塘、稻田等; 结构简单, 由叶状体和假根(紫萍属、少根紫萍属、青萍属)或仅由叶状体(扁无根萍属、芜萍属)组成; 易于培养, 无性繁殖生长快(生物量约24 h翻一倍), 很早就被用作生物学研究的常用植物材料. 浮萍高淀粉和高蛋白含量也是良好的食品、饲料和生物质基质来源[2~5]; 另外, 浮萍也是毒理检测和生态修复中的常用水生植物类群(图2). 近年来, 越来越多浮萍组学数据的完善和遗传转化体系的建立、利用组学和分子生物学技术开展浮萍基础生物学研究, 以及挖掘浮萍中具有重要应用前景的功能基因进行浮萍品质改良等研究逐渐深入[5~8]. 本文对浮萍的分类和进化、形态解剖学、生理学、遗传转化、组学以及在生物能源、污染修复等方面的研究和应用进行综述.

1 浮萍的起源、分类和进化

1.1 浮萍的起源及分布

浮萍科起源于约五千万年前(从晚白垩世到中新世), 曾广泛分布于北美和欧亚大陆, 其中美洲地区的浮萍物种最为丰富. 浮萍5个属的起源和地理分布不同,

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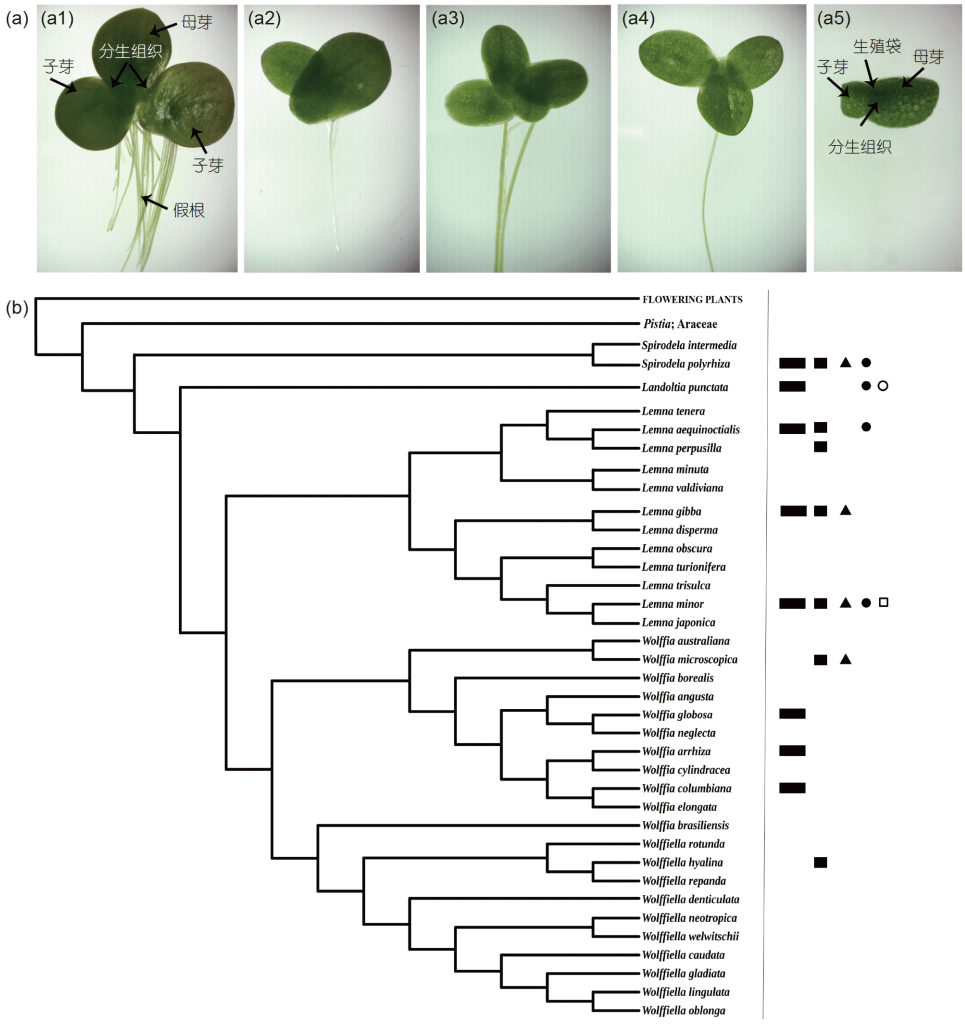

图 1 (网络版彩色)常见5个浮萍物种的外部形态(a)及浮萍科系统发育树(b). (a1) 紫背浮萍(*Spirodela polyrhiza*), 约5~11个根; (a2) 少根紫萍(*Landoltia punctata*), 仅1个根; (a3) 青萍(*Lemna minor*), 仅1个根; (a4) 稀脉浮萍(*Lemna aequinoctialis*), 仅1个根; (a5) 芜萍(*Wolffia globosa*), 无根. (b) 浮萍的进化、分类, 共5个属, 36个物种, 系统树构建使用叶绿体DNA(*mat*K/*trn*K+*rbc*L+*rpl*16)通过最大似然法构建, Bootstrap replication为1000. 实心长方形表示已经建立遗传转化体系的浮萍物种, 实心正方形表示已经实现开花诱导的浮萍物种, 实心三角形表示具有核基因组数据的浮萍物种, 实心圆表示具有转录组数据的浮萍物种, 空心圆表示具有蛋白质组数据的浮萍物种, 空心正方形表示具有代谢组数据的浮萍物种

Figure 1 (Color online) The morphology of five common duckweed species (a) and the phylogenetic tree of Lemnaceae (b). (a1) *Spirodela polyrhiza*, with 5−11 rhizoids; (a2) *Landoltia punctata*, with one rhizoid; (a3) *Lemna minor*, with one rhizoid; (a4) *Lemna aequinoctialis*, with one rhizoid; (a5) the rootless *Wolffia globosa*. (b) The evolution and classification of duckweeds, including 5 genera and 37 species. The maximum likelihood phylogenetic tree is conducted using 1000 replicates on data of published chloroplast DNA data for *mat*K/*trn*K, *rbc*L and *rpl*16. The solid rectangle indicates the duckweed species with established genetic transformation system, the solid square indicates the duckweed species that have achieved flowering induction, the solid triangle indicates the duckweed species with nuclear genome data, and the solid circular indicates the duckweed species with transcriptome data, the hollow circle indicates the duckweed species with proteome data, and the hollow square indicates the duckweed species with metabolomic data

其中青萍属起源于北美洲, 扁无根萍属起源于美洲和非洲[9], 芜萍属中仅*Wolffia angusta*和*Wolffia globosa*两个物种起源于亚洲或大洋洲, 其他芜萍物种可能起源于美洲和南美洲, 也可能独立分散于澳大利亚或东亚地区. 研究者通过大量的分子证据和化石校准点推测紫萍属分化出的时间是35.5 Ma, 少根紫萍属分化出的时间是56.8 Ma, 青萍属分化出的时间是54.4 Ma. 由于芜萍属和扁无根萍属系统发育关系的不确定性, 目前推测出的扁无根萍属的分化时间约为15.6 Ma, 芜萍属的分化时间约为8.0 Ma[1,10]. 紫萍属包含*Spirodela poly-*

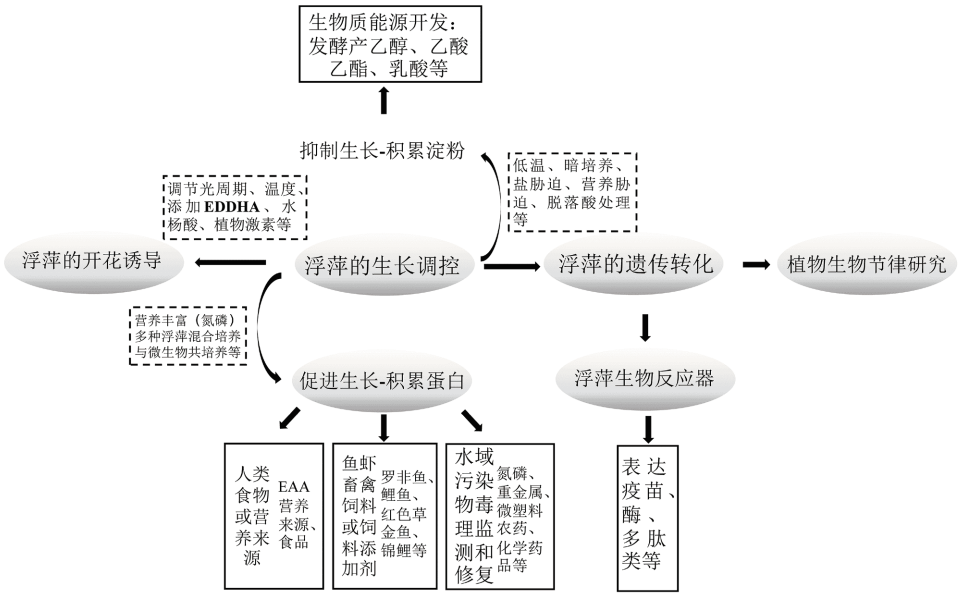

图 2 浮萍的生长调控及应用
Figure 2 The regulation of growth in duckweeds and their applications

*rhiza*和*Spirodela intermedia*两个物种, 其中*S. polyrhiza*广泛分布于热带、亚热带和温带地区, 部分高纬度地区和近北极圈的芬兰地区也有分布, 而*S. intermedia*仅分布在南美洲的热带地区. 少根紫萍属仅有*Landoltia punctata*一个物种, 广泛分布于热带和亚热带地区, 在非洲地区的分布尚不清楚. 青萍(*Lemna minor*)和青萍(*Lemna gibba*)两个物种的分布十分广泛, 包括亚热带和温带地区, 最南端到达新西兰(45°S), 北部到达芬兰、挪威(60°N)和加拿大萨斯卡通地区. 扁无根萍属只集中分布在南美洲、北美洲和非洲地区, 芜萍属在除南极洲之外的地区都有分布, 且扁无根萍和芜萍都成纬度连续分布.

1.2 浮萍的分类和进化

Hartog和Plas[11]及Landolt[9]在早期浮萍种质资源的收集、分类和命名工作中发挥了重要作用, 他们主要根据浮萍的形态学特征进行了物种鉴定和分类, 许多关键的浮萍物种被鉴定出. 也有学者根据代谢产物, 如黄酮类、酚类物质和花青素等对一些从形态学上较难区分的浮萍进行物种鉴定[12,13], 如青萍*Lemna valdiviana*和*Lemna minuta*[14,15]. 近年来, DNA多态性检测技术, 包括RAPD(random amplified polymorphic DNA)[16]、ISSR(inter-simple sequence repeat)[17]、SSR (simple sequence repeats)[18]、AFLP(amplified fragment length polymorphism)[19,20], 以及DNA条形码分子遗传标记(DNA barcoding)[1,21,22]被广泛应用到浮萍的分类和鉴定中. DNA条形码分子遗传标记具有扩增可靠性高、可直接进行序列比对、种间和种内多态性高的优势, 在浮萍分类和鉴定中具有较大的应用前景, 利用该方法至少能够鉴定和区分30多个浮萍物种[22]. 对于同一个浮萍物种的不同生态型, 由于DNA条形码序列也基本相同, 需要利用AFLP技术或AFLP与DNA条码相结合的方法进行鉴定[19,20]. 前期的研究普遍支持将浮萍划分为5个属, 37个种, 包括紫萍属2个物种、少根紫萍属1个物种、青萍属13个物种、扁无根萍属10个物种, 以及芜萍属11个物种, 其中紫萍是最古老、个体最大的浮萍物种, 芜萍是个体最小、最后进化出的浮萍物种[23]. 近期, Bog等人[24]综合定量形态测定法、代谢组学、AFLP以及叶绿分子标记鉴定出*Lemna yungensis*与*Lemna valdiviana*应为同一个物种, 至此浮萍科被认为由36个物种组成(图1(b)). 目前, 罗格斯大学(Rutgers University)和中国科学院成都生物研究所的浮萍资源库收集了全世界绝大多数的浮萍资源, 中国科学院植物研究所、中国科学院水生生物研究所、中国科学院青岛能源所以及海南大学也保存了一些浮萍资源.

浮萍科的分类地位目前还存在一些争议，系统发育分析和系统发育基因组学研究表明，早白垩世时期(104 Ma)天南星科中开始出现浮萍的最早分支[25]，化石分析和大量的形态学证据也支持浮萍植物与天南星科植物具有较近的亲缘关系[21,26~30]. 因此，研究者将浮萍科(Lemnaceae)划分为与天南星科并列且同属于天南星目(Arales), 而天南星目与包含众种水生植物, 如水鳖科、眼子菜科的泽泻目(Alismatales)亲缘关系很近[31]. 但Cabrera等人[32]、Cusimano等人[33]以及Nauheimer等人[25]综合AFLP和DNA条形码分析结果推测, 浮萍应是属于天南星科的一个亚科, 即浮萍亚科, 例如与浮萍形态差异较大的天南星科的麻菖蒲属(*Gymnostachys*)、金棒芋属(*Orontium*)以及臭菘属(*Symplocarpus*)植物都与浮萍亲缘关系较近. 随着越来越多浮萍基因组序列的公布，通过结合核基因组和质体基因组的分子证据, 浮萍系统发育分析中的不确定性将得到逐步解决.

2 浮萍的形态解剖学

Landolt[9]较全面地对浮萍的外部形态进行了研究, 揭示从紫萍属到芜萍属根器官逐渐退化至消失，扁平状的叶状体逐渐变小最后进化为半球状(图1(a)). Anderson等人[34]、White和Wise[35]分析了3种芜萍的显微和超微结构, 他们认为芜萍退化的程度可能被高估: 虽然芜萍个体较小、外部形态退化，但仍具有高等植物所有的细胞器；芜萍通过位于生殖袋基部的唯一分生组织快速繁殖后代，一个母芽体内通常能够包含6~7个处于不同发育阶段的子芽，正是这种快速无性繁殖方式使芜萍得以迅速积累生物量且广泛分布于世界各地; 另外，芜萍缺乏维管组织，细胞间隙大，细胞空泡化明显, 这些结构特征与其水生漂浮生活方式相适应, 而且芜萍(*Wolffia columbiana*)的成熟表皮细胞中形成了一种转移细胞(transfer cells)结构，这对于芜萍通过叶状体进行营养物质的运输和转移非常重要.

虽然浮萍根器官在结构和功能上都发生一定程度的退化，但是仍具有一定的吸收和运输功能. Echlin等人[36]发现青萍(*L. minor*)根尖区域极度活跃，大多数离子的吸收都发生在根尖区域，而且根尖的内皮层具有凯氏带, 推测青萍(*L. minor*)的假根不仅具有吸收功能, 还可能和叶状体之间进行物质运输. 紫萍(*S. polyrhiza*)根细胞之间含有大量的胞间连丝，假根与叶状体之间可能通过共质体途径来转运离子和代谢产物，且根尖细胞的细胞质密度大，皮层空泡化明显，形成类似“钟摆”式的结构应对水体波动，保证叶状体稳定漂浮[37]. Lemon和Posluszny[38]对紫萍(*S. polyrhiza*)、青萍(*L. minor*)、芜萍(*Wolffia borealis*)的发育过程进行了详细观察，发现3种浮萍个体发育过程中并未出现具有明显分区的顶端分生组织, 紫萍(*S. polyrhiza*)和青萍(*L. minor*)具有发育出假根和叶状体的2个分生组织区(meristem area, MA), 但芜萍(*W. borealis*)只有一个分生组织区(称为“生长点”)，这与Anderson等人[34]、White和Wise[35]以及仇雪珍[39]的研究结果一致. 虽然研究者在浮萍根器官的结构和功能研究方面取得了一些成果, 并对其结构与水生漂浮生活的适应性机制有一定程度的了解，但这些研究仍处于形态和生理层面. 目前，只有An等人[40]通过挖掘根发育相关基因和根系营养元素吸收能力研究初步揭示, 由于紫萍(*S. polyrhiza*)不需要强烈依赖根系吸收营养从而导致浮萍根结构和功能的退化，但相关根发育相关的基因在调控浮萍根器官退化中的作用和分子机制还需要深入研究.

浮萍能够形成休眠体以度过不良环境，休眠体中积累了大量积累淀粉为休眠期提供充足的营养，同时休眠体能够沉入水底以度过不良环境. 黄青[41]通过石蜡切片观察到芜萍(*W. globosa*)休眠个体中充满了淀粉粒，细胞个体小且紧密排列，几乎没有细胞间隙. 紫萍(*S. polyrhiza*)叶状体进入休眠时也发生气室数目减少和淀粉积累[42]. 特定条件下浮萍会进入有性生殖, 但有关野外条件下浮萍开花的报道很少，目前也没有关于浮萍种子的报道. 孟海奇[43]报道在武汉植物园采集到开花的芜萍(*W. globosa*)个体，进行有性生殖的芜萍仅一个雄蕊和雌蕊, 从叶状体背部发育形成, 无花瓣、花萼等结构，而且芜萍可同时进行有性生殖和无性繁殖, 但有性生殖和无性繁殖是否由一个生长点发育形成还需要研究.

3 浮萍的生理学

3.1 浮萍的生长调控

自然界中，浮萍几乎以无性繁殖的方式繁殖后代, 约1.34~4.54 d生物量可翻一倍[44]. 浮萍的生长受各种环境因子，包括营养盐、pH、温度、光照和CO_2浓度等影响. 例如，青萍(*L. minor*)能够生长的氨氮浓度为7~84 mg/L, 最佳氨氮浓度为28 mg/L, 氨氮浓度过高会抑制其生长[45]. 同一营养背景下, 青萍(*L. minor*)和少根紫萍(*L. punctata*)的生长随磷浓度的升高先增加后减

少, 且青萍(*L. minor*)的生长速度快于紫萍(*S. polyrhiza*)和少根紫萍(*L. punctata*), 表明青萍(*L. minor*)对环境的适应能力强于其他两种浮萍[46]. 浮萍可生长的pH范围是4.0~10.0, 当生长环境的pH偏离最佳值时, 浮萍的生长会迅速下降[47]. 额外施加植物激素也能明显影响浮萍的生长, 例如, 低温和脱落酸结合处理能够诱导紫萍(*S. polyrhiza*)产生休眠芽[48,49]; 乙烯合成前体(1-氨基环丙烷羧酸)和茉莉酸对浮萍的生长起一定的促进作用, 但乙烯合成抑制剂(氨氧乙基乙烯基甘氨酸)和脱落酸则明显抑制浮萍的生长[50].

浮萍的生长还受到周围环境中微生物的影响, Toyama等人[51]从稀脉浮萍(*Lemna aequinoctialis*)上分离到一种醋酸钙不动杆菌(*Acinetobacter calcoaceticus* P23), 当它与浮萍共培养时能够分别将稀脉浮萍(*L. aequinoctialis*)、青萍(*L. minor*)和紫萍(*S. polyrhiza*)的生物量提高3.8~4.3、2.3~3.3和1.4~1.5倍. 日本北海道大学(Hokkaido University)Masaaid Morikawa实验室从青萍(*L. minor*)上分离出短小芽孢杆菌(*Bacillus pumilus* MRB-10)、不动杆菌(*Acinetobacter calcaaceticus* P23)和黄褐假单胞菌(*Pseudomonas fulva* Ps6), 当它们与青萍(*L. minor*)共培养时能够将其生长提高2.5~3倍. 另外, 他们还从共生的短小芽孢杆菌(*Bacillus pumilus* MRB-10)和不动杆菌(*Acinetobacter calcaaceticus* P23)中提取到成分分别为蛋白(50 kD)和胞外多糖的新型植物生长调节剂(plant growth factors, PGFs). 当在浮萍培养中添加浓度为50 μg/mL的PGFs时, 浮萍的叶状体增殖率提高了近40%. 目前相关研究成果已经在日本申请专利保护(专利号JP2016069474A和WO2017002929A1).

3.2 浮萍的开花诱导

虽然自然条件下浮萍较少进入有性生殖, 但目前已经能够实现人工诱导浮萍开花(表1)[52~72]. 在早期的研究中, 研究者发现施加EDTA(ethylene diamine tetra-acetic acid)、玉米素、Fe^{2+}、Fe-EDDHA(Fe ethylene-diamine-N,N′-*bis*(2-hydroxyphenylacetic acid))、8-羟基喹啉等物质能够诱导浮萍开花[53,70~75]. 近期, 黄猛等人[60]发现培养基的状态, 即分别使用固体培养和液体培养也能够影响浮萍的开花, 且固体培养基比液体培养基更有利于诱导浮萍开花; 他们分别使用固体和液体培养基同时添加20 umol/L的水杨酸都能够诱导青萍(*L. gibba*)开花, 但使用固体培养条件下的开花率(45%)高于液体培养条件下的开花率(39.37%). Fu等人[61]利用改良霍格兰培养基诱导了青萍(*L. gibba* 7741)和青萍(*L. gibba* 5504)开花, 额外添加20 μmol/L水杨酸时, 两种青萍的开花率分别提高到73.5%和54.1%, 且单独使用固体培养基培养就能够诱导青萍(*L. gibba* 5504)开花, 但他们未能成功诱导青萍(*L. minor* 7210)开花. 另外, 他们发现青萍(*L. gibba* 5504)为雄性不育系. 2019年9月在以色列举办的第5届国际浮萍大会上, 日本东京大学Tokitaka Oyama实验室报道, 持续光照、短日照(9/15 h)和长日照(15/9 h)条件下添加5 μmol/L的水杨酸都能诱导扁无根萍(*Wolffiella hyaline* 9525)开花, 而扁无根萍(*W. hyaline* 7378)只在短日照条件下添加5 μmol/L水杨酸处理时开花; 另外, 长日照条件下氮胁迫和水杨酸处理都能够诱导青萍(*L. minor* 5512)开花. 罗格斯大学(Rutgers University)Joachim Messing实验室报道, 添加75 μmol/L的EDDHA且每周更换一次新鲜培养基能够诱导芜萍(*W. microscopica*)开花, 开花诱导率为29%; 另外, 温和振荡培养青萍(*L.gibba* G3)时开花率可达到100%, 还能够产生种子, 且在培养第3天就有70%的个体产生种子.

3.3 浮萍是植物生物节律研究的新模型

浮萍个体微小、生长快速和叶状体浮水生长的优势, 便于在有限的环境中进行培养和生长监测; 另外, 高效的遗传转化(基因枪和农杆菌介导)体系, 以及成熟浮萍个体的叶状体维管组织稳定而便于长时间监测的特性, 使浮萍成为植物生物节律研究的新模型. Miwa等人[76]首次构建拟南芥生物钟核心振荡器关键因子*CCA1*(*Circadian Clock Associated 1*)基因启动子融合荧光素酶的植物表达载体, 并瞬时转化长日照浮萍植物-青萍(*L. gibba* G3), 持续光照条件下4 d内都能够观察到青萍(*L. gibba* G3)*CCA1*基因出现清晰的近24 h昼夜节律, 与拟南芥一致; 而短日照浮萍植物-青萍(*Lemna paucicostata* 6746)的生物节律稳定性虽然与青萍(*L. gibba* G3)存在一定的差异, 但整体的生物节律特征仍然保守; 青萍中*GI*(*GIGANTEA*)、*ELF3*(*EARLY FLOWERING 3*)、*ELF4*(*EARLY FLOWERING 3*)和*PRRs*(*Pseudo-Response Regulators*)同源基因在特定的光周期条件下也表现出与拟南芥类似的生物节律特征, 但*LHY/CCA1*基因数目和表达在浮萍、水稻和拟南芥中存在一定的差异, 这表明植物生物节律的核心元件不仅在不同浮萍物种之间是保守的, 而且在单子叶植物与双子叶植物之间也存在保守型. Miwa等人[76]的研究

表 1　浮萍的开花诱导[a)]

Table 1　The induction of flowering in duckweeds

浮萍物种	关键诱导条件	开花率	文献
Lemna gibba G3	持续光照培养至少3 d	76%	[52]
Lemna gibba G3	14/10 h光周期条件培养17 d	22%	[53]
Lemna gibba G3	3.2 μmol/L水杨酸+2 μmol/L $K_3Fe(CN)_6$, 8/16 h光周期条件培养8 d后持续光照培养3 d	32.8%	[54]
Lemna gibba G3	3.2 μmol/L水杨酸, 持续光照培养	超过80%	[55]
Lemna gibba G3	半强度Hunter's培养基(缺NH_4^+), 持续光照培养4~11 d	超过80%	[56]
Lemna gibba G3	1 μmol/L苯并(1,2,3)噻唑-7-硫代甲酸甲酯	超过70%	[57]
Lemna gibba G3	热处理的去甲肾上腺素+0.1 mg fw/mL茄子水提取物	约20%	[58]
Lemna gibba G3	温和振荡培养	100%	Joachim Messing, Rutgers University
Lemna gibba 7007	14/10 h, 处理17 d	59%	[53]
Lemna gibba (Obrov)	20.25 μmol/L EDDHA, 16/8 h光周期培养	40%	[59]
Lemna gibba	固体培养基培养, 添加20 μmol/L的水杨酸	45%	[60]
Lemna gibba 5504	改良霍格兰培养基(含80 mmol/L NH_4NO_3), 20 μmol/L水杨酸	54.1%	[61]
Lemna gibba 7741	改良霍格兰培养基(含80 mmol/L NH_4NO_3), 20 μmol/L水杨酸	73.5%	[61]
Lemna perpusilla	25 mL 1/10强度的Hunter's 培养基(不添加蔗糖), 8/16 h光周期培养	57.4%	[62]
Lemna perpusilla 6746	23°C, (13)0.25 (10.5)0.25 h光周期培养	超过40%	[63]
Lemna paucicostata 6746	3.2 μmol/L水杨酸, 10/14 h, 27°C条件下培养	超过60%	[55]
Lemna paucicostat 151	3 μmol/L苯并(1,2,3)噻唑-7-硫代甲酸甲酯	超过70%	[57]
Lemna paucicostata 6746 (synonym Lemna aequinoctialis)	热处理的去甲肾上腺素+0.1 mg fw/mL茄子水提取物	约30%	[58]
Lemna paucicostata 441	热处理的去甲肾上腺素+0.1 mg fw/mL茄子水提取物	超过45%	[58]
Lemna paucicostat 151	热处理的去甲肾上腺素+0.1 mg fw/mL茄子水提取物	约50%	[58]
Lemna paucicostat 151	30 μmol/L热/碱处理去甲肾上腺素	超过60%	[64]
Lemna paucicostat LP6	pH 4.4, 3.2 μmol/L苯甲酸+0.32 μmol/L 6-苄基氨基嘌培养基pH 4.4, 16/8 h光周期	93.4%	[65]
Lemna paucicostat 6746	10^{-4}儿茶酚胺, 8/16 h光周期	超过70%	[66]
Lemna paucicostat 6746	3×10^{-5} mol/L水杨酸, 自来水培养, 16/8 h光周期	42%	[67]
Lemna minor 6573	14/10 h光周期条件下培养26 d	33%	[53]
Lemna minor (Dokleovje)	20.25 μmol/L EDDHA, 16/8 h光周期	44%	[59]
Lemna minor (Barje)	20.5 μmol/L EDDHA + 47.5 nmol/L茉莉酸, 16/8 h光周期	62.2%	[68]
Lemna minor 5512	氮胁迫(KNO_3浓度低于5 mmol/L), 持续光照条件培养	–	Tokitaka Oyama, Kyoto University
Spirodela polyrhiza (Zvirce)	20.25 μmol/L EDDHA, 16/8 h光周期	28%	[59]
Spirodela polyrhiza (Dobrnic)	20.25 μmol/L EDDHA, 16/8 h光周期	30%	[59]
Spirodela polyrhiza	3×10^{-5} G/Ml赤霉素, 持续光照培养27 d	13.3%	[69]
Wolffia microscopica	100 μg/L 玉米素, 20/4 h光周期	63%	[70]
Wolffia microscopica	玉米素+10 mg/L Fe-EDDHA	92%	[71]
Wolffia microscopica	10^{-6} mol/L 8-羟基喹啉, 16/8 h光周期	75%	[72]
Wolffia microscopica	75 μmol/L的EDDHA, 每周更换一次新鲜培养基	29%	罗格斯大学(Rutgers University)Joachim Messing实验室
Wolffiella hyaline 7378	5 μmol/L水杨酸, 短日照	–	日本东京大学Tokitaka Oyama实验室

a) "–"表示文中未有相关数据报道

为利用浮萍开展植物生物节律研究奠定了基础.

之后, 日本东京大学Tokitaka Oyama实验室利用浮萍作为植物生物节律研究模型, 开展了一系列的研究工作. Serikawa等人[77]通过反向遗传学技术验证了青萍(*L. gibba*)*LgLHYH1*、*LgLHYH2*、*LgGIH1*和*LgELF3H1*基因的功能保守性. 他们发现, 过表达这4个基因都能影响其中一个或者多个基因的生物节律; 抑制表达时除*LgLHYH2*外都能够影响所有基因的节律性, 且*LgGIH1*受抑制时几乎终止青萍(*L. gibba*)的生物节律, 这种影响甚至比拟南芥*LHYH2*功能缺失更加强烈. 为了研究和比较不同浮萍物种之间生物节律的差异性, Muranaka等人[78]对5种浮萍, 包括紫萍(*S. polyrhiza*)、少根紫萍(*L. punctata*)、青萍(*L. gibba*)、稀脉浮萍(*L. aequinoctialis*)、芜萍(*W. columbiana*)9个株系的生物节律进行了观察, 分别将拟南芥*AtCCA1*启动子、玉米*ZmUBQ1*或*CaMV35S*启动子融合荧光素酶构建的植物表达载体转入9个株系的浮萍中, 12/12 h光周期条件下9个浮萍株系中*AtCCA1::luc*$^+$都表现出与拟南芥相似的生物节律, 但9个浮萍株系中*ZmUBQ1::luc*$^+$都未出现清晰的昼夜节律; 持续光照条件下, 不同浮萍株系中*AtCCA1::luc*$^+$、*ZmUBQ1::luc*$^+$和*CaMV35S::luc*$^+$的昼夜节律都发生了变化, 且不同株系之间的生物节律特性也存在差异. 例如, 青萍(*L. gibba* G3)和青萍(*L. gibba* p8L)的*AtCCA1::luc*$^+$昼夜节律特征保持一致, 但稀脉浮萍(*L. aequinoctialis* 6746)和稀脉浮萍(*L. aequinoctialis* Nd)两个株系的*AtCCA1::luc*$^+$和*ZmUBQ1::luc*$^+$生物节律出现差异, 表现为稀脉浮萍(*L. aequinoctialis* 6746)的*AtCCA1::luc*$^+$昼夜节律严重衰减且只有一个最高峰, 但稀脉浮萍(*L. aequinoctialis* Nd)的*AtCCA1::luc*$^+$昼夜节律呈逐渐衰减的趋势且有5个最高峰. 上述研究表明不同浮萍物种甚至同一物种的不同株系生物钟机制的多样化.

基于浮萍便于观察和监测的优势, Muranaka等人[79]利用青萍(*L. gibba*)作为研究模型开发了单细胞生物发光成像系统, 该系统通过基因枪转化青萍(*L. gibba*)叶状体(主要是上表皮细胞和叶肉细胞)从而表达荧光素酶基因或荧光蛋白(green fluorescent protein, GFP)基因, 再通过冷却电子倍增电荷耦合器件(EM-CCD)相机检测叶状体上的发光点, 实现完整植株中单个细胞的生物节律长时程监测. 利用单细胞生物发光成像系统, Muranaka和Oyama[80]发现持续光照条件下, 完整植株中的细胞作为单独的生物钟振荡器独立响应外部环境, 甚至相邻细胞之间会出现生物节律的不同步性; 当光周期调整为黑暗交替时, 持续光照引起的细胞生物节律不同步性会得到修正, 以便与植物的整体生物节律保持一致性. 该项研究将植物生物节律的研究和监测推入了细胞水平. 之后, Isoda和Oyama[81]进一步利用扁平、无根的浮萍-扁无根萍(*Wolffiella hyalina*)作为植物材料, 实现了单细胞水平的整体植株生物节律监测.

4 浮萍的遗传转化

目前, 有近10个浮萍物种的遗传转化体系已经建立(表2)[8,82~99]. 浮萍的遗传转化效率主要受外植体材料、农杆菌种类和菌液浓度、乙酰丁香酮浓度、共培养培养基pH、共培养时间等条件的影响, 其中青萍(*L. minor*)和青萍(*L. gibba*)遗传转化方法最为成熟. Yamamoto等人[82]首次建立了农杆菌介导(C58-Z707)的青萍(*L. minor*)和青萍(*L. gibba*)的遗传转化体系, 他们使用未完全脱分化成愈伤组织的一种组织(报道为"Nodules")作为侵染材料, 并在农杆菌菌液和共培养培养基中添加100 μmol/L的乙酰丁香酮(acetosyringone, AS)来提高农杆菌的侵染活性. 基于Yamamoto等人[82]的方法, Cox等人[83]、Sun等人[84]和Nguyen等人[85]分别在青萍(*L. minor*)中表达了人单克隆抗体(mAbs)、嗜热内切葡聚糖酶E1和禽流感病毒血凝素(*Haemagglutinin*, *HA*)基因. Chhabra等人[86]在青萍(*L. minor*)遗传转化过程中将共培养培养基的pH调整为5.2, 共培养时间缩短到3 d, 并添加0.2%的非离子表面活性剂(吐温20)促进农杆菌的侵染, 将青萍(*L. minor*)的遗传转化效率提高到3.8%. Cantó-Pastor等人[87]和Firsov等人[88]进一步改进青萍(*L. minor*)遗传转化方法, 研究中均使用愈伤组织作为侵染材料, 但Firsov 等人[88]并未使用乙酰丁香酮, 而Cantó-Pastor等人[87]添加200 μmol/L的乙酰丁香酮用于活化菌液, 并将选择培养和再生培养同时进行, 使整个遗传转化周期从6~7周缩短到5周, 获得了59%的转化效率. Gasdaska等人[89]专门开发了一套浮萍表达系统(Lemna Expression System或者"LEX SystemSM")用于表达和生产再生蛋白, Bertran等人[90]利用该系统成功表达了H5血凝素疫苗抗原.

Rival等人[91]和Vunsh等人[92]也利用愈伤组织作为侵染材料建立了少根紫萍(*L. punctata*)的遗传转化方法. Rival等人[91]首先利用基因枪轰击愈伤组织后再将其与农杆菌共培养, 遗传转化效率超过25%. Yang等人[6]建立了农杆菌介导的紫萍(*S. polyrhzia*)稳定遗传转

表 2　浮萍的遗传转化

Table 2　The genetic transformation of duckweeds

浮萍物种	稳定/瞬时转化	转化方法	侵染材料	外源基因	文献
Spirodela polyrhzia	稳定	根癌农杆菌*Agrobacterium tumefaciens* (LBA4404)	Callus	Auxin reporter gene, cytokinin reporter gene	[6]
Lemna aequinoctialis	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Frond	Phytoene desaturase gene (*LaPDS*)	[8]
Lemna gibba	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (C58-Z707)	Nodules	*uidA* gene	[82]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (C58-Z707)	Nodules	*uidA* gene	[82]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Frond	Cytochrome P450 710A11	[8]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (C58-z707)	Nodules	Human monoclonal antibodies (mAbs) MDX-060 synthetic gene	[83]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (C58-z707)	Nodules	E1 endoglucanase gene	[84]
Lemna minor	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (C58-z707)	Nodules	Avian influenza hemagglutinin *HA* gene	[85]
	稳定 瞬时	根癌农杆菌*Agrobacterium tumefaciens* (EHA105)	Nodules	*uidA* gene	[86]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (GV3101)	Callus	*gfp* gene	[87]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (CBE21)	Callus	M130-β-glucuronidase gene	[88]
	稳定	Lemna Expression System (LEX SystemTM, Biolex Therapeutics, Pittsboro, NC)		Hemagglutinin (*HA*) gene	[89,90]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Frond	Porcine epidemic diarrhea virus (PEDV) spike protein 1 gene	[96]
	稳定	根癌农杆菌*Agrobacterium tumefaciens* (EHA105)	Frond	*The sterol 22-desaturase gene* (*CPY710A11*)	[98]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Callus	Glyoxylate aminotransferase (SGAT) *AtAGT1*	[99]
Spirodela oligorrhiza	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Callus	Aprotinin synthetic gene	[91]
	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Callus	*gfp* gene	[92]
Spirodela punctata	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)		Antitnfα-scFv (anti-tumor necrosis factor alpha single-chain variable fragment) gene	[97]
Wolffia arrhiza	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Cluster	*uidA* gene	[93]
Wolffia globosa	稳定	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105)	Cluster	Cytokinin reporter gene	[94]
	瞬时	根癌农杆菌 *Agrobacterium tumefaciens* (EHA105、LBA4404)	Frond	Auxin reporter gene, cytokinin reporter gene	[94]
	瞬时	基因枪	Frond	*gfp* gene	[93,95]
Wolffia columbiana	瞬时	根癌农杆菌 *Agrobacterium tumefaciens* (LBA4404)	Frond	*uidA* gene	
	瞬时	基因枪	Frond	*uidA* gene, reporter gene	[95]

化体系, 该研究以紫萍(*S. polyrhzia*)愈伤组织为侵染材料, 最高遗传转化效率达13%. Khvatkov等人[93]和Heenatigala等人[94]分别建立了芜萍(*Wolffia arrhiza*)和芜萍(*W. globosa*)的遗传转化方法, 使用未完全脱分化为愈伤组织的一种组织(报道为"Cluster")作为侵染材料, 但由于获得"Cluster"的周期至少需要4个月, 因此芜萍的稳定遗传转化周期较长, 转化效率也较低. Boehm等人[95]还建立了芜萍(*W. columbiana*)瞬时转化体系(基因枪介导), 认为基因枪转化芜萍的遗传转化效率比农杆菌介导法要高, 但最高的转化效率也只有3.9%. 由于浮萍遗传转化效率和转化周期基本依赖于高效的组织培养体系, Ko等人[96]和Balaji等人[97]尝试直接使用叶状体作为侵染材料, 分别获得了青萍(*L. minor*)和少根紫萍(*L. punctata*)的转化植株. 另外, Ko等人[96]对叶状体进行受伤处理来促进农杆菌的侵染, 而Balaji等人[97]直接使用完整叶状体作为侵染材料就达到95%的转化率, 而且他们发现经受伤处理的叶状体在选择培养过程中几乎全部死亡. Yang等人[98]进一步建立了成熟、高效的浮萍叶状体转化法, 通过将叶状体的子芽从母芽中剥离从而暴露出母芽的分生组织, 达到促进农杆菌菌液侵染分生组织的目的. Liu等人[8]通过叶状体转化首次建立了CRISPR/Cas9介导的稀脉浮萍(*L. aequinoctialis*)基因编辑实验体系, 整个转化周期为5~6周, 转化率高达94%. 稳定、高效的遗传转化体系为开展浮萍植物的基因功能研究, 以及利用植物生物技术进行浮萍品质改良奠定了基础, 而叶状体转化法不仅能够避免不同生态型对转化效率的影响, 还大大缩短了遗传转化周期, 未来在浮萍的研究中具有较大的应用前景.

5 浮萍的组学

5.1 浮萍基因组

基因组最小(约158 Mbp)的紫萍(*S. polyrhiza*)是第一个完成基因组测序的浮萍物种, 基因组组装也最为完整(表3)[100~117]. Wang等人[100]利用二代测序平台对紫萍(*S. polyrhiza* 7498)进行了全基因组测序, 由于二代测序读长短, 本次测序产生了10%的缺失, 大部分的缺失可能由重复序列导致. 为比较不同生态型浮萍基因组的差异, Michael等人[101]对另一个生态型的紫萍(*S. polyrhiza* 9509)进行了基因组测序, 组装到染色体水平的scaffold N50提高到7.6 Mb. 两种生态型的紫萍(*S. polyrhiza*)在编码基因数目、结构变异、基因组甲基化程度等都存在一定的差异, 例如编码基因数目相差近1000个, 存在96个高度可信的结构变异. 研究还发现, 紫萍(*S. polyrhiza* 7498)中与木质素合成相关基因的拷贝数仅70个, 远低于水稻和高粱中的141和156个; 调控氮吸收的谷氨酸合酶基因的拷贝数比拟南芥和水稻多4倍; 对开花和个体成熟起关键负调控作用的*miRNA159*的拷贝数也显著增多, 这些都与紫萍退化的维管组织、氮磷吸收能力强以及浮水生活方式相适应. An等人[40]利用三代PacBio测序平台对紫萍(*S. polyrhiza* 7498)进行了基因组重测序, 与前期版本相比, 连续性提高了44倍, 填补了前期版本中的95.4%的序列丢失区域; 重测序的基因注释率为74.6%, 共注释出18708个编码基因, 平均基因长度增加了24.5%.

青萍(*L. minor*)和青萍(*L. gibba*)分布极为广泛, 它们也是生物学研究中的常用实验材料, 研究者首先对这两种青萍进行了基因组测序. Van-Hoeck等人[102]对青萍(*L. minor* 5500, 约481 Mbp)进行了基因组测序, 共注释出22382个编码基因, 平均基因长度为2738 bp, 重复序列占比较高, 为62%(紫萍为17%). 另外, 青萍(*L. minor*)基因组中与环境适应、生物量积累、非生物胁迫响应相关的基因具有较高的丰度. 例如, 编码谷氨酰胺合成酶(GSs)和谷氨酸合成酶(GOGATs)的基因分别扩大到12和21个, 高于紫萍(*S. polyrhiza*, 7498)中的7和11个, 表明青萍(*L. minor*)在氮素吸收和生物量积累方面比紫萍(*S. polyrhiza*)更有优势. 冷泉港实验室(The Cold Spring Harbor Laboratory, CSHL)的Rob Martienssen实验室对青萍(*L. minor* 8627, 约800 Mb)、青萍(*L. gibba* 7742a, 约450 Mb)和芜萍(*Wolffia australiana* 8730)也进行了基因组测序, 已有的基因组数据公布在https://www. Lemna.org网站上.

5.2 浮萍转录组

浮萍的转录组数据也较为丰富, 调控其生长发育、淀粉和蛋白合成以及与抗逆相关的许多关键基因被挖掘. 水杨酸处理能够诱导紫萍(*S. polyrhiza*)休眠, 且在休眠过程中与碳水化合物合成、种子脱水以及次级代谢等相关基因显著表达[103]. 烯效唑处理能够调节少根紫萍(*L. punctata*)中内源激素的水平, 从而影响淀粉代谢相关基因和叶绿素生物合成关键酶的表达, 使少根紫萍(*L. punctata*)大量积累淀粉. 转录组研究发现, 烯效唑处理主要是引起内源性激素-脱落酸和细胞分裂素水平的升高, 从而促进ADP葡萄糖(ADP-glucose pyro-

表 3　浮萍组学数据汇总[a)]

Table 3　The genome and transcriptome data of duckweeds

	浮萍物种	株系	胁迫处理	文献
核基因组	紫萍(*S. polyrhiza*)	7498		[100]
	紫萍(*S. polyrhiza*)	9509		[101]
	紫萍(*S. polyrhiza*)	7498		[40]
	青萍(*L. minor*)	5500		[102]
	青萍(*L. minor*)	8627		Rob Martienssen
	青萍(*L. gibba*)	7742a		Rob Martienssen
	芜萍(*W. australiana*)	8730		Rob Martienssen
转录组	紫萍(*S. polyrhiza*)	7498	水杨酸处理	[103]
	少根紫萍(*L. punctata*)	0202	烯效唑处理	[104,105]
	少根紫萍(*L. punctata*)	0202	低氮胁迫	[106]
	少根紫萍(*L. punctata*)	0202	低氮胁迫	[107]
	稀脉浮萍(*L. aequinoctialis*)	6000	低氮胁迫	[108]
	紫萍(*S. polyrhiza*)	7498	盐胁迫	[109]
	青萍(*L. minor*)	–	高氨氮胁迫	[110]
	青萍(*L. minor*)	5500	辐射胁迫	[111]
	少根紫萍(*L. punctata*)	6001	重金属镉胁迫	[112]
蛋白质组	少根紫萍(*L. punctata*)	0202	营养胁迫	[113]
	少根紫萍(*L. punctata*)	0202	烯效唑处理	[114]
	青萍(*L. minor*)	–	铝胁迫	[115]
代谢组	青萍(*L. minor*)	–	钴胁迫	[116]
	青萍(*L. minor*)		除草剂处理	[117]

a) “–”表示文中未有相关数据报道

phosphorylase，AGPase))的活性和叶绿素水平上升，同时赤霉素含量降低使α-淀粉酶失活[104,105]．低氮胁迫能够诱导少根紫萍(*L. punctata*)中参与淀粉和类黄酮生物合成关键酶的基因表达上调，而参与光合作用的酶和木质化速率限制酶的表达却下调[106,107]．低氮胁迫也能诱导稀脉浮萍(*L. aequinoctialis*)中碳水化合物生物合成的基因表达的变化，同时硝酸还原酶、谷氨酰胺合成酶和谷氨酸合成酶基因表达下调；另外，低氮胁迫下植物腺苷二磷酸葡萄糖焦磷酸化酶(AGPase)随着淀粉的积累其含量也相应增加，表明低氮条件下稀脉浮萍中的淀粉合成是糖异生和糖异生途径累积的结果[108]．盐胁迫下紫萍(*S. polyrhzia*)大量吸收钠离子，降低对钾离子和钙离子的吸收，同时鲜重、Rubisco和AGPase酶活性及淀粉含量在胁迫第1天显著降低；盐胁迫下紫萍(*S. polyrhzia*)中与乙烯代谢、脂质降解、氮代谢、类黄酮代谢、非生物胁迫相关的基因显著增加，而与细胞壁、ATP合成的线粒体电子传递、光合作用光反应、生长素代谢等相关的基因受到显著抑制；盐胁迫还显著影响一些参与非生物胁迫和细胞分化的转录因子的表达．这些结果表明，盐胁迫下细胞中的离子稳态受到强烈影响[109]．此外，高氨氮胁迫、重金属处理以及辐射胁迫(γ和β辐射)都能诱导浮萍中与DNA修复、ROS代谢以及抗氧化通路的基因差异表达[110~112]．

5.3　浮萍蛋白质组和代谢组

Huang等人[113]利用蛋白组学分析发现，营养胁迫下少根紫萍(*L. punctata* 0202)中参与淀粉生物合成的酶表达上调，参与淀粉降解的酶表达差异不显著；另外，参与类黄酮合成的关键酶表达上调，木质素生物合成相关酶的表达丰度较低，表明营养胁迫下酶表达和代谢通量的变化调控导致淀粉积累和木质素降低，这与转录组、酶定量和定性分析结果一致．蛋白质组学的研究结果有助于了解浮萍高淀粉积累和低木质素含量的分子机制，促进浮萍作为一种生物能源作物的发

展. Huang等人[114]还利用蛋白质组学研究了烯效唑处理诱导少根紫萍(*L. punctata* 0202)积累淀粉的机制, 他们发现烯效唑处理下大多数参与脱落酸(ABA)生物合成的酶表达上调, 内源性ABA含量增加进而上调ADP葡萄糖焦磷酸化酶的表达, 最终促进淀粉的积累. 他们的研究揭示了激素调控淀粉代谢响应胁迫的途径. Su等人[115]结合蛋白质组学和形态学、生理学三方面评价了青萍(*L. minor*)应对铝胁迫的响应, 铝胁迫下青萍(*L. minor*)中与柠檬酸循环和氨基酸代谢有关的蛋白质表达上调, 而与能量代谢、乙醛酸和二羧酸代谢相关的蛋白质表达下调; 另外, 抗氧化酶在青萍(*L. minor*)应对铝胁迫的响应中起重要作用, 该研究提供了一个较全面的青萍(*L. minor*)耐受铝胁迫的生理和分子机制. 青萍(*L. minor*)能够超量富集钴, 但青萍(*L. minor*)富集和耐受钴的生理和代谢机制尚不清楚. Hu等人[116]利用代谢组学分析发现, 钴胁迫下特定氨基酸和有机酸含量显著增加, 柠檬酸含量增加了约15倍; 柠檬酸合成酶、苹果酸脱氢酶和磷酸烯醇丙酮酸羧化酶活性增加, 与有机酸代谢相关的异柠檬酸脱氢酶活性降低, 表明青萍(*L. minor*)能够通过积累有机物、氨基酸和抗氧化物质来抵抗钴胁迫. 此外, 在利用浮萍进行毒性评估时, 特定浓度的毒性物质并不一定能够对浮萍造成肉眼可见的毒性症状, 限制了利用浮萍进行毒理监测和评估, 而利用核磁共振(^{1}H NMR)能够灵敏地检测毒性胁迫下青萍(*L. minor*)特征性的代谢变化, 代谢组学的应用为开展浮萍毒理学研究提供了一种便捷的途径[117].

6 浮萍在食品/饲料开发和生物能源中的应用

6.1 浮萍应用于食品、饲料开发

浮萍是一类安全且营养丰富的水生植物, 富含淀粉、蛋白、维生素等营养物[118], 在食品、饲料和生物能源方面具有巨大的应用潜力. 调节浮萍的生长环境能够改变浮萍中营养物质(主要是淀粉和蛋白)的含量, 例如, 生长环境营养匮乏时浮萍中的蛋白含量约9%~20%, 营养丰富时可达24%~41%[119]. 调节浮萍生长环境的pH、光周期、温度、营养、激素处理、盐胁迫以及不同浮萍物种混合培养都能影响浮萍淀粉含量[112,120~124]. 例如, 物激素脱落酸处理14 d后, 少根紫萍(*L. punctata*)的淀粉含量从2.29%增加到46.18%[121]. 在培养温度为5°C条件下, 紫萍(*S. polyrhiza*)中的淀粉含量比在25°C高114%, 且所有温度条件下紫萍(*S. polyrhiza*)中的淀粉含量都随光照时间的延长而增加[123]. 将氯化钠的浓度从10 mmol/L增加到30 mmol/L, 紫萍(*S. polyrhiza*)中的淀粉含量从13.4%增加到18.7%[124]. 不同生态型的浮萍生物量积累速率和营养物质含量差异也较大. Ma等人[5]报道了20个生态型的稀脉浮萍(*L. aequinoctialis*)生物量、淀粉含量和淀粉产量差异较大, 并鉴定出稀脉浮萍(*L. aequinoctialis* 6000)是一个生物量积累快和淀粉含量较高的株系, 淀粉含量为28.68%±1.10%, 淀粉产量为4.39 ± 0.25 g/m^2.

营养丰富的浮萍很早就被直接用作鱼虾、畜禽饵料或者作为优质蛋白质源替换传统饲料中的豆粕成分. 例如, 直接使用新鲜浮萍投喂罗非鱼和鲤鱼时能够明显提高鱼产量[125,126], 而且使用浮萍饲养鲤鱼的生长和饲料转换效率明显高于豆粕[127]. 浮萍也可以用作饲料添加剂, 显著提高鱼体的蛋白和脂肪含量[128]. 黄斌等人[129]在红色草金鱼的基础饲料中加入10%~30%的芜萍干粉, 红色草金鱼的日增质量、相对生长率随着芜萍干粉添加量的增加而逐渐上升, 添加比例为30%时鱼体的日增质量、相对生长率最快, 分别为对照组的2.7和2.66倍; 投喂芜萍干粉还能显著改善红色草金鱼的体色, 使体色更加艳丽, 且红色草金鱼皮肤中的类胡萝卜素含量与芜萍干粉添加量呈正相关. 张植元等人[130]利用浮萍替代饲料中的菜粕成分对黄金锦鲤(*Cyprinus carpio*)生长性能、抗氧化和消化能力的影响发现, 随着浮萍替代水平的升高黄金锦鲤的生长性能呈上升的趋势, 当饲料中浮萍的添加水平为14%时十分有利于黄金锦鲤的生长, 并能提高鱼体抗氧化能力与消化酶活力. 利用标准化的池塘培养浮萍时, 其相对生长速率约0.422~0.073 g/(g d), 总产量约702.5 kg/(ha 月)(干重)[2], 因此可将浮萍培养池设置于养鱼塘旁, 从而直接使用新鲜浮萍饲喂, 避免干燥饲料产生的昂贵成本. 用于废水处理的浮萍由于吸收了大量的氮磷营养物质, 生物量和蛋白含量较高, 也可以直接用作鱼饲料且不会对鱼体产生危害[131].

浮萍在开发作为人类食物和营养来源方面也具有巨大的价值和潜力, 荷兰地区甚至已经开始将浮萍作为食品或开发为可食用的产品. 2019年9月在以色列举办的第5届国际浮萍大会上, 瓦格宁根大学(Wageningen University)科学家Jurriaan Mes报道了摄入青萍(*L. minor*)对人体安全性和营养指标的影响, 发现人体能够吸收青萍(*L. minor*)中的必需氨基酸(Essential amino acids,

EAA), 并在摄入2 h左右达到最大值; 摄食青萍(*L. minor*)也未对人体的血压、心率、体温、肠道健康等指标产生显著影响, 而且人体可以长期摄食浮萍. 最小的浮萍物种芜萍(*W. globosa*)富含蛋白和淀粉、微量元素(铁、锌等)、维生素、膳食纤维、多酚等营养物质, 且不会对人体产生毒害[132]. 以色列Hinoman公司正在尝试开发芜萍(*W. globosa*)作为食品, 以及评估芜萍是否适合作为人类获得必需氨基酸(essential amino acids, EAA)的营养来源, 将芜萍(*W. globosa*)命名为"Mankai"用于未来的食品推广.

6.2 浮萍应用于生物能源开发

浮萍超高的生物量积累速率、短的繁殖周期、浮水生长和易于收获的优势也是开发生物能源, 如乙醇、丁醇和沼气等的优质原料. 利用厌氧消化处理的浮萍生产甲烷的产量可达390±0.1 mL CH_4/g[133]. 处理污水后的稀脉浮萍(*L. aequinoctialis* 6000)富集了大量的淀粉, 经发酵处理后95%的糖类能够被发酵为酒精, 得率为0.17 g/g[134]. Shen等人[135]利用丁二酸放线杆菌GXAS137发酵浮萍生产琥珀酸, 在1.3 L搅拌式生物反应器中进行分批发酵时, 能够产生约57.85 g/L的琥珀酸. 用酶预处理和半同步糖化发酵工艺在2 L生物反应器中反应56 h后, 少根紫萍(*L. punctata*)水解生产琥珀酸的产量约为75.46 g/L, 产率为82.87%. 通过烯效唑处理能够诱导青萍(*L. minor*)大量合成淀粉(约50.4%), 之后高温(180°C)反应较短时间内葡萄糖得率可达93.4%, 生成的葡萄糖转化为乙酰丙酸乙酯的产率为55.2%(400.6 g/kg), 额外使用$[C_3H_6SO_3HPy]HSO_4$作为催化剂时能够将工艺效率提高到81.8%[136]. Calicioglu等人[137]将乙醇发酵、产酸消化和产甲烷消化中的生物过程依次整合, 最大限度地将处理废水后的浮萍转化为生物能源, 而且在厌氧发酵中结合使用浮萍与微生物进行发酵, 比单独使用浮萍发酵时甲烷的产量提高了51.2%. 对浮萍暗发酵还能进行生物制氢, 最大产量为169.30 mL/g(干重), 且利用发酵中产生的废料与小球藻混养生产油脂的产脂量比单独使用小球藻的产脂量提高了33倍[138]. 赖烦等人[139]研究了浮萍发酵产乳酸过程中的降黏工艺, 他们利用干酪乳杆菌(*Lactobacillus casei* CICC 23184)为发酵菌种进行发酵产乳酸, 整个发酵只以浮萍为单一底物, 并将降黏酶处理和补料分批发酵相结合的方式在高底物浓度下发酵获得了高浓度的乳酸, 为以浮萍为原料发酵生产乳酸的实用化工艺开发奠定了基础.

7 浮萍在污染修复中的应用研究

浮萍也是生态毒理学研究中常用植物材料, 不仅可作为毒理监测中的指示植物, 还广泛应用于氮磷、重金属、有机污染物等的污染修复. 浮萍能够将吸收的氮磷转化为自身的生物量累积, 约0.3~1.0 g/L的浮萍可以去除城市污水中20~50 mg/L的总溶解无机氮, 与青萍(*L. minor*)、青萍(*L. gibba*)和少根紫萍(*L. punctata*)相比, 紫萍(*S. polyrhiza*)对氮的去除率最高, 约2.0~10.8 mg/(L d)[140]. 利用少根紫萍(*L. punctata*, 0202)进行微污染地表水体的深度处理时, 经处理的Ⅴ类、劣Ⅴ类水体中氨氮和总磷含量在3 d内能够达到地表Ⅱ类水标准, 氮、磷去除率均达到98.5%和82.9%以上[141]. 浮萍能够富集和吸收镉(Cd)、砷(As)、铅(Pb)、汞(Hg)、硼(B)、锌(Zn)、铜(Cu)、钴(Co)等金属离子[142,143]. 例如, 唐利萍等人[144]筛选出对镉耐受性和富集量较高的少根紫萍(*L. punctata*, ZH0049)株系, 当镉浓度为3 mg/L时, 其对镉的吸收率和富集率能达到66.74%和72.43%; 青萍(*L. minor*)能够超量富集钴(Co), 富集量达到5000 μg/g(鲜重)时仍未对浮萍产生明显的毒害作用[116]; 青萍(*L. valdiviana*)对砷的富集量可达到1190 mg/kg(干重)[145]; 紫萍(*S. polyrhiza*)对锰的耐受性高达70 mg/L且生长几乎不受影响[146]. Türker等人[147]开发了一套基于浮萍消纳的灌溉水体硼去除装置, 当青萍(*L. gibba*)暴露于4 mg/L的硼溶液中时可去除71%的硼, 而且还能同时进行生物发电, 最高的生物发电量为1.04 V, 功率为17783 mW/m^2. 浮萍还能在短期内用作沼液还田模式下水体和土壤重金属污染控制的有效手段, 在一定程度上控制沼液施用中土壤、水稻籽粒和秸秆中的重金属(Cu和Pb)含量[148]. 浮萍还能吸附或吸收水体中的微塑料[149]、农药[150]、药品[151]以及一些工业化学品[152].

水环境是影响浮萍分布的最主要因素[153], 不同浮萍物种甚至不同生态型的浮萍对污染物的敏感度和富集能力差异较大. 王香莲等人[154]研究了鄱阳湖流域的浮萍种质资源分布及生长环境, 发现青萍(*L. minor*)富集锌和铅、紫萍(*S. polyrhiza*)富集铬和铅、少根紫萍(*L. punctata*)富集锰的能力较强. Yang等人[7]比较了青萍(*L. minor*, 6580)、青萍(*L. gibba* 6745)和紫萍(*S. polyrhiza* 5543)对汞的耐受性和富集能力的差异, 发现紫萍(*S. polyrhiza* 5543)对汞富集能力最高, 青萍(*L.*

gibba, 6745)对汞毒性最为敏感. Chen等人[155]比较了采集自中国南方地区30个生态型的紫背浮萍(*S. polyrhiza*)应对镉胁迫(1 μmol/L)的响应, 发现镉胁迫处理1周后不同生态型浮萍的生长、光合色素和抗氧化酶的活性差异较大. 因此, 不同浮萍物种混养不仅可提高水体净化效果, 还能促进生物质的积累, 从而加强植物修复效果[156]. 这主要由于不同浮萍物种混养时微生物活群落性较高, 改变了碳源的利用率, 提高了浮萍的生长和抗氧化酶活性, 进而降低污染物对浮萍的毒性[157].

Ishizawa等人[158]在青萍(*L. minor*)的生长环境中加入醋酸钙不动杆菌(acinetobacter calcoaceticus P23)共培养, 使青萍(*L. minor*)的生物量提高了1.9~2.3倍, 并显著提高青萍(*L. minor*)对氮磷的吸收率. 日本北海道大学Masaaid Morikawa实验室分离出对生长起促进作用的微生物, 称为"PGPB(plant growth-promoting bacteria)", 主要是假单胞菌和气单胞菌属与浮萍共培养用于食品工厂的废水处理, 不仅积累了更多的浮萍生物量用于生物能源开发, 而且工厂废水也得到较好的净化处理. 藻类与浮萍共培养时也能影响浮萍的净化效果, 例如, 铜绿微囊藻和少根紫萍(*L. punctata*)共培养时, 较低浓度的铜绿微囊藻能够促进少根紫萍(*L. punctata*)有效地吸收培养液中的藻毒素、总氮和总磷[159], 而高浓度的铜绿微囊藻在生存竞争中处于有利位置, 会抑制少根紫萍(*L. punctata*)的生长, 导致其代谢能力降低甚至死亡[160]. 因此, 在利用浮萍进行污染物修复时应该考虑不同浮萍物种和生态型对污染物净化能力的差异, 以及利用微生物、藻类与浮萍共培养产生的优势, 通过不同浮萍物/品种组合或浮萍-菌/藻组合以达到最佳的净化效果.

浮萍还能够被加工成干粉作为一种潜在的新型吸附剂[161]. 聂小琴等人[162]比较了少根紫萍活体和干粉对U(Ⅵ)溶液的去除率, 发现pH 5时1.25 g/L(DW, 干重)干粉对5g/L U(Ⅵ)溶液的去除率可达95.55%, 高于2.5 g/L(FW, 鲜重)活体处理下的去除率(78.70%); 他们还发现, 干粉对U(Ⅵ)的吸附主要通过静电吸引、离子交换、络合配位等方式实现, 而活体对U(Ⅵ)的吸附还存在还原和矿化的过程. Yang等人[99]通过基因工程技术在青萍(*Lemna turionifera* 5511)中过表达Na^+/H^+逆向转运蛋白(*Na:H antiporter*, *NHX1*), 显著提高了青萍(*L. turionifera* 5511)对镉胁迫和盐胁迫的耐受性, 过表达*NHX1*主要是通过抑制Cd^{2+}的流入从而减少浮萍中Cd^{2+}的含量; 反之, *NHX1*表达受抑制时, 浮萍体内的Cd^{2+}的流入增加, 流出受抑制延迟.

8 结论与展望

综上所述, 浮萍不仅是植物发育和植物生物节律等生物学研究的适合模型, 在开发作为食物、饲料、生物能源以及生物反应器用于表达蛋白、酶、疫苗等方面也具有巨大的潜力, 更是水体污染监测和修复的常用植物材料. 浮萍生物能源的开发依赖于高效表达淀粉, 但目前缺乏对浮萍代谢机制的深入研究, 未来需要对浮萍淀粉代谢和生物质积累开展深入的研究, 并借助浮萍表达系统来提高浮萍的淀粉表达量和产量. 用于污水净化的浮萍不仅能够高效富集氮磷、重金属、有机污染物等, 还能快速繁殖并积累淀粉, 而且还不会产生毒性. 因此, 作为一种非粮食作物的优质生物质原料和水体净化植物, 浮萍将在实现生物能源开发和污染修复共赢中发生巨大的效益. 但开发和推广浮萍作为饲料添加剂尤其是食品方面还存在较多的困难, 需要开展更多的营养评估等方面的研究. 浮萍作为最简单的高等植物, 结构简单, 生长繁殖快, 利用浮萍作为生物反应器具有生产周期短、产量高、表达产物便于分离和纯化、安全性高、成本低、表达品系可稳定遗传以及便于培养和采收等优势; 另外, 浮萍个体微小和可食用性的特点也具有开发口服疫苗的潜力. 未来需要在浮萍表达系统的表达载体优化、提高浮萍遗传转化效率和稳定性, 以及浮萍大规模培养装置研发方面开展研究, 进而获得成本低、稳定、高产的表达产物, 实现浮萍生物反应器的产业化. 随着浮萍组学数据的丰富以及越来越多浮萍遗传转化体系的建立, 尤其是叶状体转化法使得浮萍的遗传转化效率显著提高, 未来将能够利用基因组、转录组和生物信息学挖掘调控浮萍形态建成、发育和生殖、淀粉代谢和积累, 以及环境适应性的关键基因, 并借助高效的遗传转化体系和反向遗传学手段解析相应的分子调控机制, 开展相关的基础研究和应用基础研究, 为开展浮萍资源化利用提供数据支撑.

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Summary for “浮萍的研究及应用”

Research and application in duckweeds: A review

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The free-floating aquatic duckweeds consist of the five genera, *Spirodela*, *Landoltia*, *Lemna*, *Wolffiella* and *Wolffia*, comprising 36 species within the monocot order of Alismatales. They are strongly adaptable to various environments and are widely distributed in a variety of climates all over the world except that *Wolffiella* species are restricted to the Americas and Africa. Among them, *Spirodela* species have large a flattened frond with 5−11 rhizoids while the rootless *Wolffia* species form an oval-shaped frond and are the smallest flowering plants. Duckweeds seldom flower and their rapid asexual propagation allows them to accumulate biomass quickly. They usually bud new generations (daughter fronds) from their reproductive pouches at the base of the mother fronds and the frond number almost doubles within 24 hunder good growth conditions yielding the fastest-growing flowering plants. They tend to be associated with nutrient-rich or eutrophic freshwater environments with slow-moving flow such as ponds, lakes, ditches, and paddy fields. While duckweeds become more reduced from *Spirodela* to *Wolffia*, their genome size varies 13-fold, ranging from 150 Mb in *Spirodela polyrhiza* to 1881 Mb in *Wolffia arrhiza*. With the development of long-read sequencing technology, the genomes of common duckweed species including *S. polyrhiza*, *Lemna minor*, *Lemna gibba* and *Wolffia australiana* have been sequenced and assembled. The underlying molecular mechanisms and regulatory networks in duckweeds under different treatments such as starvation, salt stress, heavy metal and radiation have also been revealed by transcriptome, proteome and metabolome analyses for the genera *Spirodela*, *Landoltia*, and *Lemna* enhancing their potential applications in the fields of phytoremediation and bioenergy. Furthermore, genetic transformation systems for some common duckweeds such as *L. minor*, *Lemna aequinoctialis*, *S. polyrhiza* and *Wolffia globosa* have been established, and especially the frond transformation systems have significantly improved transformation efficiencies (more than 90%). These characteristics have made duckweeds model systems for several decades for the study of plant physiology, biochemistry, ecotoxicology, and have contributed to knowledge of auxin biosynthesis, plant flowering and the circadian system. Duckweeds have also been widely used for the treatment of agricultural, municipal, and even industrial wastewater because they can uptake nitrogen and phosphorus as well as quickly accumulate heavy metals and organic pollutants. Besides, their accumulated biomass is rich in starch and protein and therefore can be used for feed applications and biofuels. In this review, we introduce the origin, classification and evolution of duckweeds, as well as research in morphology and anatomy, physiology, genetic transformation and omics studies. We further discuss the application of duckweeds in food, feed, bioenergy, and bioremediation. Finally, we highlight the challenges and future directions of duckweed research, providing a reference for basic biological research and resource utilization.

duckweed, evolution, physiology, omics, bioenergy, bioremediation

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