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# The Transcriptome in *Landoltia punctata*

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**Abstract**

Research on *Lemnaceae* (duckweeds) is hot spot recently due to its application potential in bioenergy production and phytoremediation. Among the five genera of duckweed, *Landoltia punctata* has great potential in starch production and heavy metal bio-extraction. Although starch accumulation and heavy metal absorption by *L. punctata* have been studied at biochemical and physiological levels for many years, the underlying mechanism has not been subjected to omics analysis until recently. Transcriptomics plays an important role in understanding gene expression regulation as response to changing environment. Several studies have been carried out to investigate the transcriptomic expression profiles of *L. punctata* to illustrate the mechanisms of sugar high starch content biosynthesis, cadmium hyperaccumulation, and flavonoids biosynthesis. Here, we reviewed the progress in transcriptome analysis of *L. punctata* to set framework and give the readers insights into the current status and future perspectives in researches and application potential of *L. punctata*.

## 12.1 Introduction

*Lemnaceae* (duckweed) is the smallest and fastest-growing aquatic flowering monocotyledonous plant in the world. It spreads a broad range of climates with 5 genera (*Spirodela*, *Landoltia*, *Lemna*, *Wolffiella*, and *Wolffia*), comprising 37 species around the world (Cao et al. 2014). It is able to reach a very high biomass yield (55 tons/ha/year dry weight) due to its asexual reproduction and rapid propagation (Zhao et al. 2012). Duckweed has been gained increasing attention due to its application potential in starch production, protein production, and phytoremediation.

Genus *Landoltia* is one of the widely distributed and practically applied duckweeds. It consists of one species, namely *Landoltia punctata*. Previously, *L. punctata* was a member of genus *Spirodela*, with old nomenclatures of *Spirodela oligorrhiza* and *Spirodela punctata* (Sree et al. 2016). It was recognized as a new genus since 1999 by Les. This generic name

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X. H. Cao et al. (eds.), *The Duckweed Genomes*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-030-11045-1_12

*Landoltia* commemorates Elias Landoltia for his outstanding contributions to systematics and biology of *Lemnaceae*. *L. punctata* possesses 2–7 roots per frond, perforating the prophyllum. Its frond is ovate to lanceolate with 3–7 veins, and a red dorsal surface is often observed. Unlike *Spirodela*, *Landoltia* is turion-absent (Les and Crawford 1999).

The most application field of *L. punctata* is starch production. In 1970, Reid and Bieleski reported that *L. punctata* accumulated starch content to approximately 30% (dry weight) in 30 days cultivation on phosphorus-deficient complete mineral nutrient medium. The starch content was sharply increased to 75% (dry weight) with the presence of glucose (1%) in the medium (Reid and Bieleski 1970). In our previous studies, we evaluated various approaches, like nutrient starvation, uniconazole supplementation, and heavy metal (cobalt and nickel) stress, to induce starch accumulation by *L. punctata* (Tao et al. 2013; Liu et al. 2015b; Guo et al. 2017). The three approaches evaluated induced starch accumulation and resulted in approximately 50% (dry weight) of starch content in 10 days cultivation. Phytoremediation is another important application field for *L. punctata*. *L. punctata* is able to uptake nitrogen (N) and phosphorus (P) from water very quickly, even under relatively low N/P concentration. It is widely used to purify wastewater and eutrophic water bodies (Fang et al. 2007). Besides, *L. punctata* is resistant to heavy metals to some extent and is able to accumulate heavy metals like cadmium, cobalt, nickel, lead, uranium, and silver (Guo et al. 2017; Nie et al. 2016; Stegemeier et al. 2017; Tang et al. 2017; Fang et al. 2007). Also, it is reported that *L. punctata* is potential for phytoremediation on petroleum hydrocarbons (Ertekin et al. 2015). *L. punctata* is rich in flavonoids (Wang et al. 2014) and is used in traditional Chinese medicine. It is also potential for pharmaceutical drugs.

To further release and improve the potential applications, it is critical to interpret the physiological mechanism on how *L. punctata* response to changing environment. General central dogma states as “DNA makes RNA and RNA makes protein”. RNA sequencing plays an important role in understanding gene expression regulation, and transcriptome analysis is able to reflect the global regulation. With the decreasing cost of next-generation sequencing, transcriptome analysis is accessible for individual laboratory and a few duckweed transcriptome studies were carried out in recent years (Table 12.1). Applying this deep-sequencing technology will set framework and stimulate novel potential of duckweeds. In this chapter, we introduced the primary researches on *L. punctata* and its transcriptome analysis. This will give the readers insights into the current status and future perspectives in researches and application potential of *L. punctata*.

## 12.2 Starch Production and Transcriptome Analysis

*L. punctata* is a potential bioenergy crop with high starch productivity and low lignin content. Several transcriptome studies in *L. punctata* had been carried out for understanding the mechanism of high starch content and low lignin content under abiotic stress.

The comparative transcriptome analysis was conducted to reveal the mechanism of high starch accumulation of *L. punctata* 0202 under nutrient starvation. *L. punctata* 0202 was transferred from nutrient-rich solution to distilled water and sampled in time course. Physiological measurements revealed that the activity of the key enzyme of starch biosynthesis, ADP-glucose pyrophosphorylase (AGPase), as well as the starch content increased continuously in *L. punctata* 0202 under nutrient starvation condition. Samples harvested at 0, 2, and 24 h were used for RNA-Seq, respectively. A comprehensive transcriptome, containing 74,797 contigs, was obtained by a de novo assembly of the RNA-Seq reads. Gene expression profiling showed that transcripts encoding key enzymes responsible for starch biosynthesis were up-regulated. Inversely, the expression of transcripts encoding enzymes involved in starch consumption and some photosynthesis-related transcripts were down-regulated. Specifically,

**Table 12.1** Summary of transcriptome studies in duckweeds

| Species | Condition | Time points | Main findings | References |
|---|---|---|---|---|
| *Landoltia punctata* 0202 | Nutrient starvation | 0, 2 and 24 h | Nutrient starvation down-regulated the global metabolic status, redirects metabolic flux of fixed $CO_2$ into starch synthesis branch in *L. punctata* | Tao et al. (2013) |
| *Spirodela polyrhiza* 7498 | ABA treatment | 3 days | Generated a model summarizing the signal transduction leading to *Spirodela* dormancy by comparing the transcriptome between fronds and developing turions | Wang et al. (2014) |
| *Landoltia punctata* 0202 | Uniconazole treatment | 0, 2, 5, 72, and 240 h | Uniconazole treatment altered endogenous hormone levels and enhanced chlorophyll content and net photosynthetic rate by regulating key enzymes involved in endogenous hormone and chlorophyll biosynthesis | Liu et al. (2015a) |
| *Landoltia punctata* 0202 | Uniconazole treatment | 0, 2, 5, 72, and 240 h | Uniconazole treatment altered endogenous hormone levels, thus resulted in starch accumulation | Liu et al. (2015b) |
| *Lemna minor* | Ammonium treatment | 7 days | The antioxidant enzyme system was activated under $NH4^+$ toxicity for ROS scavenging. The increased lignin biosynthesis might play an important role in $NH4^+$ toxicity resistance | Wang et al. (2016) |
| *Landoltia punctata* 0202 | NS, UT, FN | NS (0, 2, 24 h); UT (0, 2, 5, 72, 240 h); FN (2, 5, 72, 240 h) | Nutrient starvation is the best option to obtain high starch and flavonoid accumulation simultaneously in a short time | Tao et al. (2017) |
| *Lemna minor* | Ionising radiation | 7 days | Duckweeds shift from acclimation responses toward survival responses at increasing dose rates of ionising radiation | Van Hoeck et al. (2017) |
| *Lemna aequinoctialis* 6000 | Nitrogen starvation (applied sucrose) | 0, 3, and 7 days | Nitrogen starvation increased ADP-glucose and starch contents by regulating the gluconeogenesis and TCA pathways and lipids and pectin biosynthesis | Yu et al. (2017) |
| *Landoltia punctata* 6001 | $CdCl_2$ treatment | 0,1, 3, 6 days | Cd stress could affect the processes from DNA to protein metabolism, metabolic flux of carbohydrate, sulfur and ROS metabolism, and tonoplast transporter expression to cope with Cd cytotoxicity, tolerance, and detoxification | Xu et al. (2018) |

*Note NS* Nutrient starvation; *UT* Uniconazole treatment; *FN* Full nutrient (1/6 Hoagland solution)

the transcripts of some transporters were up-regulated within the first 2 h. Additionally, the expression of most transcripts encoding key enzymes involved in flavonoid biosynthesis was up-regulated drastically regardless of starvation, while the expression of the last rate-limiting enzyme of lignification, laccase, presented very low in all the three samples. Furthermore, the expression of genes involved in starch biosynthesis was confirmed by quantitative reverse transcription PCR (qRT-PCR) in *L. punctata* 0202. Another independent research on qRT-PCR assay for the expression of key starch biosynthesis enzymes, including AGPase, soluble starch synthase (SSS), starch degradation enzymes (SDE), and alpha- and beta-amylase, under nitrogen (N) and phosphorus (P) deficiency condition was consistent with this RNA-Seq data (Zhao et al. 2015). Those studies provided a comprehensive transcriptome analysis of *L. punctata* 0202 under nutrient starvation, which indicated that nutrient starvation down-regulated the global metabolic status and redirected metabolic flux of fixed $CO_2$ to starch biosynthesis pathway. It provided a valuable genomic resource for duckweed and paved the way for further molecular biological studies and the application of duckweed as a bioenergy crop (Tao et al. 2013).

Not only nutrient starvation but also uniconazole can improve the starch content. Uniconazole is a plant growth retardant, which can increase starch and biomass accumulation of *L. punctata* simultaneously under eutrophic conditions. The result of transcriptome sequencing of uniconazole application on fronds of *L. punctata* 0202 revealed the expression of genes involved in endogenous hormones and chlorophyll biosynthesis pathway changed responsively. The dry weight following the uniconazole treatment increased by 10% compared to the controls at 240 h, resulting from the uniconazole affecting endogenous hormones content, chlorophyll content, and the net photosynthetic rate. The dry weight starch content increased up to 48% compared to 15.7% in the control group after 240 h growth. Transcriptome sequencing revealed that the expression of regulatory elements of hormone signaling pathways that are involved in chlorophyll and starch metabolism changed correspondingly. Importantly, the expression of key enzymes responsible for starch biosynthesis was up-regulated, and transcript-encoding enzymes involved in starch degradation and other carbohydrate metabolic branches were down-regulated (Liu et al. 2015a, b).

According to current research on *L. punctata* 0202 starch accumulation, starch content could reach up to approximately 45% (dry weight) within 7 days under nutrient starvation condition (Tao et al. 2013; Huang et al. 2014) and approximately 48% (dry weight) within 10 days under uniconazole treatment (Liu et al. 2015a, b). A combined treatment of nutrient starvation and uniconazole application or others to *L. punctata* 0202 is considered a promising method to further improve the starch content. To support this, more tests should be carried out to verify the hypothesis in the future. According to the latest study, the starch content can reach 60% under nitrogen starvation in the presence of exogenously applied sucrose condition in *Lemna aequinoctialis* 6000 after treatment for 9 days. In this study, a combined transcriptome and metabolites analysis was carried out for metabolic flux in starch accumulation. The researchers evaluated expression of the genes involved in nitrogen metabolism, protein and amino acid metabolism, starch and sucrose metabolism, and lipid metabolism by sampling 7-day in time course under nitrogen starvation condition. The expression of genes encoding nitrate reductase, glutamine synthetase, and glutamate synthase was down-regulated; the expression of genes encoding enzymes involved in gluconeogenesis was up-regulated, whereas the majority of unigenes involved in glycolysis were down-regulated. The metabolome analysis revealed that more ADP-Glc was accumulated and lower levels of UDP-Glc were accumulated, which was consistent with the transcriptome results. The activity of AGPase involved in starch biosynthesis was significantly increased while the activity of UGPase was dramatically decreased. This study serves as an excellent candidate for functional transcriptome study and metabolic engineering to improve the production of next-generation biofuels in duckweeds (Yu et al. 2017).

## 12.3 Heavy Metal Accumulation and Transcriptome Analysis

Except for transcriptome analysis for starch accumulation, the gene expression response to cadmium stress in *L. punctata* 6001 was analyzed via RNA-Seq technique by Xu et al. *L. punctata* 6001, which is considered a promising candidate for Cd phytoremediation, was isolated by large-scale screening of over 200 duckweed clones. To understand the molecular mechanisms of Cd hyperaccumulation, a comprehensive transcriptome analysis was performed by RNA-Seq for samples treated with 20 μM $CdCl_2$ for 0, 1, 3, and 6 days. Xu et al. revealed that genes involved in DNA repair acted as an early response to Cd, and RNA and protein metabolism would be likely to respond as well. Furthermore, the carbohydrate metabolic flux tended to be modulated in response to Cd stress. Up-regulated genes involved in sulfur and reactive oxygen species (ROS) metabolism might contribute to Cd tolerance. Vacuolar sequestration most likely played an important role in Cd detoxification in *L. punctata* 6001. The novel findings provided important clues for molecular-assisted screening and breeding of Cd hyper-accumulating cultivars for phytoremediation (Xu et al. 2018).

## 12.4 Flavonoids Accumulation and Transcriptome Analysis

Flavonoids belong to phenolic compounds and are widely existed secondary metabolites in plants. It can be potentially exploited in the food and drug. The flavonoid content of duckweed can reach up to approximately 5.56%, and at least, 20 flavonoid compounds were found in duckweed (Tao et al. 2017).

Nutrient starvation also triggers high flavonoid accumulation in *L. punctata* 0202. A combined omics study was performed to investigate the biosynthesis of flavonoid and the metabolic flux changes in *L. punctata* 0202 grown in different culture media. To understand the global flavonoid and starch metabolite-related molecular response to nutrient starvation, *L. punctata* 0202 was cultivated in nutrient starvation, uniconazole treatment, and full nutrient, respectively. RNA-Seq analysis for three groups sample was carried out, and the proteome data obtained from iTRAQ-LC-MS/MS technology of previous studies was re-analyzed using the new transcriptome data as a reference database. The abundance of the most detected flavonoid-related proteins, including the phenylalanine ammonia-lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-hydroxycinnamoyl-CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone 3-hydroxylase (F3H), and anthocyanidin synthase (ANS) were detected to be improved in *L. punctata* 0202 when grown in distilled water. The proteome data was consistent with the transcriptome data in this study. Flavonoid content was measured, and purple coloration accumulation which is regarded as anthocyanin was observed on the dorsal fronds. The metabolome and morphology further verified the results of transcriptome and proteome analysis (Tao et al. 2017).

## 12.5 Other Transcriptomes Analysis

Several transcriptome studies in other duckweed species were also reported currently. *Lemna minor* can grow well in the high $NH_4^+$ environment but to some extent can also suffer toxic effects. To study the toxicity and tolerance of $NH_4^+$, the transcriptome study using RNA-Seq was reported in *L. minor*. The *L. minor* was cultured in the Hoagland solution of control and treated with 84 mg/L $NH_4^+$ and 840 mg/L $NH_4^+$. RNA-Seq generated 6.62 G nucleotides from the three distinct libraries. Bioinformatic analysis identified 70,728 unigenes and 14,207 differentially expressed genes (DEGs), most of which were down-regulated under $NH_4^+$ toxicity. Lignin biosynthesis-related genes in the phenylpropanoid biosynthesis pathway were up-regulated to enhance $NH_4^+$ toxicity resistance. The accumulation of ROS induced by $NH_4^+$ toxicity can cause oxidative damage leading to cell death in *L. minor*. The antioxidant enzyme system was also activated to scavenge

ROS and reduce the toxicity. The transcriptomic and physiological research of *L. minor* responding to high $NH_4^+$ may provide us a better understanding not only of toxic processed but also tolerance mechanisms (Wang et al. 2016).

Ionising radiation (IR) in the environment is considered harmful to plants and animals when conferring extremely high dose rates. To better understand the physiological response to plant exposure to ionising radiation, RNA-seq in *L. minor* treated ionising radiation for seven days in a dose rate-dependent manner was carried out. The gene expression data revealed that *L. minor* plants exposed at lower dose rates can tolerate the exposure by triggering acclimation responses. In contrast, the genes related to antioxidative defense systems in terms of DNA repair and cell cycle were high expressed at the highest dose rate. It indicated that plants can shift from acclimation responses toward survival responses at increasing dose rates of ionising radiation. Importantly, the photosynthetic process seems to be unaffected in *L. minor* plants among the tested dose rates (Van Hoeck et al. 2017).

## 12.6 Conclusion

In summary, in the absence of genomic data, RNA-seq of *L. punctata* was powerful in uncovering molecular mechanism under different treatments or stresses. Especially, the transcriptome of starch metabolism mapped the transcriptional profiles of high starch, high flavonoids, and low lignin metabolism pathways and revealed the molecular mechanisms of high starch accumulation under the particular conditions in duckweed. It provides new ideas for the study of gene regulation and genetic manipulation of starch metabolism in plants.

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