Journal of Applied Plant Biology Vol3, No1, 2026 pp: 1-۸ 1

doi 10.22034/JAPB.2025.248

Biology and Biotechnology of Duckweeds: Emerging Champions of Sustainable Bioproduction

Seyed Javad Davarpanah*

Applied Biotechnology Research Center, New Health Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.

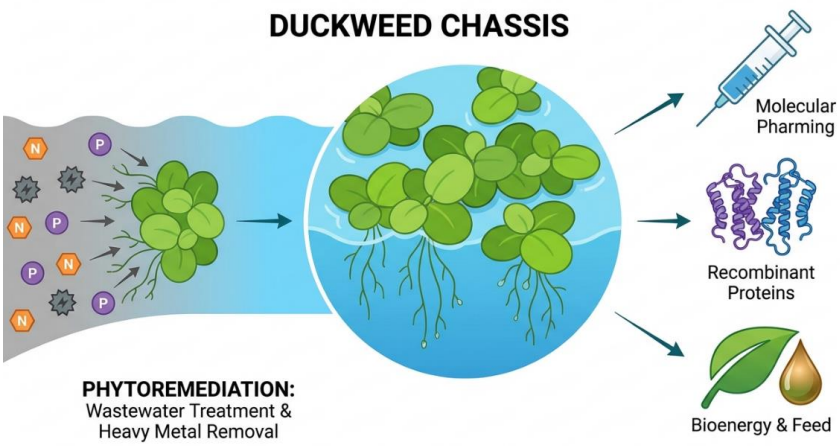
**Corresponding author Email: davarpanah@bmsu.ac.ir*

Article Revised: [date], Received: [date], Accepted: [date]

Abstract

Background: Duckweeds (Lemnaceae family), the smallest and fastest-growing flowering plants, are gaining prominence as sustainable bioproduction platforms. Their rapid growth, aquatic habit, and high protein content make them uniquely suited for applications in molecular pharming, phytoremediation, bioenergy, and nutrition. **Methods:** This narrative review synthesizes peer-reviewed literature on duckweed systematics, genomics, genetic transformation, and biotechnological applications, with particular emphasis on molecular pharming and phytoremediation. **Results:** Advances in genomics, such as the sequencing of the *Spirodela polyrhiza* genome, have provided critical insights into their streamlined architecture and physiological adaptations. Efficient *Agrobacterium*-mediated transformation protocols have been established for genera including *Lemna*, *Spirodela*, and *Wolffia*, enabling high-yield production of recombinant proteins like vaccines and antibodies. Concurrently, duckweeds demonstrate remarkable efficacy in phytoremediation, efficiently removing nutrients, heavy metals, and organic micropollutants from wastewater. **Conclusion:** The integration of duckweed biology with modern biotechnological tools positions these plants as powerful, scalable, and sustainable solutions to global challenges in health, environment, and food security. Future research focused on precision breeding and integrated biorefinery models will further unlock their potential.

Keywords: Duckweed, *Lemna*, *Spirodela*, Molecular Pharming, Phytoremediation, Genetic Transformation, Sustainable Bioproduction, Biosafety



1. Introduction

The escalating pressures of climate change, environmental pollution, and resource scarcity necessitate the development of innovative, sustainable technologies. In this context, duckweeds (family Lemnaceae) have emerged from obscurity to become a focal point of multidisciplinary research. These minute, free-floating aquatic plants, recognized as the smallest and fastest-growing flowering plants on Earth, possess a suite of characteristics ideal for biotechnological exploitation (4, 10). Their simple body plan, consisting primarily of a frond and a root, presents a complex physiology capable of rapid biomass accumulation, efficient nutrient assimilation, and high protein synthesis (40).

Historically valued as a model for plant physiology, duckweeds have been revived in the post-genomic era. The landmark sequencing of the *Spirodela polyrhiza* genome provided the first genetic blueprint for this family, revealing a streamlined genome and unique evolutionary adaptations (52). This genomic foundation has accelerated research into their diverse applications. This review synthesizes the current understanding of duckweed biology and genomics, and critically examines their transformative applications, with a particular emphasis on species-specific advancements in molecular pharming and phytoremediation, underscoring their role as a next-generation sustainable bioproduction platform, and highlights the regulatory and biosafety considerations that will shape their translation from laboratory systems into commercial platforms. Recent

calls to unify duckweed research across disciplines further underscore the timeliness of this synthesis (58).

2. Systematics, Biology, and Genomics

2.1. Systematics and Morphology

Duckweeds comprise five genera—*Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia* which form a monophyletic group within the Araceae family (37). This lineage exhibits a clear trend of morphological reduction, from *Spirodela* with multiple roots to the rootless, globular *Wolffia*, the world's smallest angiosperm. They reproduce predominantly through clonal vegetative budding, facilitating exponential growth. Certain species also form turions, dormant buds that allow survival in unfavorable conditions (43).

2.2. Genomic and Cytogenetic Features

A striking feature of duckweeds is the immense 12-fold variation in genome size across the family, from a compact 158 Mbp in *Spirodela polyrhiza* to 1,881 Mbp in *Wolffia arrhiza* (10, 26). Despite this variation, chromosome numbers are relatively conserved, suggesting complex processes of genome expansion and contraction, largely driven by transposable element activity (10). Sequenced genomes reveal a reduced repertoire of protein-coding genes, with losses in families related to root development and pathogen defense, reflecting their specialized niche (52). Furthermore, duckweeds exhibit exceptionally low global DNA methylation levels, a trait potentially linked to their asexual lifestyle and rapid growth (10).

2.3. Genetic Diversity and Barcoding

Morphological simplicity complicates species identification, making DNA barcoding essential. The plastid spacers *atpF-atpH* and *psbK-psbI* constitute a highly effective two-marker system for discriminating most species (8). While intra-specific genetic diversity is often low, phenotypic variation among ecotypes in traits like growth rate and starch accumulation is significant, highlighting the value of germplasm collections such as the Landolt Duckweed Collection (10, 49).

3. Advanced Genetic Transformation and Tool Development

3.1. Stable Nuclear Transformation

Agrobacterium tumefaciens-mediated transformation is the most established method. Early protocols relied on callus induction, a process that was lengthy and genotype-dependent (38, 55). Recent innovations have overcome these hurdles. For example, a novel turion transformation system for *Spirodela polyrhiza* achieved up to 90% efficiency by exploiting the natural resilience of these dormant organs (11). Similarly, a Frond Transformation System (FTS) for *Lemna minor* bypasses the callus phase, yielding stable transgenic lines in approximately three months (56). These advances have enabled high-yield production, as demonstrated in *Spirodela oligorrhiza*, where Green Fluorescent Protein (GFP) accumulated to more than 25% of Total Soluble Protein (TSP) (51). The transformation toolkit has since been extended to the rootless genus *Wolffia* as well: efficient *Agrobacterium*-mediated protocols for both stable and transient transformation have been established in *Wolffia globosa*, broadening the range of duckweed genera available as bioproduction chassis (24).

3.2. Transient Expression Systems

For rapid, high-level protein production, transient systems are superior. A robust *Agrobacterium*-mediated transient system in *S. polyrhiza* and *Landoltia punctata* utilized a Potato Virus X (PVX)-based vector to achieve GFP yields exceeding 1 mg/g fresh weight, rivaling established platforms like *Nicotiana benthamiana* (44).

3.3. Enabling Genetic Tools

The development of endogenous genetic tools is crucial. The LpSUT2 promoter from *Landoltia punctata* was shown to outperform the conventional CaMV 35S promoter under antibiotic stress by resisting methylation-induced silencing (53). Furthermore, CRISPR-Cas9-mediated genome editing has also been established in *Lemna aequinoctialis*, opening opportunities for targeted metabolic engineering (39).

4. Duckweeds in Molecular Pharming and High-Value Products

4.1. Pharmaceutical Proteins and Edible Vaccines

Lemna minor is a leading platform for pharmaceuticals. It has been engineered to express an M2e peptide from Avian Influenza Virus as a GUS fusion, accumulating to 1.96% of TSP, demonstrating potential for edible vaccine development (17). In a groundbreaking application, freeze-dried *L. minor* biomass expressing Chicken Interleukin-17B was used as an oral mucosal vaccine adjuvant, significantly enhancing protective immunity in chickens (50). The production of human Granulocyte Colony-Stimulating Factor (hG-CSF) in controlled bioreactors further underscores the industrial scalability of this system (34).

The rootless genus *Wolffia* has likewise been validated as an edible vaccine chassis: *Wolffia globosa* engineered to express the LamB outer-membrane antigen of *Vibrio alginolyticus* conferred significantly improved survival in fish challenged with the pathogen, demonstrating the feasibility of a fully edible, extraction-free oral vaccine (25). Because *Wolffia* biomass can be consumed directly without downstream purification, such systems illustrate a distinctive advantage of duckweed-based molecular pharming over non-edible hosts such as tobacco for oral antigen delivery (58).

4.2. Industrial Enzymes and Technical Proteins

Beyond pharmaceuticals, duckweeds have been engineered to produce industrial enzymes. The *Acidothermus cellulolyticus* E1 endoglucanase has been expressed in *Lemna minor*, demonstrating its potential as a cost-effective production system for biofuel and paper industries (48).

4.3. Biofortification and Valuable Metabolites

Metabolic engineering can enhance native profiles. Expression of a sterol 22-desaturase in *Lemna minor* via FTS resulted in a 397% increase in stigmasterol, a valuable phytosterol (56). The rootless *Wolffia* genus has also been explored as a contained bioreactor, with

successful production of the anticoagulant peptide hirudin in *Wolffia arrhiza* (35).

Table 1. Selected recombinant proteins and metabolites produced in transgenic duckweeds

Host species	Product	Expression system	Yield / outcome	Application	Reference
Lemna minor	M2e peptide (avian influenza)	Stable nuclear (GUS fusion)	1.96% TSP	Edible/mucosal vaccine candidate	17
Lemna minor	Chicken interleukin-17B	Stable nuclear	Enhanced protective immunity	Oral mucosal vaccine adjuvant	50
Wolffia arrhiza	Human G-CSF	Stable, controlled bioreactor	Industrial-scale production	Biopharmaceutical	34
Lemna minor	*Acidothermus cellulolyticus* E1 endoglucanase	Stable nuclear	—	Industrial enzyme (biofuel, paper)	48
Lemna minor	Sterol 22-desaturase (stigmasterol)	FTS (stable)	397% increase in stigmasterol	Biofortification	56
Wolffia arrhiza	Hirudin	Stable, contained bioreactor	—	Anticoagulant peptide	35
Wolffia globosa	LamB antigen (*V. alginolyticus*)	Stable nuclear	Improved fish survival post-challenge	Edible fish vaccine	25
Spirodela oligorrhiza	GFP	Stable nuclear	>25% TSP	Reporter / yield benchmark	51
S. polyrhiza / *Landoltia punctata*	GFP (PVX vector)	Transient (*Agrobacterium*)	>1 mg/g fresh weight	Rapid protein production	44

Abbreviations: TSP, total soluble protein; GUS, β-glucuronidase; G-CSF, granulocyte colony-stimulating factor; PVX, potato virus X; FTS, frond transformation system; —, not reported.

5. Duckweed versus Established Bioproduction Platforms

5.1. Duckweed in comparison with *Nicotiana benthamiana*

Nicotiana benthamiana has become one of the most established plant hosts for transient molecular farming because of its high susceptibility to *Agrobacterium*-mediated infiltration, rapid recombinant-protein accumulation, and the extensive development of viral and non-viral expression systems. Modern plant molecular farming increasingly combines transient expression with optimized genetic circuits, viral replicons, host engineering, and glycoengineering to improve recombinant-protein yield, quality, and consistency (6, 14). These developments have made *N. benthamiana* particularly attractive for rapid production and evaluation of vaccine antigens, antibodies, enzymes, and other complex recombinant proteins. Beritza et al. (6) emphasized that optimization of agroinfiltration, host responses, and intracellular protein stability remains an important route for further increasing transient expression in *N. benthamiana*.

Duckweeds offer a substantially different production strategy. Rather than relying primarily on transient infiltration of mature leaves, duckweeds combine rapid vegetative multiplication with stable transformation and cultivation in soil-free aquatic systems. Their ability to generate biomass through clonal propagation, together with relatively simple cultivation requirements, represents a potentially attractive feature for continuous recombinant biomass production. Recent reviews have emphasized that duckweed species possess diverse genetic resources and increasingly effective transformation systems, while recombinant vaccines and therapeutic proteins have already been produced in several genera, including *Lemna*, *Spirodela*, and *Wolffia* (33). Thus, the principal advantage of duckweed may not necessarily be a higher instantaneous expression level than *N. benthamiana*, but rather the combination of rapid biomass multiplication, stable propagation, aquatic cultivation, and the possibility of integrating production with other biomass-based applications (33).

An informative example is provided by recombinant influenza vaccine antigens. Bertran et al. (7) demonstrated that hemagglutinin produced in transgenic *Lemna minor* could generate a protective immune response against highly pathogenic H5N1 influenza virus in chickens. Earlier work also demonstrated high-level production of influenza M2e antigen in transgenic *L. minor*, with accumulation reaching 1.96% of total soluble protein (17). These findings establish that stable duckweed systems can generate biologically functional vaccine antigens rather than merely reporter proteins. In comparison, *N. benthamiana* has been extensively exploited for transient expression of influenza antigens and numerous other recombinant vaccine candidates, benefiting from the maturity of agroinfiltration and transient-expression technologies (6, 14).

The two platforms should therefore be regarded as complementary rather than directly substitutable. *N. benthamiana* currently has a clear technological advantage for rapid transient expression, construct screening, and short-cycle recombinant-protein production. Duckweed, in contrast, is particularly attractive when stable biomass generation, clonal propagation, soil-free cultivation, and potentially contained aquatic production are important considerations (33). This distinction is particularly relevant when selecting a host according to the intended product and production strategy rather than simply comparing maximum expression levels.

Another relevant distinction concerns the transition from laboratory expression to an integrated manufacturing process. Although plant molecular farming offers

important advantages in speed and production flexibility, it remains less horizontally integrated than established microbial and mammalian platforms, particularly with respect to standardized process development, analytical workflows, and routine clinical translation (9). Duckweed therefore faces not only the general challenges of plant molecular farming but also species-specific limitations related to transformation efficiency, cultivation standardization, biomass harvesting, downstream processing, and regulatory acceptance (33).

5.2. Duckweed versus microbial and mammalian expression systems

Compared with *Escherichia coli*, duckweed possesses an important biological advantage for recombinant proteins that require eukaryotic folding, disulfide-bond formation, secretion, or post-translational processing. *E. coli* remains one of the most widely used and cost-effective hosts for recombinant protein production because of its rapid growth, well-established fermentation technology, extensive genetic toolbox, and relatively simple downstream processing. However, bacterial expression can be constrained by protein aggregation, toxicity, inefficient folding, and the absence of many eukaryotic post-translational modifications (28). Duckweed, as a eukaryotic plant system, can potentially overcome some of these limitations for selected proteins, although plant-specific glycosylation patterns must also be considered when the target is a therapeutic glycoprotein (14).

Yeast systems, particularly *Komagataella phaffii* (*Pichia pastoris*), occupy an important intermediate position between bacterial and higher-eukaryotic production platforms. They combine the scalability and process control of microbial fermentation with eukaryotic protein-folding machinery and secretion capabilities. Consequently, yeast can be highly effective for secreted enzymes and selected therapeutic proteins. Duckweed instead offers the possibility of biomass-based cultivation and plant-specific biological processing, but its industrial bioprocessing infrastructure is considerably less mature than microbial fermentation (9, 33).

Mammalian cell systems, particularly Chinese hamster ovary (CHO) cells, remain the benchmark for many therapeutic glycoproteins because of their capacity to generate post-translational modifications that are highly compatible with human proteins and because of decades of development in cell-line engineering, bioreactor operation, quality control, and regulatory manufacturing. Geng et al. (18) highlighted both the importance of CHO cells for recombinant therapeutic proteins and the extensive engineering strategies available to overcome intracellular degradation and improve product quality. Plant systems can offer advantages in production flexibility and potentially lower upstream costs, but they still face challenges associated with product consistency, purification, process standardization, and regulatory translation (9, 14).

Microalgae represent another potentially relevant platform because they combine photosynthetic biomass production with the possibility of stable genetic engineering and cultivation in controlled photobioreactor systems. Recent work has emphasized the increasing potential of microalgae for production of biopharmaceuticals, although transformation efficiency, expression stability, cultivation requirements, and species-specific limitations remain important barriers to broader application (19). In this context, duckweed occupies an intermediate and potentially distinctive position: like microalgae, it can be cultivated without agricultural soil and can utilize aquatic production systems, while, like higher plants, it possesses a complex eukaryotic cellular machinery suitable for production of recombinant proteins and metabolites (33).

The most defensible positioning of duckweed is therefore not as a universal replacement for *E. coli*, yeast, CHO cells, *N. benthamiana*, or microalgae. Rather, duckweed represents a complementary biological chassis with a distinctive combination of rapid clonal biomass production, aquatic cultivation, genetic tractability, and potential integration with food/feed, environmental remediation, and biorefinery processes (33). Its commercial competitiveness will ultimately depend on whether these biological advantages can be translated into reproducible biomass productivity, high product recovery, acceptable downstream-processing costs, effective containment, and regulatory approval.

Table 2. Comparative characteristics of major platforms for recombinant protein production

Feature	Duckweed (*Lemna*, *Spirodela*, *Wolffia*)	*Nicotiana benthamiana*	*E. coli*	*K. phaffii*	CHO cells	Microalgae
Primary production strategy	Stable transformation; emerging transient systems	Transient agroinfiltration; stable systems also available	Fermentation	Fermentation	Mammalian cell culture	Stable/transient expression; photobioreactors
Growth rate	Rapid clonal biomass multiplication	Rapid terrestrial biomass production	Very rapid	Rapid	Relatively slow	Rapid, species-dependent
Soil requirement	No	Generally yes/controlled terrestrial cultivation	No	No	No	No
Genetic toolbox	Developing; species-dependent	Highly developed	Very highly developed	Highly developed	Highly developed	Developing; species-dependent
Transient expression maturity	Moderate/emerging	Very high	Very high	High	High	Emerging/ moderate
Stable recombinant lines	Well demonstrated in several species	Demonstrated	Routine	Routine	Routine	Species-dependent
Eukaryotic folding/PTMs	Yes	Yes	Limited	Yes	Excellent	Yes
Human-compatible glycosylation	Requires engineering/ characterization	Requires engineering for some products	Generally not suitable	Requires engineering for selected products	Strong benchmark	Species-dependent
Complex recombinant proteins	Demonstrated	Extensively demonstrated	Frequently challenging	Demonstrated	Excellent	Demonstrated for selected proteins
Cultivation infrastructure	Relatively simple aquatic systems	Greenhouse/controlled environment	Fermenter	Fermenter	Advanced bioreactor	Photobioreactor
Land requirement	Potentially minimal	Higher than aquatic systems	None	None	None	None
Containment potential	High in appropriately closed aquatic systems	High in controlled production systems	Very high	Very high	Very high	High in closed systems
Whole-biomass applications	Potential advantage	Limited	No	No	No	Potential
Phytoremediation integration	Major unique advantage	Limited	No	No	No	Potential
Process-development maturity	Emerging	Advanced plant platform	Very high	Very high	Very high	Emerging
Major competitive strength	Rapid clonal biomass + aquatic/contained cultivation + multifunctionality	Rapid transient expression and rapid prototyping	Low cost + rapid growth + mature fermentation	Secretion + scalable fermentation	Product quality + mature bioprocessing	Photosynthetic production + aquatic cultivation
Major limitation	Scale-up, standardization, downstream processing, regulatory maturity	Dependence on transient-expression infrastructure and plant biomass processing	Limited eukaryotic PTMs	Product-specific PTM limitations	High cost and process complexity	Genetic and process limitations are species-dependent

Abbreviations: PTM, post-translational modification; CHO, Chinese hamster ovary.

6. Duckweeds in Phytoremediation and Environmental Management

6.1. Nutrient Recovery and Heavy Metal Removal

Duckweeds are exceptionally efficient at scavenging nitrogen and phosphorus from wastewater, mitigating eutrophication while producing protein-rich biomass (22, 40). They also act as biofilters for heavy metals like cadmium and arsenic, hyperaccumulating them through chelation and vacuolar sequestration (15). At the molecular level, genome-wide characterization of the phosphorus- and nitrogen-responsive SPX and GARP transcription-factor superfamilies in *Spirodela polyrhiza* has begun to reveal the regulatory architecture underlying this efficient nutrient scavenging, identifying candidate genes for engineering enhanced phytoremediation capacity (57, 61).

6.2. Degradation of Organic Micropollutants

Duckweeds effectively degrade complex organic molecules. *Lemna aoukikusa* and *Lemna aequinoctialis* remediates pharmaceutical wastewater and swine wastewater contaminated with the antibiotic oxytetracycline, with research identifying twelve degradation intermediates (23, 27). Furthermore, *Lemna minor* has been shown to remove pharmaceuticals and personal care products (PPCPs), such as diclofenac, from urban wastewater (41, 59). The efficacy of duckweed-based systems is often enhanced by its associated microbiome, which can improve stress tolerance and directly contribute to the degradation of hydrocarbons and other pollutants (29, 30, 31, 40).

7. Other Biotechnological Applications

7.1. Bioenergy Production

Under nutrient starvation, duckweeds accumulate high levels of starch (up to 50% dry weight), making them a promising non-food feedstock for bioethanol production (3). The whole biomass can be utilized for biogas production via anaerobic digestion.

7.2. Sustainable Feed and Human Nutrition

With a protein content of 25-45% dry weight and a favorable amino acid profile, duckweed biomass is a sustainable feed supplement in aquaculture and poultry farming (1). *Wolffia* species are consumed as a vegetable in Southeast Asia and are being commercialized as a nutrient-dense superfood (2, 10).

7.3. Carbon Sequestration and Space Applications

Their rapid growth makes duckweeds efficient at capturing atmospheric CO_2. Their potential extends to space exploration, where they are investigated for Bioregenerative Life Support Systems (BLSS) to treat wastewater, produce oxygen, and provide fresh food for astronauts (10, 46).

8. Regulatory and Biosafety Considerations

As duckweed-based platforms move toward commercial and clinical application, regulatory and biosafety considerations warrant explicit attention. Plants used for phytoremediation and bioproduction carry biological risks independent of their transgenic status, including the potential for establishment, spread, and introgression of transgenes into related populations (21).

Duckweed's obligate aquatic habit, highly reduced root system, clonal propagation, and rare flowering under standard cultivation substantially limit its capacity to establish in terrestrial ecosystems and reduce the likelihood of pollen-mediated transgene flow (5, 16, 45, 62); however, these built-in features are contingent on appropriate physical containment (13, 54) and do not remove the need for site-specific risk assessment, particularly for open-pond cultivation which is reinforced by regulatory frameworks emphasizing that gene flow pathways, ecological consequences, and local environmental conditions must be evaluated case by case (Dolezel et al. 2025).

Rare flowering under standard cultivation is well-established, with multiple studies noting that flower induction requires specific experimental protocols and occurs infrequently in nature (36). The male sterility of interspecific hybrids further constrains pollen-mediated gene flow (36, 47). However, hybridization does occur in nature — *L.* × *mediterranea* was detected across 4200 km² in southern Italy and that clonal lineages can disperse over 130 km via waterfowl (47), demonstrating that biological barriers are not absolute.

Molecular containment strategies developed for other transgenic crops — including inducible kill-switches, marker-free transformation, and chloroplast-level containment — offer additional safeguards that merit systematic evaluation in duckweed systems (12). Regulatory pathways for plant-made pharmaceuticals and industrial proteins also differ substantially between jurisdictions, and early engagement with regulatory and biosafety authorities will be essential for translating laboratory-scale duckweed platforms into approved products. Integrating biosafety governance into the design of transformation systems, rather than treating it as a downstream hurdle, would strengthen the credibility and pace of duckweed-based bioproduction.

9. Challenges and Future Prospects

Despite significant progress, challenges remain, including optimizing transformation for all species and managing metal-laden biomass from phytoremediation. Future prospects are bright and they will focus on:

1. Precision Breeding: Widespread adoption of CRISPR-Cas9 for creating tailor-made lines.
2. Multi-Omics Integration: Using systems biology for predictive engineering.
3. Consortium-Based Remediation: Designing synthetic microbial communities to enhance pollutant degradation.
4. Biorefinery Concepts: Developing integrated processes to fractionate biomass into protein, starch, and metabolites, maximizing value and sustainability.
5. Regulatory Harmonization: Engaging biosafety and regulatory agencies early to establish clear approval pathways for duckweed-derived pharmaceuticals, industrial proteins, and food/feed products.

10. Conclusion

Duckweeds are increasingly being recognized as promising platforms for sustainable biotechnology, although substantial technical and regulatory barriers remain before broad industrial adoption. Their unique biology, advanced genomic resources, and sophisticated genetic tools position them as powerful contributors to a sustainable future. Through species-specific applications—from producing pharmaceuticals in *Lemna minor* to purifying wastewater with *Spirodela polyrhiza*—duckweeds offer versatile solutions across multiple industries. As research continues to unlock their full potential, they are poised to become a cornerstone of the global bioeconomy. Realizing this promise fully will require parallel investment in scale-up bioprocess engineering, regulatory science, and public acceptance, alongside continued advances in genetic tool development.

Funding

This research received no external funding.

Conflicts of Interest

The author declares no conflict of interest.

References

1. Appenroth K-J, Sree KS, Böhm V, et al. Nutritional value of duckweeds (Lemnaceae) as human food. Food Chem. 2017; 217: 266–273. https://doi.org/10.1016/j.foodchem.2016.08.116
2. Appenroth K-J, Sree KS, Bog M, et al. Nutritional value of the duckweed species of the genus *Wolffia* (Lemnaceae) as human food. Front Chem. 2018; 6: 483. https://doi.org/10.3389/fchem.2018.00483

3. Appenroth K-J, Ziegler P, Sree KS. Accumulation of starch in duckweeds (Lemnaceae), potential energy plants. Physiol Mol Biol Plants. 2021; 27(11): 2621–2633. https://doi.org/10.1007/s12298-021-01100-4
4. Baek G, Saeed M, Choi H-K. Duckweeds: their utilization, metabolites and cultivation. Appl Biol Chem. 2021; 64(1): 73. https://doi.org/10.1186/s13765-021-00644-z
5. Barragán-Borrero V, de Santana Lopes A, Batista EDR, Höfer M, Elias R, Chakraborty A, et al. Strain, procedures, and tools for reproducible genetic transformation and genome editing of the emerging plant model *Spirodela polyrhiza*. New Phytol. 2025; 250: 735–756. https://doi.org/10.1111/nph.70919
6. Beritza K, Watts EC, van der Hoorn RAL. Improving transient protein expression in agroinfiltrated *Nicotiana benthamiana*. New Phytol. 2024; 243: 846–850. https://doi.org/10.1111/nph.19894
7. Bertran K, Thomas C, Guo X, et al. Expression of H5 hemagglutinin vaccine antigen in common duckweed (*Lemna minor*) protects against H5N1 high pathogenicity avian influenza virus challenge in immunized chickens. Vaccine. 2015; 33: 3456–3462. https://doi.org/10.1016/j.vaccine.2015.05.076
8. Bog M, Appenroth K-J, Sree KS. Duckweed (Lemnaceae): its molecular taxonomy. Front Sustain Food Syst. 2019; 3: 117. https://doi.org/10.3389/fsufs.2019.00117
9. Buyel JF. Towards a seamless product and process development workflow for recombinant proteins produced by plant molecular farming. Biotechnol Adv. 2024; 75: 108403. https://doi.org/10.1016/j.biotechadv.2024.108403
10. Cao XH, Fourounjian P, Wang W, editors. The duckweed genomes. Compendium of Plant Genomes. Springer International Publishing; 2020. https://doi.org/10.1007/978-3-030-11045-1
11. Chanroj S, Jaiprasert A, Issaro N. A novel technique for recombinant protein expression in duckweed (*Spirodela polyrhiza*) turions. J Plant Biotechnol. 2021; 48(3): 156–164. https://doi.org/10.5010/JPB.2021.48.3.156
12. Daniell H. Molecular strategies for gene containment in transgenic crops. Nat Biotechnol. 2002; 20(6): 581–586. https://doi.org/10.1038/nbt0602-581
13. Deepika C, Hankamer B, Ross I. Can microalgal omega-3 PUFA production systems remain viable as GM plant sources emerge? Bioresour Technol. 2025; 133525. https://doi.org/10.1016/j.biortech.2025.133525
14. Eidenberger L, Kogelmann B, Steinkellner H. Plant-based biopharmaceutical engineering. Nat Rev Bioeng. 2023; 1: 426–439. https://doi.org/10.1038/s44222-023-00044-6
15. Ekperusi AO, Sikoki FD, Nwachukwu EO. Application of common duckweed (*Lemna minor*) in phytoremediation of chemicals in the environment: state and future perspective. Chemosphere. 2019; 223: 285–309. https://doi.org/10.1016/j.chemosphere.2019.02.025
16. Ernst E, Abramson B, Acosta K, Hoang PT, Mateo-Elizalde C, Schubert V, et al. Duckweed genomes and epigenomes underlie triploid hybridization and clonal reproduction. Curr Biol. 2025; 35: 1828–1847.e9. https://doi.org/10.1016/j.cub.2025.03.013
17. Firsov A, Tarasenko I, Mitiouchkina T, et al. High-yield expression of M2e peptide of avian influenza virus H5N1 in transgenic duckweed plants. Mol Biotechnol. 2015; 57(7): 653–661. https://doi.org/10.1007/s12033-015-9855-4
18. Geng SL, Zhao XJ, Zhang X, et al. Recombinant therapeutic proteins degradation and overcoming strategies in CHO cells. Appl Microbiol Biotechnol. 2024; 108: 182. https://doi.org/10.1007/s00253-024-13008-6
19. Gerszberg A, Hnatuszko-Konka K, et al. In vitro culture, genetic transformation and the production of biopharmaceuticals in microalgae. Int J Mol Sci. 2025; 26: 3890. https://doi.org/10.3390/ijms26083890
20. Golubova D, Tansley C, Su H, Patron NJ. Engineering *Nicotiana benthamiana* as a platform for natural product biosynthesis. Curr Opin Plant Biol. 2024; 81: 102611. https://doi.org/10.1016/j.pbi.2024.102611
21. Gressel J, Al-Ahmad H. Assessing and managing biological risks of plants used for bioremediation, including risks of transgene flow. Z Naturforsch C. 2005; 60(3–4): 154–165. https://doi.org/10.1515/znc-2005-3-402
22. Gupta C, Prakash D. Duckweed: an effective tool for phyto-remediation. Toxicol Environ Chem. 2013; 95(8): 1256–1266. https://doi.org/10.1080/02772248.2013.879309
23. Habaki H, Thyagarajan N, Li Z, Wang S, Zhang J, Egashira R. Removal of antibiotics from pharmaceutical wastewater using *Lemna aoukikusa* (duckweed). Sep Sci Technol. 2023; 58(8): 1491–1501. https://doi.org/10.1080/01496395.2023.2195544
24. Heenatigala PPM, Yang J, Bishopp A, Sun Z, Li G, Kumar S, et al. Development of efficient protocols for stable and transient gene transformation for *Wolffia globosa* using *Agrobacterium*. Front Chem. 2018; 6: 227. https://doi.org/10.3389/fchem.2018.00227
25. Heenatigala PPM, Sun Z, Yang J, Zhao X, Hou H. Expression of LamB vaccine antigen in *Wolffia globosa* (duck weed) against fish vibriosis. Front Immunol. 2020; 11: 1857. https://doi.org/10.3389/fimmu.2020.01857
26. Hoang PTN, Fiebig A, Novák P, et al. Chromosome-scale genome assembly for the duckweed *Spirodela intermedia*, integrating cytogenetic maps, PacBio and Oxford Nanopore libraries. Sci Rep. 2020; 10(1): 19230. https://doi.org/10.1038/s41598-020-75728-9
27. Hu H, Zhou Q, Li X, et al. Phytoremediation of anaerobically digested swine wastewater contaminated by oxytetracycline via *Lemna aequinoctialis*: nutrient removal, growth characteristics and degradation pathways. Bioresour Technol. 2019; 291: 121853. https://doi.org/10.1016/j.biortech.2019.121853
28. İncir İ, Kaplan Ö. *Escherichia coli* as a versatile cell factory: advances and challenges in recombinant protein production. Protein Expr Purif. 2024; 219: 106463. https://doi.org/10.1016/j.pep.2024.106463
29. Ishizawa H, Kuroda M, Inoue K, Inoue D, Morikawa M, Ike M. Colonization and competition dynamics of plant growth-promoting/inhibiting bacteria in the phytosphere of the duckweed *Lemna minor*. Microb Ecol. 2019; 77(2): 440–450. https://doi.org/10.1007/s00248-018-1306-x
30. Ishizawa H, Kuroda M, Inoue D, Morikawa M, Ike M. Community dynamics of duckweed-associated bacteria upon inoculation of plant growth-promoting bacteria. FEMS Microbiol Ecol. 2020; 96(7): fiaa101. https://doi.org/10.1093/femsec/fiaa101
31. Ishizawa H, Saimee Y, Sugiyama T, et al. Duckweed as an emerging model system for plant–microbiome interactions. Environ Microbiol. 2025; 27(9): e70181. https://doi.org/10.1111/1462-2920.70181
32. Kanagarajan S, Tolf C, Lundgren A, Waldenström J, Brodelius PE. Transient expression of hemagglutinin antigen from low pathogenic avian influenza A (H7N7) in *Nicotiana benthamiana*. PLoS ONE. 2012; 7: e33010. https://doi.org/10.1371/journal.pone.0033010
33. Kang J, Kim K, Do THT, Han M, Lee Y. Duckweeds for plant molecular farming: advances, challenges, and future directions. J Plant Biol. 2025; 68: 77–91. https://doi.org/10.1007/s12374-025-09458-8
34. Khvatkov P, Firsov A, Shvedova A, et al. Development of *Wolffia arrhiza* as a producer for recombinant human granulocyte colony-stimulating factor. Front Chem. 2018; 6: 304. https://doi.org/10.3389/fchem.2018.00304
35. Khvatkov P, Firsov A, Shvedova A, et al. *Wolffia arrhiza* as a promising producer of recombinant hirudin. 3 Biotech. 2021; 11(5): 209. https://doi.org/10.1007/s13205-021-02762-3
36. Lee Y, Braglia L, Stepanenko A, Fuchs J, Schubert V, Gianì S, et al. Hybridity of mainly asexually propagating duckweeds in genus *Lemna* – dead end or breakthrough? New Phytol. 2025; 250: 629–647. https://doi.org/10.1111/nph.70748
37. Les DH, Landolt E, Crawford DJ. Systematics of the Lemnaceae (duckweeds): inferences from micromolecular and morphological data. Plant Syst Evol. 1997; 204(3–4): 161–177. https://doi.org/10.1007/BF00989203

38. Li J, Jain M, Vunsh R, et al. Callus induction and regeneration in *Spirodela* and *Lemna*. Plant Cell Rep. 2004; 22(7): 457–464. https://doi.org/10.1007/s00299-003-0724-4
39. Liu Y, Wang Y, Xu S, et al. Efficient genetic transformation and CRISPR/Cas9-mediated genome editing in *Lemna aequinoctialis*. Plant Biotechnol J. 2019; 17(11): 2143–2152. https://doi.org/10.1111/pbi.13128
40. Liu Y, Xu H, Yu C, Zhou G. Multifaceted roles of duckweed in aquatic phytoremediation and bioproducts synthesis. GCB Bioenergy. 2021; 13(1): 70–82. https://doi.org/10.1111/gcbb.12747
41. Matamoros V, Nguyen LX, Arias CA, Salvadó V, Brix H. Evaluation of aquatic plants for removing polar microcontaminants: a microcosm experiment. Chemosphere. 2012; 88(10): 1257–1264. https://doi.org/10.1016/j.chemosphere.2012.04.004
42. Pan Y, Yang J, Wu J, Yang L, Fang H. Current advances of *Pichia pastoris* as cell factories for production of recombinant proteins. Front Microbiol. 2022; 13: 1059777. https://doi.org/10.3389/fmicb.2022.1059777
43. Pasaribu B, Acosta K, Aylward A, et al. Genomics of turions from the greater duckweed reveal its pathways for dormancy and re-emergence strategy. New Phytol. 2023; 239(1): 116–131. https://doi.org/10.1111/nph.18941
44. Peterson A, Kishchenko O, Zhou Y, et al. Robust *Agrobacterium*-mediated transient expression in two duckweed species (Lemnaceae) directed by non-replicating, replicating, and cell-to-cell spreading vectors. Front Bioeng Biotechnol. 2021; 9: 761073. https://doi.org/10.3389/fbioe.2021.761073
45. Redmond C, O'Mahoney R, Blanchard M, Coughlan NE. Varied growth media necessitate different light regimes for indoor duckweed cultivation. Plants. 2025; 14(3): 397. https://doi.org/10.3390/plants14030397
46. Romano LE, Aronne G. The key role of duckweed (Lemnaceae) in human space flight. Paper presented at: 116° Congresso della Società Botanica Italiana, VII International Plant Science Conference; 2021; Italy. https://drive.google.com/file/d/1I7I5NRyYWbvhsuWwEi8cD0AJpH24kO1k/view?pli=1
47. Romano LE, Braglia L, Iannelli M, Lee Y, Gianì S, Gavazzi F, et al. A survey of duckweed species in Southern Italy provided first distribution records of the hybrid *Lemna* × *mediterranea* in nature. bioRxiv. 2025. https://doi.org/10.1101/2024.08.09.607168
48. Sun Y, Cheng JJ, Himmel ME, et al. Expression and characterization of *Acidothermus cellulolyticus* E1 endoglucanase in transgenic duckweed *Lemna minor* 8627. Bioresour Technol. 2007; 98(15): 2866–2872. https://doi.org/10.1016/j.biortech.2006.09.055
49. Taghipour E, Bog M, Frootan F, et al. DNA barcoding and biomass accumulation rates of native Iranian duckweed species for biotechnological applications. Front Plant Sci. 2022; 13: 1034238. https://doi.org/10.3389/fpls.2022.1034238
50. Tan X, Chen S, Fang Y, et al. Rapid and highly efficient genetic transformation and application of interleukin-17B expressed in duckweed as mucosal vaccine adjuvant. Biomolecules. 2022; 12(12): 1881. https://doi.org/10.3390/biom12121881
51. Vunsh R, Li J, Hanania U, et al. High expression of transgene protein in *Spirodela*. Plant Cell Rep. 2007; 26(9): 1511–1519. https://doi.org/10.1007/s00299-007-0361-4
52. Wang W, Haberer G, Gundlach H, et al. The *Spirodela polyrhiza* genome reveals insights into its neotenous reduction fast growth and aquatic lifestyle. Nat Commun. 2014; 5(1): 3311. https://doi.org/10.1038/ncomms4311
53. Wei C, Hu Z, Wang S, et al. An endogenous promoter LpSUT2 discovered in duckweed: a promising transgenic tool for plants. Front Plant Sci. 2024; 15: 1368284. https://doi.org/10.3389/fpls.2024.1368284
54. Xu B, Liu F, Zhang Q, Ni C, Gao J, Huang X. Practical algal control in Lower Yangtze reservoirs using composite microfiltration physical enclosure. Membranes. 2025; 15(10): 311. https://doi.org/10.3390/membranes15100311
55. Yamamoto YT, Rajbhandari N, Lin X, Bergmann BA, Nishimura Y, Stomp A-M. Genetic transformation of duckweed *Lemna gibba* and *Lemna minor*. In Vitro Cell Dev Biol Plant. 2001; 37(3): 349–353. https://doi.org/10.1007/s11627-001-0062-6
56. Yang G-L, Fang Y, Xu Y-L, et al. Frond transformation system mediated by *Agrobacterium tumefaciens* for *Lemna minor*. Plant Mol Biol. 2018; 98(4–5): 319–331. https://doi.org/10.1007/s11103-018-0778-x
57. Yang J, Zhao X, Chen Y, Li G, Li X, Xia M, et al. Identification, structural, and expression analyses of *SPX* genes in giant duckweed (*Spirodela polyrhiza*) reveals its role in response to low phosphorus and nitrogen stresses. Cells. 2022; 11(7): 1167. https://doi.org/10.3390/cells11071167
58. Yang J, Ishizawa H, Hou H. Rediscovering duckweed's value: uniting to advance its research system and applications. J Exp Bot. 2025; eraf409. https://doi.org/10.1093/jxb/eraf409
59. Zhang D, Gersberg RM, Ng WJ, Tan SK. Removal of pharmaceuticals and personal care products in aquatic plant-based systems: a review. Environ Pollut. 2014; 184: 620–639. https://doi.org/10.1016/j.envpol.2013.09.009
60. Zhang Y, Yin G-M, Yang L, Li S, et al. Duckweeds: from fundamental biology to a sustainable plant chassis for biotechnology. Adv Biotechnol. 2026; 4(2): 16. https://doi.org/10.1007/s44307-026-00110-1
61. Zhao X, Yang J, Li X, Li G, Sun Z, Chen Y, et al. Identification and expression analysis of GARP superfamily genes in response to nitrogen and phosphorus stress in *Spirodela polyrhiza*. BMC Plant Biol. 2022; 22: 308. https://doi.org/10.1186/s12870-022-03696-5
62. Ziegler P. Developments in toxicity testing with duckweeds. J Xenobiot. 2025; 15(2): 48. https://doi.org/10.3390/jox15020048