2026, 66(4): 1569-1584
CSTR: 32112.14.j.AMS.20250780
DOI: 10.13343/j.cnki.wsxb.20250780

微生物学报
Acta Microbiologica Sinica
http://journals.im.ac.cn/actamicrocn

Review 综述

多技术融合驱动的重离子辐射诱变育种：从机制到应用

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郭晓鹏, 张书翰, 贾承霖, 张苗苗, 陆栋. 多技术融合驱动的重离子辐射诱变育种：从机制到应用[J]. 微生物学报, 2026, 66(4): 1569-1584.
GUO Xiaopeng, ZHANG Shuhan, JIA Chenglin, ZHANG Miaomiao, LU Dong. Heavy ion radiation-based mutation breeding driven by multi-technology integration: from mechanism to application[J]. Acta Microbiologica Sinica, 2026, 66(4): 1569-1584.

摘　要：重离子辐射具有诱变率高、诱变谱宽、突变体稳定等独特优势，在微生物菌种选育中发挥着重要作用。然而，辐射诱变育种固有的随机性限制了其效率与质量的进一步提升，这已成为研究者亟待突破的核心难题。根据重离子辐射诱变育种的流程，本文提出一套串联策略以推动高效、高质量的重离子辐射诱变育种实践。该策略包括：从立体维度优化辐射参数，调控细胞辐射损伤敏感性与修复能力；结合重离子辐射、实验室适应性进化及其他诱变剂开展渐进式辐射处理；并通过高通量筛选及后续的鉴定、验证和整合，提高诱变率、筛选效率和正向突变的利用率。同时，针对重离子辐射诱变育种的完整闭环，本文展望了整合系列策略的诱变育种工作站，为基于重离子辐射的优良微生物资源创制提供重要参考。

关键词：重离子辐射；诱变育种；高效诱变；高通量筛选；正向突变挖掘；集成工作站

资助项目：国家自然科学基金(12205132, 12575386, 12575382)；甘肃省重点研发计划(25YFGA008)；中国博士后科学基金第77批面上项目资助(2025MD774059)；甘肃省联合基金一般项目(25JRRA1148)
This work was supported by the National Natural Science Foundation of China (12205132, 12575386, 12575382), the Key Research and Development Project of Gansu Province (25YFGA008), the 77th Batch of General Funding from China Postdoctoral Science Foundation (2025MD774059), and the Joint Fund General Project of Gansu Province (25JRRA1148).

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Received: 2025-10-17; Accepted: 2025-12-16; Published online: 2026-01-09

Heavy ion radiation-based mutation breeding driven by multi-technology integration: from mechanism to application

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Abstract: Heavy ion radiation (HIR) is effective for generating new germplasm in plants and microorganisms due to its high mutation induction rate, broad mutagenesis spectrum, and excellent stability of mutants. However, the random mutagenesis induced by radiation limits the efficiency and quality of HIR-based mutation breeding, which has become a key problem to be tackled. According to the process of heavy ion radiation-based mutation breeding, this review proposes a set of tandem strategies to enable efficient and high-quality HIR-based mutation breeding practices. These strategies include adjusting the radiation parameters from multiple dimensions, regulating cellular sensitivity to radiation damage and damage repair capacity, combining heavy ion radiation with adaptive laboratory evolution, integrating heavy ion radiation with other mutagenic agents, adopting progressive radiation, formulating high-throughput screening schemes for mutants, and efficiently identifying, verifying, and integrating positive mutations. These strategies aim to improve the mutagenesis rate, screening efficiency, and utilization of positive mutations. Meanwhile, we envision a mutation breeding workstation that integrates a series of strategies to form a complete cycle for heavy-ion radiation-based mutation breeding. This study is expected to provide valuable insights for creating high-quality microbial resources through heavy-ion radiation.

Keywords: heavy ion radiation; mutation breeding; efficient mutation induction; high-throughput screening; positive mutation mining; integrated workstation

随着科技的创新发展，重离子辐射(heavy ion radiation, HIR)、伽马射线、常压室温等离子体(atmospheric and room temperature plasma, ARTP)等辐射育种方法在生物种质资源创制领域得到了广泛应用[1-5]。其中，HIR 是指通过离子加速器将比质子重的带电粒子加速至接近光速，并利用电磁聚焦等技术精确控制形成的束流，根据能量不同可将离子束划分为低能离子束、中能离子束和高能离子束[5]。HIR 具有高传能线密度(linear energy transfer, LET)、质量和能量沉积、动量转换、歧离散射小、损伤截面大、贯穿能力强(中高能重离子辐射)等独特的物理学特征[6-7]，其应用于诱变育种表现出其他育种方法无法比拟的诱变率高、诱变谱宽和突变体易稳定等优势[8]。利用重离子辐射诱变育种技术已选育出大量优良突变体，涉及模式微生物、工业微生物、模式植物、大宗农作物以及观赏植物等[9-16]。这些突变体一方面在工业、农业、医学、环境等各个领域产生了巨大的经济效益，另一方面在基础研究方面为解答生命科学问题提供了优良的研究材料[17-20]。长期以来，中国科学院合肥物质科学研究院、中国科学院近代物理研究所、日本量子科学技术研究开发机构以及日本理化所等在重离子辐射诱变育种领域

的研究较为深入[21]。据统计，已报道通过重离子辐射诱变获得了种属覆盖度较大的上百种优良微生物，这些菌种表现出产量高、逆境适应性强和生产周期短等特性[10,15-16]。

近年来，随着生命科学技术对微观世界探测能力的不断提升，重离子辐射诱变效应已在性状水平得到充分验证。同时，基于突变株在细胞器结构和功能、DNA 序列、基因表达、酶活性及代谢谱等方面的显著变化，该技术从性状、亚细胞至分子层面均被证明是高效获得优良突变体的重要技术[10-11,22-25]。此外，许多非典型物种的转化和基因编辑体系尚不成熟，且很多亟待改善的数量性状通常由多基因控制，其正向突变位点及变异方向未知，基因编辑等遗传操作技术的实施和奏效难度较大[26]。HIR 在此方面具有明显优势，它极易诱导包含多基因变异的突变体，可基于单碱基替换(single nucleotide polymorphism, SNP)和小片段插入缺失(insertion-deletion, InDel)优化基因调控区或编码区的序列结构，通过多靶点综合改善决定性状的信号途径和代谢网络，诱导全新变异[9,23]。因此，利用重离子辐射诱变技术可丰富遗传多样性、获取优质种质资源。基于重离子辐射诱导的突变体，利用正向遗传学和反向遗传学思路解码基因结构和功能，仍将是辐射生物学、育种学和分子生物学等相关学科的研究热点[12-13,18,26]。

尽管 HIR 的诸多优势使其较传统诱变技术更容易获得优良突变体，但诱变技术普遍存在的随机性仍然是重离子辐射诱变不可回避的问题，且是限制优良目标突变体快速获取的主要原因[27]。同时，基于 HIR 诱变率高和诱变谱宽的技术特性，大容量诱变群体必然包含大量的正向突变以及相应的基因结构信息[23,28-30]。然而，针对目标突变体的获取必定要经历大基数的测试和多轮筛选，只有当筛选基数远大于特定突变率的倒数时优良突变体的获取才由随机事件变为必然事件[27]。较低的效率极大地限制着重离子辐射诱变育种的应用。此外，相对于突变体的获取和应用，性状改善的机理研究相对不足，正向突变位点的开发利用鲜有报道。目前，生产实践对整体性能全面提升的种质资源的需求，以及工业微生物应用对更短育种周期的要求，对重离子辐射诱变同样提出了挑战。一些重离子辐射诱变相关研究已致力于改善或解决诱变实践中突变体获取通量低、突变体机理研究不够深入的问题[8,18,30-33]。

提高诱变育种的效率总体上有 3 个着力点：提升突变体发生频率、提高筛选效率以及有效利用突变体和突变基因。目前，许多不同工作都在尝试使用某一种策略以提升目标突变体发生频率、提高筛选效率或有效定位突变体的正向突变位点，如优化辐射参数[34-35]、调节细胞内在状态[25,32]、使用高通量筛选体系[29,31]、联合其他育种方法[36-37]、联合组学和遗传操作等[9,19]。

综合重离子辐射诱变育种现有的理论和技术研究成果，本文总结了提升重离子辐射诱变育种的策略(表 1)，并讨论了在其他育种方法中使用的值得借鉴的方式，涉及从样品制备、辐射处理、目标突变体筛选、优良基因元件的定位，到突变体和突变位点应用等对诱变效果产生影响的各个环节，旨在使研究者可以更全面地把握、权衡和组合这些策略，最终提升重离子辐射诱变育种的效率。

1 优化辐射参数

与其他诱变剂相比，HIR 具有更多维度的辐射参数，包括剂量、能量、离子种类、LET 和剂量率，这些辐射参数均会对生物学效应(包括诱变效应)产生显著影响[11,35,39,50-51]。其中，剂量是几乎每项重离子辐射诱变育种工作都会关注的因素。目前，重离子辐射植物和微生物的剂量-突变率曲线提供了一定的经验和参考[11]。对于微生物模型，在中高剂量范围对应于存活分数为 10%–40% 之间的剂量点的正向突变率较

表1　重离子辐射诱变技术在生物改良中的应用策略及案例

Table 1　Application strategy and case of heavy ion radiation mutagenesis in biological improvement

Biological/ Strain name	Strategy	Core results	Technical highlights and innovations	References
Bacillus subtilis	Carbon ion (80 MeV/u, 10−160 Gy)	Increased production of nattokinase	Screening high-yielding mutants in a wide dose range	[38]
Neurospora crassa	Carbon ion (30 keV/μm), iron ion (641−646 keV/μm), argon ion (287 keV/μm)	Determination of biological effect ranking	Comparison of biological efficacy and mutational spectrum of different ion types	[39]
Oryza sativa	Argon ion (92 keV/μm), carbon ion (13 keV/μm), neon ion (31 keV/μm)	Different structural variation patterns	Mechanism of structural variation (SV) formation induced by different LET ions	[40-41]
Petunia	High expression of anthocyanin gene coupled with carbon ion beam	The number of color mutants increased by 2.5 times	High gene expression and chromatin remodeling enhance mutagenic efficiency	[32]
Arabidopsis	Carbon ion irradiation for drying seeds *vs*. seedlings	The mutation frequency of dry seeds was 1.4−1.9 times that of seedlings	Comparison of mutagenic efficiency of materials at different developmental stages	[25]
Chrysanthemum	Ion beam irradiation of petal *vs*. leaf cloning	The mutation rate of petal clones was 6.47%, and that of leaf clones was 3.89%	Effect of tissue origin selection on the production of flower color mutants	[42]
Saccharomyces cerevisiae	Combination of HIR and adaptive laboratory evolution (ALE)	Tolerance to 1.6 g/L vanillin	Heavy ion radiation combined with adaptive laboratory evolution	[43]
Flavobacterium sp.	Combination of nitrogen ion implantation and γ-ray	Improved substrate tolerance and increased yield of methyl naphthoquinone	Compound mutagenesis using ion implantation and γ-ray	[37]
Trichoderma longibrachiatum	Combination of nitrogen ion implantation and ultraviolet irradiation	The yield of alkaloids increased by 2.62 times	Combined mutagenesis to improve the yield of endogenous bacterial secondary metabolites	[44]
Trichoderma viride	Combination of carbon ion irradiation and electron beam radiation	The activity of soluble protein and cellulase doubled	Enhancing enzymatic performance by combined radiation mutagenesis	[45]
Clostridium tyrobutyricum	Multiple-round heavy-ion irradiation	Robust organic acid production in extreme acidic environment	Improving industrial environmental adaptability through multiple-round heavy-ion irradiation	[46]
Lecanicillium attenuatum	Five rounds of nitrogen ion implantation	The propamocarb EC_{50} value increased 2.54-fold	Multiple rounds of progressive irradiation to improve the tolerance of fungicides	[28]
Saccharomyces cerevisiae	Multiple rounds of progressive radiation combined with ALE	Tolerance to 4.5 g/L furfural	Multiple rounds of progressive radiation combined with ALE	[47]
Euglena gracilis	Combination of iron ion beam radiation and flow cytometry screening	Oil content increased by 40%	Fluorescence-labeled flow cytometry screening of high-yield oil-producing algae strains	[48]
Chlamydomonas sp.	Combination of heavy ion irradiation, high nitrate culture, and fluorescence sorting	High oil yield under high nitrate conditions	Cultivation and screening under specific stress conditions	[49]

高，继续增加剂量，尽管突变率会增加，但正向突变率开始下降[11,31]。Lu 等[52]利用不同剂量的 85 MeV/u 氖离子辐照酿酒酵母(*Saccharomyces cerevisiae*)菌株，发现当剂量达到 40 Gy 时正向突变率较高。Sheng 等[38]通过 80 MeV/u 碳离子在 10−160 Gy 宽剂量范围内辐照枯草芽孢杆菌(*Bacillus subtilis*)，成功筛选出高产纳豆激酶的突变菌株。对于植物模型，一些研究同样表明中高剂量是合适的选择，但许多育种实践中，中低剂量，即 50% 存活分数左右对应的剂量点也被证明是诱导丰富遗传变异的最佳剂量选择[53]。突变源于错误的损伤修复，突变率是损伤和修复之间的一种平衡。因此，剂量的选择本质上是寻求有利于正向突变的损伤和修复的平衡点。未来，更多的突变率的剂量依赖性结果将给出更多物种在更多情形下的数据以供参考。

除了剂量，离子种类和 LET 也是备受关注的辐射参数[24,39,51,54-56]。在诱变育种实践中通常采用不同的离子种类并设置不同的 LET 进行尝试，以诱导不同的表型(诱变效果)突变谱和分子突变谱[24,35,39-41,56-57]。Ma 等[39]用 30 keV/μm 的碳离子、641－646 keV/μm 的铁离子和 287 keV/μm 的氩离子辐照模式丝状真菌脉孢菌(*Neurospora crassa*)，确定了相对生物学有效性的排列顺序为氩离子>铁离子>碳离子，并观察到 3 种离子类型诱导 *ad-3* 基因座正向突变频率和 *ad-3B* 基因突变谱的差异。Zheng 等[40-41]考察了 92 keV/μm 的氩离子、13 keV/μm 的碳离子和 31 keV/μm 的氖离子辐射水稻导致的结构变异(structural variation, SV)，表明 3 种具有不同 LET 的离子在诱导 SV 形成的过程中可能存在差异，即串联重复序列的扩展、转座因子插入和非等位基因同源重组之间的相对比例不同。针对同一种离子的辐射，酵母细胞模型表明，具有较高 LET 的碳离子束比具有较低 LET 的碳离子束更具诱变性[55]。事实上，在使用具有不同 LET 的多种离子的辐射诱变研究中都着重强调了 LET 的影响[58-60]。由于能量的变化最终会体现为 LET 的差异，对能量影响诱变效应的考量可以参考 LET。此外，关于 HIR 的剂量率对诱变效应的影响鲜有报道，但剂量率的差异是辐射暴露后风险评估模型考虑的重要因素，这提示剂量率对诱变效应的影响值得重视[61]。

目前，多数研究仅对 1 种或 2 种辐射参数进行优化，尽管在正向突变率提升方面的效果显著，但尚未充分发挥其多维参数可调控优势。在多个因素都有显著影响时，二维参数的最优值和三维及以上参数的最优值可能会有很大差距。同时，剂量、能量、离子种类、LET、剂量率并非相互独立，而是相互影响的，如不同的 LET 会具有不同的半致死剂量。综上所述，HIR 的多参数协同优化将产生多维度的生物学效应。这一特征是重离子辐射诱变的显著特点，一方面提供了丰富的辐射质量可供选择，另一方面也表明辐射参数优化的必要性。

2 细胞内在状态的调节

2.1 染色质构象调控

HIR 诱导 DNA 损伤的微观效应，包括沿离子径迹的质量、能量、电荷联合作用，并涉及径迹上的损伤截面和激发的次级效应[62-63]。这种复杂性决定了 DNA 损伤具有构象敏感性，即去浓缩化染色质中双链 DNA 断裂(double strand break, DSB)的频率高于浓缩染色质[64]。基因表达始于染色质开放和 DNA 解螺旋，随后转录因子结合启动子并启动表达；处于转录激活状态的基因座常具有疏松的染色质结构，便于转录调控因子与顺式调控元件结合以及 RNA 聚合酶在模板上的延伸；因此，转录活跃的基因由于染色质构象变化对辐射损伤更敏感。突变源于 DNA 损伤的错误修复，有研究表明目标性状相关基因转录活跃度(对应于染色质开放和 DNA 解螺旋的程度)与电离辐射诱导的突变率存在相关性[32,65-66]。在重离子辐射诱变中，Hase 等[32]

诱导矮牵牛幼苗花青素生物合成基因高表达后，耦合碳离子辐射导致花色突变体增加了 2.5 倍。这表明通过诱导性状相关基因高表达引发基因座染色质构象重塑和 DNA 解螺旋，可在性状基因座高效引入突变，从而增加目标性状突变率并提升辐射诱变育种效率。

2.2 损伤修复能力的调控

突变由损伤和修复共同决定，完全修复和无法修复都不利于突变产生，只有错误修复才产生突变。这意味着，在一定的损伤状态下，修复能力对突变的影响存在最优值(即拐点)。与常规射线相比，HIR 诱发更迅速的同源重组修复(recombinational repair, HR)途径响应，这是细胞对其诱导更多 DSB 的代偿性反应[67]。同时，与常规射线相比，HIR 在 DNA 损伤修复途径的选择上有差异。在重离子辐射下，DNA 损伤修复模式中易错修复途径占比较大，尤其是切除介导的连接，包括不依赖 Ku 蛋白的替代性非同源末端连接(non-homologous end-joining, NHEJ)修复途径和单链退火(single strand annealing, SSA)修复途径[62-63,67-68]。相比常规射线，HIR 诱导的 DNA 损伤修复在修复程度和方式上的差异，可能更有利于形成损伤-修复平衡，这是其诱变率较高的分子基础之一[54]。研究表明，损伤修复相关基因功能丧失会改变 HIR 诱导变异的敏感性及其突变谱[69]。在模式丝状真菌中，重离子辐射 HR 缺陷菌株导致的突变频率最高，而 NHEJ 修复缺陷菌株的突变频率最低[39]。总之，在重离子辐射诱变育种实践中细胞损伤修复能力的调整与损伤程度的控制同等重要，二者引起的损伤修复模式变化均应以形成有利于突变发生的损伤和修复之间的平衡为目标。

2.3 其他生理生化状态

在重离子辐射诱变育种实践中，通过调节细胞生理生化状态可获得较好的诱变效果。例如，微生物对数期(细胞周期分布)、植物种子的干湿状态以及组织源的选择等[25,42]。本质上，这些细胞生理生化状态的影响都归结于改变辐射损伤敏感性以及辐射损伤修复模式，进而对突变产生影响。例如，当采用处于对数期的微生物菌悬液进行辐照处理时，由于细胞处在 G_2/M 期，对辐射更为敏感[27]。同时，细胞倾向于采用的修复方式存在差异。在 S/G_2/M 期，酵母细胞和哺乳动物细胞更倾向于采用基于 HR 的修复途径，而易错的微同源介导的 NHEJ 和 SSA 修复途径可能会形成形式更加复杂的 DNA 损伤。在全细胞周期尺度下，哺乳动物更倾向于 NHEJ 修复途径以达到快速修复的目的[27,62,68,70]。此外，在重离子辐射植物诱变育种中植物种子的干湿状态、不同的组织源等生理状态极大地影响着诱变率和诱变谱。Hase 等[25]用碳离子辐照干燥种子和幼苗，并使用随机选择的拟南芥 M-2 植株考察突变特征，结果表明来源于干种子辐照的突变频率是幼苗辐照的 1.4−1.9 倍；在全基因组水平，干种子辐照中的插入和缺失频率是幼苗辐照的 3 倍。Okamura 等[42]研究了组织源选择结合离子束照射对菊花花色突变体产生的影响，发现离子束辐照花瓣克隆的突变频率为 6.47%，而辐照叶克隆则为 3.89%。因此，通过调节细胞生理生化状态，改变辐射损伤敏感性和细胞损伤修复能力，从而形成新的有利于突变发生的损伤-修复平衡，对提升诱变效率有重要意义(图 1)。

3 重离子辐射与其他育种策略或诱变技术的结合

3.1 辐射诱变结合实验室适应性进化

诱变能够拓展遗传多样性并产生有利突变，但具有随机性；而实验室适应性进化(adaptive laboratory evolution, ALE)则在定向压力下筛选有利突变，具有较强的定向性。ALE 在多种逆境适应性改良中表现出有效作用，包括改善底物抑制和产物抑制缓解等性状[36-37,71]。基于二者的互补性，诱变与 ALE 结合已取得良好的育种效

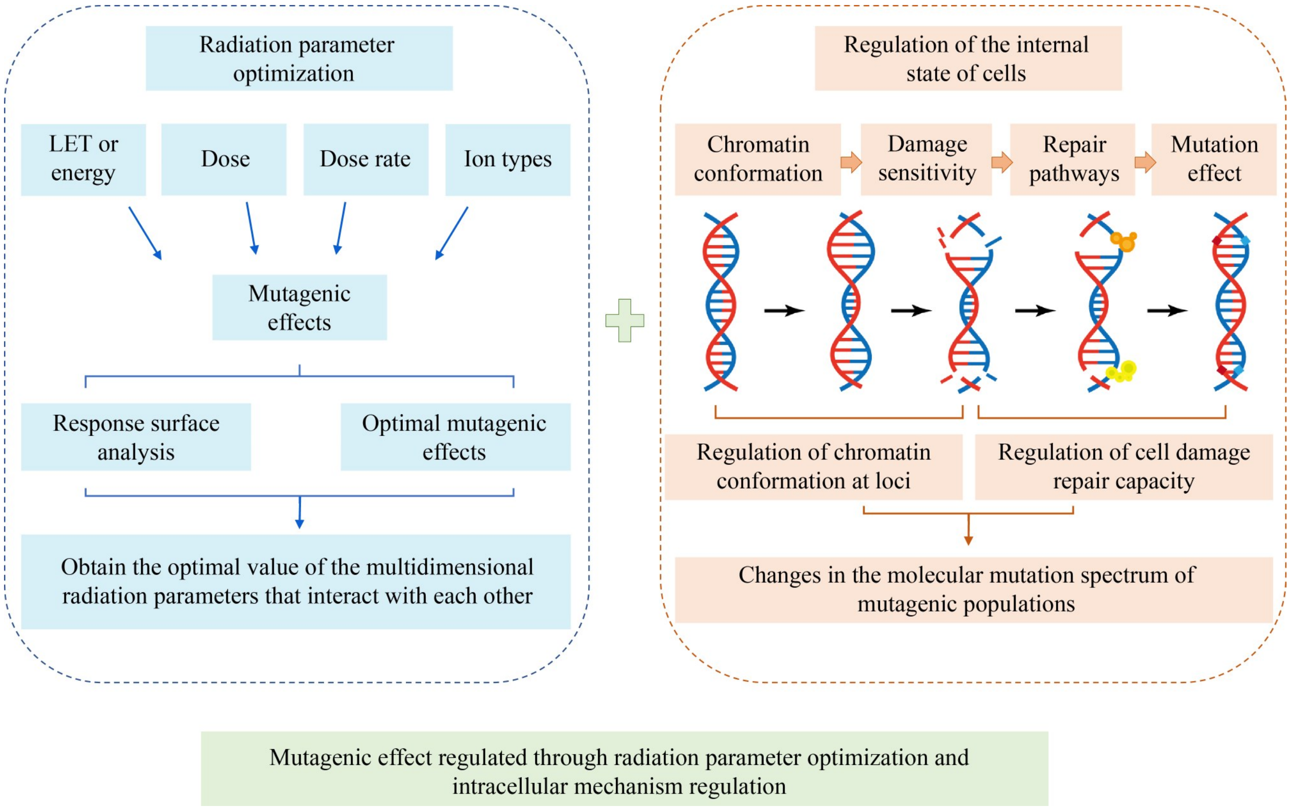

图1　基于辐照参数优化和细胞内在机制调控突变效应

Figure 1　Optimization of irradiation parameters and regulation of mutational effects through intrinsic cellular mechanisms.

果。Liu 等[71]利用 ARTP 诱变结合 ALE 提高了凝结魏茨曼氏菌(*Weizmannia coagulans*)的益生性，经过 15 s ARTP 诱变和 40 d 的 ALE 培养后获得一株对 pH 2.5 和 0.3% 胆盐耐受性增强的突变株。本课题组 Jia 等[43]通过 HIR 结合 ALE 获得 H6 等 4 株耐受高浓度香草醛(1.6 g/L)的酿酒酵母(*S. cerevisiae*)突变株。与野生型相比，这些突变体在耐受性、生长速率、遗传稳定性和发酵能力等方面表现优异。Cui 等[1]通过 ARTP 诱变结合 ALE 选育出高耐甲醇的扭托甲基红杆菌(*Methylobacterium extorquens*)，使其在发酵罐中的甲羟戊酸体积产率提高了 65%。HIR 结合适应性进化是一种极具潜力的策略，主要包括 3 种实施方式，第 1 种是先通过 HIR 得到诱变群体，再进行适应性进化，其本质是利用重离子辐射引发更多变异以供定向压力筛选；第 2 种是在重离子辐射后筛选优良突变体，再对其进行适应性进化，本质是利用 HIR 为适应性进化提供优良出发菌株；第 3 种是先通过适应性进化获得较理想的突变体，再进行重离子辐射诱变，本质是利用实验室适应性进化为重离子辐射诱变提供理想的出发菌株[36-37,71]。总之，诱变技术可以提高突变率，实验室适应性进化可以增强定向性，二者具有良好的互补性。重离子辐射诱变相较于其他诱变技术具有更高的诱变率和更宽的诱变谱，其与实验室适应性进化的结合对于提升育种效率作用显著。

3.2　联合其他诱变方式

比较研究揭示，HIR 和常规射线在突变谱上具有各自的特征，包括不同的转换和颠换比、

不同变异类型[SNP、InDel、多核苷酸变异(multiple nucleotide variant, MNV)]间的相对比例、变异在全基因组的空间分布特征以及变异位点周围的序列环境等[9,72]。基于此，2 种射线的组合可以进一步拓展突变谱。与传统高能离子辐照相比，低能离子束注入技术中的低能离子(keV 级别)穿透力弱，但其在生物材料中引起的能量沉积、质量沉积与电荷转移等复合效应能引发可遗传的突变[73]。Wu 等[37]利用氮离子注入结合伽马射线辐照甲基萘醌生产菌种黄杆菌(*Flavobacterium* sp.) F-2，有效改善了该菌株的底物耐受性，并显著提高了生物量和甲基萘醌的产量。当 HIR 结合激光、紫外线照射和化学诱变等非电离辐射的诱变方式时，由于这些方式和电离辐射基于不同的诱变机理，诱导不同的变异特征，同样可在一定程度上达到拓展突变谱的目的。尽管 HIR 辐射本身具有较高诱变率和较宽突变谱，与其他诱变方式结合时对突变谱和诱变率的改善有时并不显著，但许多重离子辐射联合其他诱变方式的实践仍获得了良好的效果。例如，Qian 等[44]对分离自石斛的产倍半萜生物碱的长枝木霉(*Trichoderma longibrachiatum*) MD33 进行氮离子注入和紫外线照射，获得突变株 UN32，其生物碱产量为 MD33 的 2.62 倍；Li 等[45]利用碳离子辐射和电子束辐照对绿色木霉(*Trichoderma viride*) GSICC 62010 进行联合诱变，获得可溶性蛋白质产量和纤维素酶活性成倍增加的突变株，由此制备的纤维素酶对锯末的水解性能是亲本菌株的 2 倍。陈奕涵等[74]先使用亚硝基甲基脲诱变，再进行氮离子注入，获得高产透明质酸的链球菌(*Streptococcus zooepidemicus*)。因此，HIR 与不同诱变剂的联合处理可产生显著协同效应，从而显著提升诱变育种效率与突变体质量。

4 多轮渐进式辐射

在多轮渐进式辐射中，出发菌株经重离子辐射诱变初步获得目标性状突变体，随后以初步获得的目标性状突变体为出发菌株进行第二轮辐照，再次筛选目标性状突变体后进行第三轮及后续多轮辐射。累进辐射策略在植物和微生物育种中均受到重视，尤其在微生物育种中应用较多。Zhou 等[46]对酪丁酸梭菌(*Clostridium tyrobutyricum*) ATCC 25755 进行重离子累进辐照，获得了在极端酸性环境下具有稳健有机酸生产能力的突变菌株。Xie 等[28]为提高渐狭蜡蚧菌(*Lecanicillium attenuatum*)对杀菌剂的耐受性，经过 5 轮氮离子注入，获得对敌霉威耐受性显著增强的突变株，其中值有效浓度(median effective concentration, EC_{50})较野生株提高了 2.54 倍。Ren 等[47]采用多轮渐进式辐射结合 ALE 技术成功选育出耐受高浓度糠醛(4.5 g/L)的酿酒酵母(*S. cerevisiae*)突变株 SCF-R4。在多轮累进辐射的过程中，每一轮均筛选目标性状突变体，属于定向筛选；而累进辐射旨在不断增加突变频率以丰富遗传多样性。综合来看，该策略通过定向选择出发菌株，不断在优良菌株中引入遗传变异，逐步实现目标性状的改善(图 2)。该策略在选育更优良的突变体时，由常规的持续扩大筛选基数转向对现有基数范围内已获得的突变体进行第二轮辐照和选育，因此涉及对扩大筛选基数和累进追加辐射的有效权衡。

5 高通量/高效率筛选

高通量/高效率的筛选技术将极大地降低诱变育种实践的盲目性。目前，在重离子辐射诱变育种相关研究中，高通量/高效率筛选技术正以“重离子辐射诱变+”模式在不断被尝试和应用。整体上，对于基于正向遗传学思路的研究，高通量/高效率筛选的实现可归纳为高效选择性培养基的设计、微孔板微量体系的应用、表型组学平台的构建以及流式分选、微流控和光谱学技术的应用[23,31,49,75-77]；对于反向遗传学思路的研究，高通量/高效率筛选的实现包括定向诱导基因组局部突变技术(targeting induced local

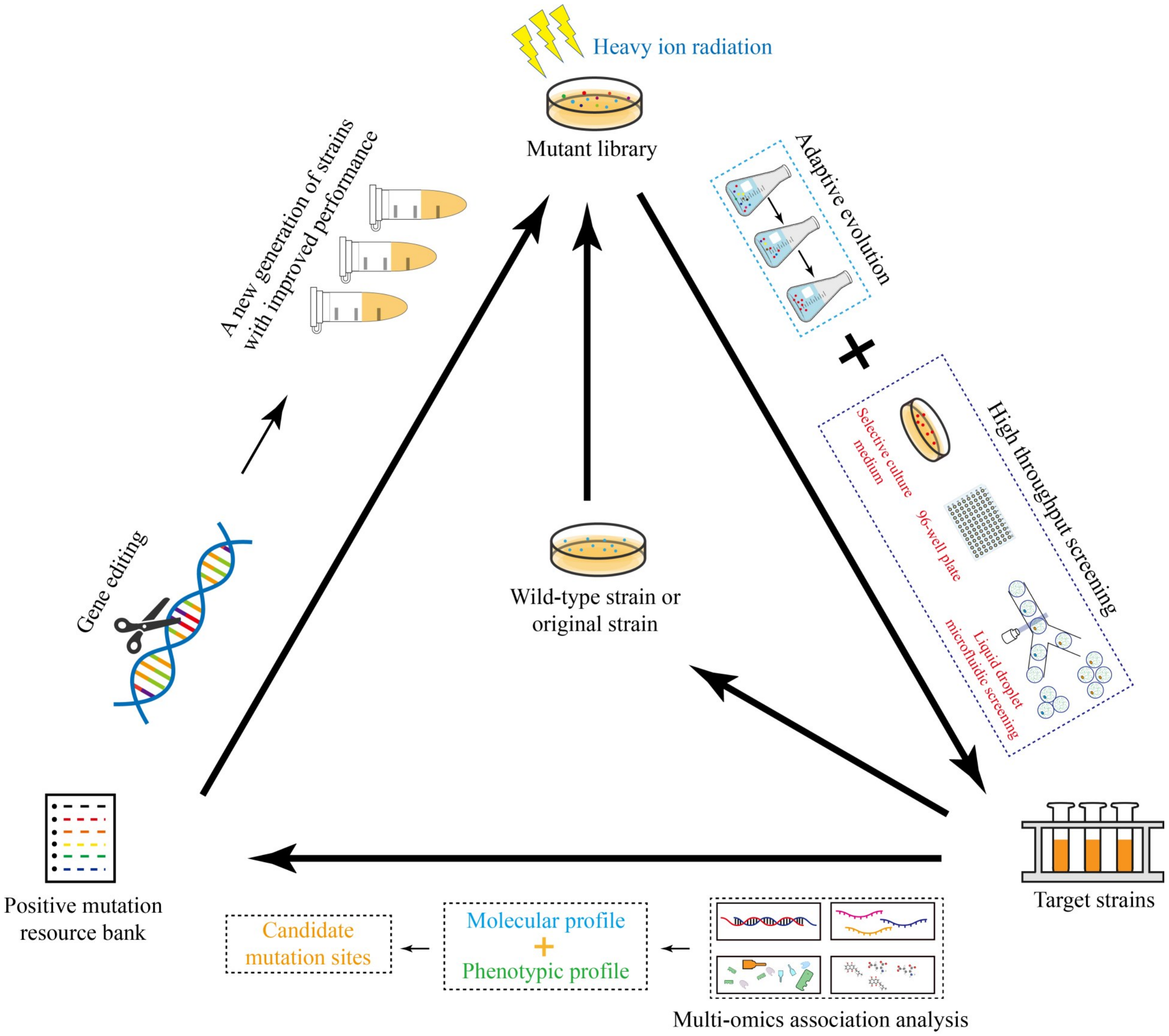

图2　辐射诱变结合遗传改造的迭代优化策略

Figure 2　Iterative optimization strategy combining radiation mutagenesis and genetic modification.

lesions in genomes, TILLING)、高分辨率溶解曲线、混池测序等[35,78-81]。

微流控技术在微生物突变体高通量筛选中的应用正是小体积、大基数思路的实践[82]。具体而言，将诱变后的活细胞悬液注入微流控筛选系统后，基于微流控芯片进行单细胞液滴分离，并自动完成液滴孵育、往复培养、液滴检测、液滴分割融合(接种)以及液滴提取和保存，最终实现高通量评估和筛选，单个运行周期的分析通量根据功能差异在数百至上万个液滴不等[83]。近年来，微流控技术在优良微生物突变体选育方面取得较多成果，例如研究者利用液滴微流控技术成功筛选出耐高浓度山梨糖的大肠杆菌(*Escherichia coli*)、高产几丁质乙酰基酶的马红球菌(*Rhodococcus equi*)突变株以及乙醇耐受性提高的异常威克汉逊酵母(*Wickerhamomyces anomalus*)等[75,84-85]。

在微流控技术的基础上结合荧光标记和分选可进一步提升突变体筛选的自动化程度。具体而言，将诱变群体细胞悬液注入分选系统后，

经过液滴发生、液滴孵育后为每个液滴进行特异性荧光染料微注入，继续孵育，最终在特定波长的激发光下接收并检测荧光信号强度以指示细胞活力或生产能力。随后基于设定的荧光阈值，收集该阈值以上的单细胞液滴至 96 孔板备份，扩大培养后进行复筛。微流控技术和流式荧光分选的结合主要基于微流控芯片的液滴生成提高了单细胞包裹率，且基于荧光染料的微注入提升了单细胞荧光染色的均匀度，同时避免了细胞之间的相互干扰[42,86]。事实上，流式分选技术广泛用于重离子辐射诱变后富油单细胞微藻突变体的筛选。Yamada 等[48]基于荧光标记的流式细胞分选技术筛选经铁离子束辐射后油脂含量增加的纤细裸藻(*Euglena gracilis*)，最终获得了油脂含量比野生型菌株高 40% 的突变体。Oyama 等[49]将重离子辐照后的衣藻(*Chlamydomonas* sp.) KOR1 在高硝酸盐的条件下培养，基于细胞的叶绿素荧光和氟硼二吡咯(boron-dipyrromethene, BODIPY)染色的荧光强度进行富脂细胞的流式分选，获得了在高硝酸盐条件下高产油脂的突变株。这些研究都显示出流式分选技术在高通量突变体筛选中潜在的应用前景。流式分选主要基于荧光光谱进行检测。除此之外，紫外-可见分光光度、傅里叶变换红外光谱、拉曼光谱等光谱学技术的应用也为重离子束辐射诱变育种实践中高通量筛选体系的建立提供了重要参考[31,53]。相较于液滴微流控技术通量高达 10^7 cells/d，96 孔板筛选的通量较低，但其具有经济性优势[87-89]。马翠等[89]开发的国产化多功能微孔板检测系统具备吸光度和荧光检测功能，结构紧凑、成本较低，可实现相对高效的自动化筛选。筛选获得的优良突变株，其表型均在实验室微量条件下验证，当菌株放大至更大规模的工业发酵罐时，必然会面临全新的环境压力。采用模拟放大筛选、阶梯式放大以及多参数验证结合的方案可以减小这种影响。此外，得到的突变株一般需经过 10 代培养以验证其稳定性。

减小体积以扩大基数的方式主要适用于微生物，而植物的高通量筛选主要基于高通量表型信息采集方法和平台的构建。例如：Awlia 等[90]结合红绿蓝图像分割(red green blue image segmentation, RGB-IS)和叶绿素荧光成像开发定量表型方案，搭建了高通量表型系统，通过该系统成功捕获了拟南芥在盐胁迫下的生长、形态、颜色和光合性能。Chang 等[91]将 γ 射线辐照处理的拟南芥种子放置在高通量表型(high-throughput phenotyping, HTP)平台上发芽生长，从 HTP 平台上拍摄的数字图像中提取的数据，可反映拟南芥 M1 幼苗在不同剂量下对 γ 辐射的细微形态反应。

基于反向遗传学思路的突变体获取建立在功能基因以及功能基因组学研究取得的进展之上，且依托于基因分型技术，向更准、更快、更高通量发展。通过识别性状关联基因或基因簇变异，有针对性地进行性状测试，进而获取目标突变株和突变位点，这对植物和微生物都具有普适性[79-81]。总之，当前新技术层出不穷，为突变体高通量筛选相关研究提供了机遇。

6　正向突变位点的高效鉴定、验证和整合

6.1　正向突变位点鉴定

优良突变体的价值不仅体现在其优良性状本身在工业应用中的潜力，更重要的是与优良性状关联的正向突变位点对种质资源的遗传改造具有重要借鉴意义。由于重离子辐射诱变可产生新的突变，而基因编辑等分子改造技术则主要基于已有突变，需要明确的靶点和变异方向。因此，对突变体进行深入的功能研究、挖掘正向突变位点，在诱变育种实践中具有重要意义[31]。基于群体的多组学分析策略，为重离子辐射诱变机理的揭示提供了新契机。首先，基于群体数据可同时获取多类群、多基因、多性状等诸多生物学信息，对于高通量挖掘有效

突变位点具有重要借鉴意义[18,25,92-93]；同时，表型组、基因组、转录组、代谢组等多组学联合策略，可进一步锁定正向突变位点，应用分子操作技术则可以对获取的表型关联位点进行功能验证和改造[9,18,94]。例如，本课题组 Lei 等[94]通过多组学方法(基因组、转录组和脂质组)发现线粒体乙醛脱氢酶编码基因 *ALD4* 是脂质代谢的核心调控靶点。在此基础上，通过重编程酿酒酵母(*S. cerevisiae*)中心碳代谢流，强化丙酮酸脱氢酶旁路，使关键前体分子乙酰辅酶 A 的含量提高了 17.10%，从而提升了脂肪酸衍生物的合成能力。Wang 等[95]通过重离子辐照处理四尾栅藻(*Scenedesmus quadricauda*)，以蛋白质组分析为主结合基因组和转录组方法发现突变株磷酸戊糖途径关键酶葡萄糖-6-磷酸-1-脱氢酶上调，显著增强了戊糖磷酸途径的流量，产生大量 NADPH，这些变化对应于碳固定和能量生成的加强。

对于非模式菌株，可先以三代测序组装的基因组图谱作为基础，进而通过转录组测序识别因突变而发生显著表达变化的关键基因；在此基础上，整合靶向或非靶向代谢组学数据，锁定与高产表型直接相关的代谢物积累变化；最后，通过关联分析将差异表达基因与差异积累代谢物映射至特定的代谢通路上，从而在多组学证据的交叉汇聚处高效缩小候选突变位点的范围[96]。

6.2 遗传操作整合突变位点

基于上述讨论，整合群体研究、多组学联合分析以及分子验证于一体的突变体分析平台，将有望在诱变育种实践中实现正向突变位点高通量、快速、准确识别的常规化(图 3)。获取的大量正向突变位点将作为遗传改造的依据，利用分子操作技术对正向突变位点进行整合，可更大程度地改善性状或集中多种优良性状[94,97]。最终实现重离子辐射诱变育种“面”的优势和基因编辑技术定点、定向的优势相结合，为新种质创制提供新思路。

7 总结与展望

基于较完整的育种流程，从选取出发菌株、确定辐射参数、制备样品、辐射处理、筛选突变体，到挖掘和利用正向突变，本文讨论了提高重离子辐射诱变育种效率的策略选择。这些策略包括在立体维度上优化辐射参数、通过调控细胞内在状态干预辐射损伤敏感性和损伤修

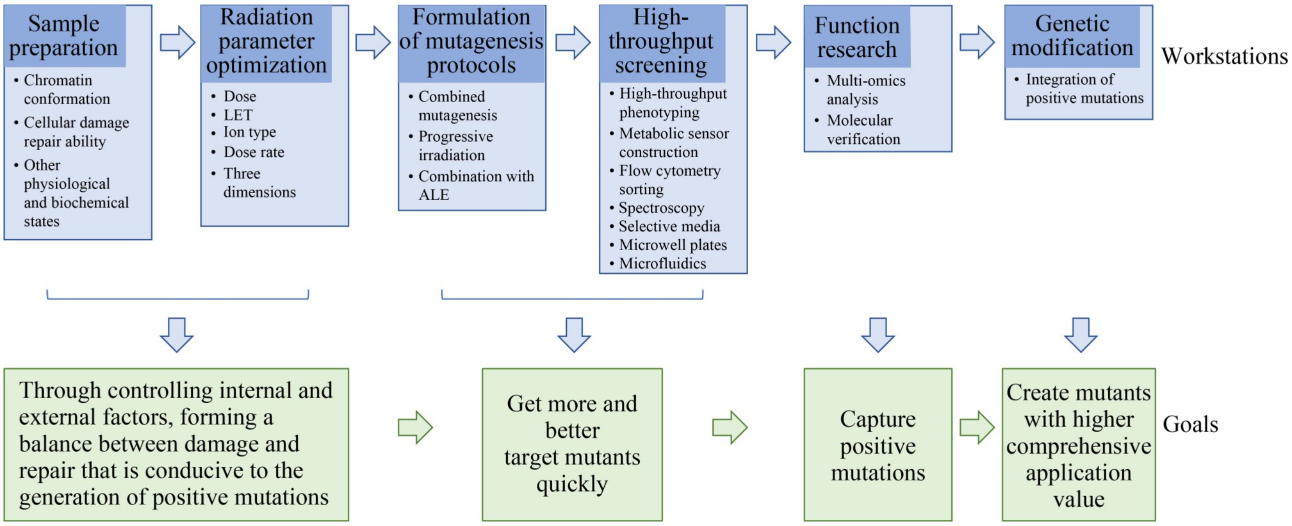

图3 通过整合多种高效策略的集成式重离子辐射育种流程

Figure 3 Integrated heavy ion radiation breeding process combining multiple efficient strategies.

复能力进而影响诱变模式、多组学联合鉴定正向突变位点和遗传操作整合正向突变位点等，这些方面仍将是重离子辐射诱变育种机理和应用的研究热点。

当在重离子辐射诱变育种的各个环节均采取相应的策略以提升育种的质量和效率时，重离子辐射诱变育种实践模式将不再是独立过程，而是由各个模块集成的工作站群，包括样品制备、辐射参数优化、诱变方案制定、高通量突变体筛选、突变体分析(功能研究)及遗传改造。未来，这一模块集成化育种实践将推动重离子辐射诱变育种的产业化应用，助力优良微生物资源高效创制。

作者贡献声明

郭晓鹏：论文构思和撰写；张书翰：论文撰写和修改；贾承霖：参与论文讨论和修改；张苗苗：文献查询和资料整理；陆栋：论文选题、写作指导和稿件修改。

作者利益冲突公开声明

作者声明不存在任何可能会影响本文所报告工作的已知经济利益或个人关系。

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