UNIVERSITI PUTRA MALAYSIA

EFFECTS OF ARSENIC ON PHYSIOLOGY, ANATOMY AND TRANSCRIPTOMES ON AQUATIC PLANTS

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FBSB 2021 19

EFFECTS OF ARSENIC ON PHYSIOLOGY, ANATOMY AND TRANSCRIPTOMES ON AQUATIC PLANTS

By

NARGES ATABAKI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

March 2021

DEDICATION

"I don't know what your destiny will be, but one thing I know; the only ones among you who will be really happy are those who have sought and have found how to serve."

-Albert Schweitzer

This humble work is dedicated to:

My beloved husband, Rambod, for your patience, love, friendship, and humor, without which I wouldn't have reached this present stage.

My parents who have supported me through all my various endeavors, **Forouz** and **Homayoun**.

My parents in law who have prayed for me, **Louis** and **MohammadReza**.

My brother, **Ali**, and my brother-in-law, **Ramin** who have encouraged inspired me.

My sisters in law, **Jila** and **Armaghan,**

My Nice, **Elena** and my nephews**, Shervin** and **Amir Sam**

Thank you for understanding that distance can help one improve his knowledge, even though 6,615 km is a long way from home.

I decided to devote my life to telling the truth, because having survived I owe something to the dead and anyone who does not remember them betrays them, my dearest brothers, **Amir** and **Navid**, who I have lost them, may their souls continue to rest in perfect peace.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

EFFECTS OF ARSENIC ON PHYSIOLOGY, ANATOMY AND TRANSCRIPTOMES ON AQUATIC PLANTS

By

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March 2021

Chairman : Noor Azmi Shaharuddin, PhD
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The presence of arsenic in groundwater has affected the water supply, especially in rural areas. Inorganic arsenic can be transferred to the food chain through water contamination and causes acute biotoxicity. Selecting appropriate aquatic plants tolerant to arsenic contamination are the key factors in promoting the practical application of phytoremediation in a polluted environment. Water hyacinth (*Eichhornia crassipes*) and water mimosa (*Neptunia oleracea*) have been widely identified as two feasible phytoremediators. In the current study, the phytoremediation potential of water hyacinth and water mimosa exposed to different concentrations of sodium heptahydrate arsenate (5, 10, 30, 50, 60, 70, 80, 90, and 100 mg/L) was tested for two weeks. Several plant physiological and growth responses as well as arsenic accumulation and removal efficacy were analyzed. It was found that there were significant differences in the level of 5% for all the traits between the arsenic treatments for both types of plants. In water hyacinth and water mimosa, there were decreasing trends of the ratio of biomass (DRB), the ratio of dry weight (DRD), photosynthetic activities, stomatal conductance, intercellular CO_2, transpiration rate, air pressure deficit and chlorophyll content after 14 days of exposure to the arsenic. In the experiment, water hyacinth had shown the highest arsenic absorption as compared to the water mimosa, which was recorded at 70 mg/L. Water mimosa showed severe necrotic symptoms at the highest concentrated condition (100 mg/L) after 14 days of treatment but was still able to accumulate 16.36 mg/Kg-1 arsenic in its roots (0.8 mg total arsenic). To have a further understanding at the molecular level, transcriptomic analysis was performed on the arsenic-treated water hyacinth using the RNA-Sequencing technique. Differential gene expressions had uncovered transcripts encoding various genes associated with the arsenic uptake, the effect on the structural constituent of the ribosome in the plant as well as heat shock tolerance mechanisms. Accordingly, the expression of intracellular ribonucleoprotein complex, suggesting a potential means for arsenic impact on RNA-binding proteins and RNA to denote intracellular compartments involved in the processing of RNA transcripts. All in all, water hyacinth and water mimosa have the potential to be a phytoremediator plant. Their response to the heavy metal contamination, coupled with

high biomass yields, makes them a considerable option for contaminated site phytoremediation.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KESAN ARSENIK TERHADAP FISIOLOGI, ANATOMI DAN TRANSKRIPTOM TUMBUHAN AKUATIK

Oleh

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Kehadiran arsenik pada air bawah tanah telah mempengaruhi bekalan air, terutama di kawasan luar bandar. Arsenik inorganik boleh dipindahkan ke rantai makanan melalui pencemaran air dan menyebabkan biotoksisiti akut. Pemilihan tanaman akuatik yang rintang terhadap pencemaran arsenik adalah faktor utama dalam mempromosikan penggunaan fitoremediasi dalam persekitaran yang tercemar. Keladi bunting (*Eichhornia crassipes*) dan keman air (*Neptunia oleracea*) telah dikenali sebagai tumbuhan fitoremediator yang baik. Dalam kajian ini, potensi fitoremediasi keladi bunting dan keman air yang didedahkan kepada kepekatan natrium heptahidrat arsenat yang berlainan (5, 10, 30, 50, 60, 70, 80, 90, dan 100 mg / L) diuji selama dua minggu. Beberapa tindak balas fisiologi dan pertumbuhan serta keberkesanan pengumpulan dan penghapusan arsenik dianalisis. Didapati bahawa terdapat perbezaan yang signifikan pada tahap 5% untuk semua ciri pertumbuhan semasa rawatan arsenik untuk kedua jenis tanaman tersebut. Dalam kedua-dua tumbuhan ini, terdapat corak penurunan nisbah biomas (DRB), nisbah berat kering (DRD), aktiviti fotosintetik, kekonduksian stomatal, CO2 antara sel, kadar transpirasi, defisit tekanan udara dan kandungan klorofil setelah 14 hari pendedahan kepada arsenik. Dalam eksperimen tersebut, keladi bunting menunjukkan penyerapan arsenik tertinggi dibandingkan dengan keman air, yang dicatat pada 70 mg / L. Keman air telah menunjukkan gejala nekrotik yang teruk pada kepekatan arsenic tertinggi (100 mg / L) setelah 14 hari rawatan tetapi masih dapat mengumpulkan 16.36 mg / Kg-1 arsenik di akarnya (0.8 mg total arsenik). Untuk mendapatkan pemahaman lebih lanjut pada peringkat molekul, analisis transkripomik dilakukan pada keladi bunting yang dirawat arsenik menggunakan teknik penjujukan RNA. Pengekspresan gen yang berbeza telah menemui transkrip yang mengekodkan pelbagai gen yang berkaitan dengan pengambilan arsenik, kesannya pada komponen struktur ribosom di dalam tumbuhan serta mekanisme toleransi kejutan haba. Oleh itu, pengekspresan kompleks ribonukleoprotein intraselular, telah mencadangkan potensi protein pengikat RNA dan RNA yang terlibat dalam pemprosesan transkrip RNA. Secara keseluruhan, keladi bunting dan keman air berpotensi menjadi tanaman fitoremediator. Tindak balas mereka terhadap pencemaran logam berat, ditambah dengan hasil biomass

yang tinggi, menjadikannya pilihan yang sangat baik untuk fitoremediasi kawasan yang tercemar.

ACKNOWLEDGEMENTS

On this very special occasion, I would like to sincerely thank Assoc. Prof. Dr. **Noor Azmi Shaharuddin** for all supports and positive energies. I also would like to thank all of the lecturers of the faculty who have taught and guided me during my study.

I would like to express my deep gratitude to Assoc. **Prof. Dr. Siti Aqlima Ahmad** and Assoc. **Prof. Dr. Rosimah Nulit** whom have given their valuable time, advice, criticism and correction to this thesis.

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

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Al	Aluminium
ALA availability	Tδ-aminolevulinic acid
Anet	Net photosynthesis rate
ANOVA	Analysis of variance
AOS	Active Oxygen Species
Arsenic MDL	Method Detection Limit
As	Arsenic
AsO_{33}^{-}	Arsenite
AsO_{34}	Inorganic Arsenate
AtChaC2-1	*Arabidopsis thaliana heat chock2-1*
Cd	Cadmium
Cfin	Final Concentration of the Synthetic Mixture.
Ci	Chlorine
Cini	Initial concentration of the synthetic mixture
CO2	Carbon dioxide
Cr	Chromium
Cu	Copper
DEG	Differentially expressed genes
DEGs	Differential gene expressions
DNA	Deoxyribonucleic acid
DRB	Decreasing ratio of biomass
DW	Dry weight biomass of samples

E	Transpiration rate
E. crassipes	*Eichhornia crassipes*
EDX	Energy Dispersive X-ray Spectrometre
Fe	Iron
FW	Wet plant biomass
GC (%)	GC content
GO	Gene Ontology
Gs	Stomatal conductance
h	Hour
H. ranunculoides	*Hydrocotyle ranunculoides*
HCl	Hydroxide chloride
Hg	Mercury
HNO	Nitroxyl
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
KEGG	Kyoto Encyclopedia of Genes and Genomes
KOG	Clusters of Orthologous
kPa	Leaf Temperature
Lsi_2	Low silicon transporter 2
MAPK	Signalling pathway
Mg	Milligram
mg/Kg^{-1}	Milligram per kilogram
mg/L	Milligram per litter
miR156	Micro RNA 156

Mlast2GO	Research tool designed with the main purpose of enabling Gene Ontology (GO) based
Mn	Manganese
N. oleracea	*Neptunia oleracea*
NGS	Next-Generation Sequencing
Ni	Nickel
Nm	Atomic Radius
Pb	Lead
$Pb(NO_3)2$	Lead(II) nitrate
PCR	Polymerase Chain Reaction
PFAM	Large collection of protein families, each represented by multiple
HMMs	sequence alignments and hidden Markov models
Ppm	Parts per million
Q20 & Q30	Calculate the percentage of the base number of Phred score greater than 20, 30 respectively accounted for the total base number
RIN	RNA Integrity Number
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
ROS	Reactive Oxygen Species
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RWC	Relative Water Content
SAS	Solve Complex Analytical Problems
SE	Standard Errors

SEM	Scanning Electron Microscope
SNARE	Interactions in Vesicular Transport
SPAS	Soil–Plant–Atmosphere System
SPAD	The Soil Plant Analysis Development (SPAD) chlorophyll meter
TI	Thallium
TOC132	Translocase of chloroplast 132, chloroplastic
UBD	Biodiversity Unit
USA	The United State of America
V	Volume
Vpdl	Air pressure deficit
VpdL	Leaf to Air Vapour Pressure Deficit
WHO	World Health Organization
WQI	Water Quality Index
Zn	Zinc
µg/L	Microgram per litre
µM	Micromule
$\mu molCO_2m^{-2s-1}$	Intrinsic water use efficiency
$\mu mol\ H_2Om^{-2s-1}$	Stomatal conductance
$\mu molm^{-2}$	Transpiration rate
%	Percentage

CHAPTER 1

INTRODUCTION

Arsenic is an extremely toxic metalloid that is carcinogenic predominantly to humans' liver, lung, kidney and bladder. It can also cause nerve damage and skin diseases (Chen *et al.*, 2019; Utermann *et al.*, 2019). This heavy metal is ubiquitous in the Earth's crust and is typical of the dietary intake of people in developed countries (Weldeslassie *et al.*, 2018). It is also present through atypical groundwater exposure in developing countries and can contaminate the entire water source (Abdul *et al.*, 2015). The presence of arsenic in groundwater has affected the water supply to rural areas in over 70 countries. It is estimated that more than 150 million people have been exposed to arsenic (Jasrotia *et al.*, 2017). Inorganic arsenic can be transferred into the food chain through water contamination, accumulation in plants grown for food, or through livestock feed (Novo *et al.*, 2018). The damages caused by this heavy metal on humans and the environment shows that the elimination of arsenic from contaminated water is urgently essential. Removing arsenic from the environment through water and its downstream pathways is reportedly a complicated procedure (Abolayo, 2019). Novel sustainable and innovative techniques can provide stable and efficient removal procedures of the metal from the water environment (Al Jaberi, 2018; Bolisetty *et al*., 2019).

Removal of environmental pollutants from contaminated water, soil, sludge and sediments using plants is known as phytoremediation (Soussi *et al*., 2016; Arabnezhad *et al.*, 2019; Ossai *et al.*, 2020). For the past two decades, phytoremediation has been developed as a green, non-invasive and economic alternative to different conventional civil engineering-based strategies for the remediation of water, soil and even residences polluted with heavy metals (Wei *et al*., 2019; Prabakaran *et al*., 2019). Aquatic plants are natural candidates to treat contaminated soil and water by accumulating heavy metals in their tissues (Shukla *et al*., 2019). The species *Eichhornia crassipes* (water hyacinth) (Chigbo *et al*., 1982) and *Neptunia oleracea* (water mimosa) (Visoottiviseth *et al*., 2002), were previously used for decontamination or reduction of various heavy metals in water in some Asian countries including Malaysia, Thailand, Indonesia, Philippines, and Vietnam (Ab Wahab *et al*., 2016).

The rapid increase in the human population, urbanization, industrial activities, deforestation, exploration and exploitation of ecosystems have caused heavy metal and metalloid pollutions in Malaysia's environment (Jayakumar *et al*., 2017). It has been reported that the concentration of arsenic is between 2.00 to 54.00 µg/L in rivers across Malaysia (Sobahan *et al*., 2013; Ab Razak *et al*., 2015; Sakai *et al*., 2017; Othman *et al*., 2018; Hwi *et al*., 2020). This is a concern as it exceeds international environment guidelines which is below 10 µg/L for drinking water samples (Fernandez-Luqueno *et al*., 2013).

Eichhornia crassipes and *Neptunia oleracea* have short life cycles and easy to grow, therefore suitable for planting in contaminated water areas (Anokhina *et al*., 2004). Naturally, these plants grow close to the water area such as pond and lake as well as the river in Malaysia. They have a good adaptation to the tropical climate, ever-green, high biomass production, cheap and simple to maintain (Syuhaida *et al*., 2014) and these traits have made them advantages for phytoremediation purposes. However, the doubt of these plant's efficiency is the main barrier to use this promising technology widely (Thakur *et al*., 2019). Therefore, morpho- and physiological investigations, anatomy adaptation alongside gene expression profiling using RNA-sequencing have facilitated a leap at the opportunity of the phytoremediation process discovering the capacity of this technology (Reuther and Monzon, 2019; Saxena *et al*., 2019; Thakur *et al*., 2019; Xu *et al*., 2019).

Despite increasing the application of phytoremediation as a green technology, their full potential and active mechanisms have not been fully explored (O'Connor *et al*., 2019). Therefore, the main objective of the current study was to elucidate the influence of different concentrations of arsenic on water hyacinth (*Eichhornia crassipes*) and water mimosa (*Neptunia oleracea*) and discover the genetic regulatory networks alongside morpho- and physiological identification and anatomy adaptation during the phytoremediation of arsenic in the plants.

The specific objectives of this project were:

1) To determine the effect of various arsenic concentrations on the morpho- and physiological traits and anatomy changes of water hyacinth (*Eichhornia crassipes*) and water mimosa (*Neptunia oleracea*)
2) To identify the arsenic-responses differentially expressed genes (DEGs) of the aquatic plant via comparative RNA-Seq transcriptomic analysis

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