Water and arsenic movement in *Salix nigra* under simulated phytoremediation conditions

by

Nicolas Perrault

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science
in
Forest Biology and Management

Department of Renewable Resources
University of Alberta

Abstract

Arsenic is a highly toxic and ubiquitous element. To this day, millions of people are exposed to arsenic contamination, which poses global health concerns. Phytoextraction performed with willows, a form of phytoremediation, is a promising means of cleaning up soils containing hazardous levels of arsenic. Plant-water relations and cell transport activity both play key roles in this decontamination process, as they explain the movement of hydrophilic contaminants from bulk soil into the plant. Both water and arsenic uptake, and their movement across cell membranes, are regulated by transport proteins. This thesis studies the processes of arsenic and water transport in willows used in a phytoremediation context. Through two greenhouse experiments using a double-compartment design, physiological activity monitoring and mRNA profiling, we investigated the movement of water and arsenic in the soil and within willows under experimental treatments of drought and soil contamination. Our results show that a process of water redistribution towards dry surface soil occurs through *Salix nigra* root system, although aquaporins activity is repressed in surface roots exposed to drought. Based on mRNA profiles, we highlight the intricate willows' root activity in response to both arsenic and drought at the gene expression level. The expression of aquaporins, phosphate transporters and ABC transporters in roots identifies key genes responsible for water and arsenic transport under stress conditions. Their expression level indicates the presence of a weak exclusion mechanism of arsenic in *Salix nigra*, allowing the easy uptake of the contaminant from deep soil. Simultaneously, the repression of aquaporin genes in surface roots blocks a possible efflux pathway, confining the arsenic inside the plant. Most importantly, adverse growth conditions caused by contamination exposure and an extended episode of drought in surface soil are more likely responsible for root decay, and induce the arsenic

redistribution to surface soil layers. This process does not seem to result from the hydraulic redistribution observed in the short-term. This process should be considered in the planning of phytoremediation experiments in the field, either by trying to prevent it with proper irrigation, or by exploiting it in rotational cultures allowing the decontamination of deep and shallow soil in succession. The activity of transporter genes identified from the mRNA profiling results needs to be investigated with further testing to uncover their specific roles in arsenic transport, for example with heterologous expression systems. This knowledge could allow for the development of more efficient plants for decontamination purposes, through genetic engineering or genotypic selection of plants favoring contaminant transport.

Preface

A version of the research article presented in section 2 of this is in preparation for publication as “Perrault, N., Grenier, V., Bedard, L., Saint-Fleur, L., Cardenas, M., Oligny, C., Lefrançois, M., Hacke U.G., Laur, J. (2023). Hydraulic Lift and Arsenic Redistribution Occur Independently in Salix nigra.”. Experimental design and testing were conducted by me, with advice from J. Laur and U.G. Hacke. I performed the data collection and analysis, with input from V. Grenier and J. Laur, and wrote the body of this thesis, with editing contributions from J. Laur and U.G. Hacke and V. Grenier.

Acknowledgements

I would first like to thank my supervisors, Dr. Joan Laur and Dr. Uwe Hacke, whose guidance, support, and expertise allowed me to complete my research project and thesis. A special thanks to Dr. Laur for answering all my questions, reassuring me during my moments of uncertainty and regularly following up on my progress. I would also like to thank my lab mentors, Vanessa Grenier and Martin Lefrançois, who allowed me to go from completely helpless to somewhat capable in terms of molecular biology lab work. Their patience and skills made the learning process a great experience for me. A big thank you to Corinne Oligny, my intern over the summer of 2022, who helped me incredibly in a very busy period of my master's, allowing me to complete my experiments and catch up on a lot of deadlines. Thank you to my favourite lab/office partners, Laurianne Bédard, Laurie Saint-Fleur and Margot Cardenas, and to all my IRBV friends in general. You supported me throughout the good and the not-so good moments of this degree, and made my master's experience unforgettable with your friendship, crooked humour, and mental support. Finally, I want to thank my mom and dad, who supported me, believed in me and pushed me to keep working when I needed it. Thank you for the weekly calls, the financial and mental support, the occasional pet sitting of Lucille, and everything else you have done for me.

Table of contents

List of Tables

List of Figures

List of Abbreviations

As(III): arsenite

As(V): arsenate

DEG: differentially expressed genes

GO: gene ontology

mRNA: messenger RNA

NIP: nodulin 26-like intrinsic protein

PIP: plasma-membrane intrinsic protein

PT/PHT/PHO: phosphate transporter

RNA-seq: mRNA sequencing

SIP: small and basic intrinsic protein

TIP: tonoplast intrinsic protein

XIP: uncategorized intrinsic protein

1. Thesis introduction

In the following review, I provide a broad description of the main biological and chemical principles at play in the process of soil decontamination using plants, called phytoremediation. More precisely, I focus on a sub-division of phytoremediation called phytoextraction, and its application using willows to decontaminate soils from arsenic. To do so, I divided this literature review into two main sections: arsenic phytoremediation and water movements in vascular plants, both of which are closely interconnected, with the prior being strongly influenced by the latter.

To provide the reader with context, and to highlight the importance of decontamination efforts, I start by describing the chemical nature of arsenic, its toxic effect on living organisms, and its presence as a contaminant in the environment. This is followed by a review of the state of knowledge on molecular processes behind arsenic accumulation in plants, an essential section for understanding the physiological mechanisms of phytoremediation. I then present phytoremediation as a means of managing contamination in general, with emphasis on the advantageous characteristics of willows for such practice. Unlike with the "hyperaccumulator" plants discussed below, the capacity of willows to extract and accumulate contaminants from the soil depends largely on a specific trait: their high transpiration capacity.

After this first part, I proceed to describe how vascular plants like willows manage to access and move large quantities of water through passive processes in what is called the soil-plant-atmosphere continuum. I first explain the theory behind long-distance or axial transport of water inside of these plants, followed by the mechanisms of water uptake by roots and radial transport towards the vascular tissues. Continuing with the focus on

root activity, I describe the process of hydraulic redistribution observed in root systems and its impacts on ecosystems. Finally, I dive into the molecular biology of plant-water relations with a section on aquaporins, membrane-associated channels well-known for their role in regulating the permeability of cells and tissues to water. I end this review with a quick summary of the principles explained and tie them together to explain the reasoning behind the experiments conducted for my research.

1.1. Thesis objectives

The literature review that I present in the following pages explains the different factors at play in the movement of both arsenic and water in trees, giving context to my research article and supporting the reasoning behind the different experiments conducted. The objectives of this thesis are the following:

- To investigate the possible relation between hydraulic redistribution and arsenic movement in the soil-plant system. This objective is based on the fact that aquaporins are known to regulate both plant-water relations and arsenic movement in plants.
- To provide a better understanding of the stress response and transport mechanisms related to arsenic exposure in willows, complementing the works of Puckett et al. (2012), Yanitch et al. (2017) and Navazas et al. (2019).
- To further describe the activity behind the hydraulic redistribution occurring in plants. This process has been studied through ecological and physiological approaches but lacks documentation at the molecular level, from controlled experiments.

1.2. Literature review

1.2.1. Arsenic phytoremediation

1.2.2. Arsenic prevalence and toxicity

Arsenic (As) is a metalloid associated to the atomic number 33 and with the relative atomic mass of 74.92. This element is found under four oxidation states: –3, 0, +3, and +5. Its two most common oxidation states are arsenite (the trivalent form, As(III)) in reducing conditions, and arsenate (the pentavalent form, As(V)) in oxidizing conditions (IARC, 2012). In soil and under aerobic conditions, arsenates (compounds containing AsO_4^{3-} ions) are stable and predominant, while under reducing conditions arsenites (compounds containing AsO_3^{3-} ions) are the most common arsenic compounds (Mandal & Suzuki, 2002). Arsenic adsorption onto soil particles is positively correlated with clay minerals and ferrous/aluminum oxide contents at various levels depending on soil pH (Goldberg, 2002). This adsorption activity with soil constituents influences the bioavailability (capacity of a substance to be absorbed by a living organism) and therefore potential deleterious effects of arsenic on living organisms. Furthermore, soil microbial activity can transform mineral As(III) and As(V) into gaseous and volatile species, causing the production of arsine gas (AsH_3) and the methylated compounds monomethylarsine (CH_3AsH_2), dimethylarsine ($(CH_3)_2AsH$) and trimethylarsine ($(CH_3)_3As$) by both bacteria and fungi under anaerobic conditions (Cullen & Reimer, 1989; Huang et al., 2014; Lomax et al., 2011). Under oxidizing conditions, these compounds react quickly with oxygen to form the arsenic acids monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide (TMAO) (Cullen & Reimer, 1989; Huang et al., 2014). These highly mobile methylated

arsenic species are however much less abundant in the environment than non-volatile inorganic arsenic (Cullen & Reimer, 1989; Mandal & Suzuki, 2002).

Arsenic is found in water, soil, air and living organisms around the world as a result of contamination from natural sources and anthropogenic activities. It naturally occurs in over 200 mineral forms throughout the earth's surface, with the majority being found as arsenopyrite (Cullen & Reimer, 1989; Mandal & Suzuki, 2002) but industrial activity strongly influences its distribution across ecosystems. Such anthropogenic sources, past and present, include mining activity, metallurgy (smelting), insecticide and herbicide application, fertilization of soils, fossil fuel combustion, as well as its use in the preparation of desiccants, wood preservatives, feed additives, drugs and poisons (Mandal & Suzuki, 2002). Arsenic is known as a group I human carcinogen, meaning its ingestion is proven to cause cancer even at low levels (IARC, 2012), and has caused poisoning episodes in human populations around the world on several occasions (Mandal and Suzuki, 2002), making it a concerning substance for global human health. Arsenic affects millions of people through the contamination of groundwater, on which many populations depend as their source of sanitation water (Nordstrom et al., 2002). On every continent, the two main sources of water contamination from arsenic are sedimentary formations and mining activity (Shaji et al., 2021). It is estimated that between 94 and 230 million humans are at risk of poisoning by As-contaminated groundwater (above the 10 ppb concentration in water recommended by the World Health Organization), across 108 countries (Nordstrom et al., 2002, Podgorski & Berg, 2020; Shaji et al., 2021). The region with the highest risk of arsenic contamination in groundwater is South Asia, but countries from every inhabited

continent, including Canada and the United States, are also affected (Podgorski & Berg, 2020; Shaji et al., 2021).

Arsenic-induced cell toxicity has been well documented in both animals and plants, mainly based on the chemical activity of arsenate and arsenite, causing a wide array of symptoms and illnesses (Finnegan & Chen, 2012; Hughes, 2002). In general, arsenic exposure is known to induce the overproduction of reactive oxygen species (ROS) causing oxidative stress in cells, damaging molecules and cell structures (Hartley-Whitaker et al., 2001). Among arsenic species, trivalent arsenic tends to be more acutely toxic than pentavalent arsenic (Hughes, 2002). Arsenite induces toxicity due to its high affinity for thiol/sulfhydryl groups, exemplified by the binding of trivalent arsenic to glutathione (Scott et al., 1993). Reaction with these functional groups in enzymes and other functional proteins can prevent vital cellular processes from happening by inducing conformational changes (Ramadan et al., 2007). Arsenate being a chemical analogue to phosphate (P_i) it replaces P_i in vital biochemical reactions (H. B. F. Dixon, 1996), potentially disrupting multiple primary metabolism processes: glycolysis, oxidative phosphorylation, phospholipid metabolism, nucleic acid metabolism and protein phosphorylation (Finnegan & Chen, 2012). Finally, among its many adverse effects, arsenate has most importantly been shown to uncouple the formation of ATP (Delnomdedieu et al., 1994), depriving the cells of their main energy source, and to cause DNA damage by oxidative stress (Lin et al., 2008).

1.2.3. Arsenic uptake and translocation in vascular plants

The mechanisms of arsenic uptake and translocation in plants have been extensively reviewed (Awasthi et al., 2017; A. Kumar et al., 2022; N. Li et al., 2016; Meharg & Hartley-

Whitaker, 2002; Tang & Zhao, 2021; Zhao et al., 2009; Zhao et al., 2010*b*) with an emphasis on the multiple membrane proteins involved in transport/diffusion of arsenic molecules through the plant cells and tissues. Arsenate enters plant roots by the same uptake system as phosphate, through the different members of the phosphate transporters family (PTs/PHTs/PHOs) as demonstrated by experiments conducted with *Arabidopsis thaliana* (Catarecha et al., 2007; Leblanc et al., 2013; Remy et al., 2012; Shin et al., 2004), *Holcus lanatus* (Meharg & Macnair, 1990; Meharg & Macnair, 1992), *Oryza sativa* (Y. Cao et al., 2017; Kamiya et al., 2013; Wang et al., 2016; Wu et al., 2011, Y. Ye et al., 2017), *Panax notoginseng* (G.-H. Cao et al., 2020), *Pteris vittata* (DiTusa et al., 2016; D. Sun et al., 2019) and *Salix spp.* (Puckett et al., 2012). These experiments have shown that overexpression of phosphate transporters tends to induce higher arsenate uptake, and conversely gene knockout limits the plant uptake capacity. Expression of these genes plays a key role in plant tolerance to arsenic and their phytoextraction potential.

Even though plants are mostly exposed to As(V) in aerobic soils, the arsenate that is absorbed by roots is rapidly and almost entirely reduced to arsenite (Dhanker et al., 2002; Dhankher et al., 2006; Pickering et al., 2000; Su et al., 2008; X. Y. Xu et al., 2007) by the activity of Arsenic Tolerance QTL (ATQ) and High Arsenic Content (HAC) arsenic reductases (Chao et al., 2014; Sánchez-Bermejo et al., 2014; Shi et al., 2016; J. Xu et al., 2017).

Arsenite, unlike arsenate, does not share the same chemical properties than phosphate. It enters the plant and moves across cell membranes via aquaporins, membrane channel-like proteins known for their role in facilitated diffusion of water and other uncharged molecules including metalloids – notably arsenic (Maurel et al., 2015). In plants,

aquaporins in the nodulin 26-like intrinsic proteins (NIPs) and plasma-membrane intrinsic proteins (PIPs) subfamilies are the ones responsible for arsenite uptake and movement between cells (Bienert et al., 2008; Y. Chen et al., 2017; Isayenkov & Maathuis, 2008; Kamiya et al., 2009; Katsuhara et al., 2014; J. F. Ma et al., 2006; J. F. Ma et al., 2008; Mosa et al., 2012; S.-K. Sun et al., 2018; W. Xu et al., 2015). This diffusion capacity has been mainly proven by heterologous expression experiments but also by gene knockout studies on NIPs (Y. Chen et al., 2017; Isayenkov & Maathuis, 2008; Kamiya et al., 2009; J. F. Ma et al., 2008; W. Xu et al., 2015), further supporting findings of arsenite transport. The involvement of PIPs in arsenite movements has been the subject of little research and should be further investigated.

Inside the plant cells, some arsenite is complexed with thiol-containing compounds like phytochelatins (PCs) and glutathione (GSH) in a detoxification effort by the plant (Mishra et al., 2017, Raab et al., 2005). These As-thiol complexes are sequestered in the cell vacuoles by ABC type C transporters located in the tonoplast (Song et al., 2010; Song et al., 2014). In rice, knockout of the *ABCC1* gene causes a lower tolerance to arsenic, indicating this transporter's role in arsenic detoxification (Song et al., 2014). Some plants show lower degrees of As(III) complexation to thiols (and the associated vacuolar sequestration), which allows for higher xylem loading and root-to-shoot transport of arsenic (Raab et al., 2007; Su et al., 2008). This is the case for the As-hyperaccumulator fern *Pteris vittata,* and possibly a key feature allowing arsenic hyperaccumulation (Su et al., 2008).

Although less prevalent than inorganic arsenic in the environment, a few studies have been conducted on transport mechanisms of methylated arsenic species. R.-Y. Li et

al. (2009) have demonstrated that the rice aquaporin Lsi1 (a member of the NIP subfamily) mediates uptake of both MMA and DMA in roots. Another study showed competition for uptake of these methylated arsenic species and glycerol, indicating that they share the same transport system across plasma membrane. This transport system relies on aquaporins of the plasma membrane of cells (Rahman et al., 2011).

Both cellular arsenite and arsenate can be loaded into the xylem and translocated towards the stems and leaves, in a process called root-to-shoot translocation. In addition to aquaporins and phosphate transporters, a few other membrane proteins have shown transport activity specific to xylem loading. A rice silicon efflux transporter, OsLsi2, has been shown to mediate radial transport of arsenite toward the stele for xylem loading. Being localized in the plasma membrane on the proximal side of both exodermis and endodermis cells of the roots, it allows arsenite to pass the casparian strips and enter the xylem for translocation (J. F. Ma et al., 2007; J. F. Ma et al., 2008). Another study reported the implication of a rice natural resistance-associated macrophage protein (NRAMP), OsNRAMP1, in xylem loading and shoot accumulation of As(III) in plants. This metal ion transporter induces higher arsenic accumulation and tolerance in plants. It is localized on plasma membrane of the endodermis and pericycle cells, allowing xylem loading of As and subsequently root-to-shoot translocation (Tiwari et al. 2014). Another ABC type C transporter, OsABCC7, possesses an efflux transport activity towards the xylem. This ABCC transporter is localized in the plasma membrane of xylem parenchymal cells of rice roots and affects root-to-shoot translocation of arsenite, seemingly by transporting As-phytochelatin and As-glutathione complexes inside xylem cells (Tang et al., 2019). The contribution of ABCC transporters to xylem loading of As is still limited, as free inorganic

As(III) and AS(V) are the main arsenic species found in xylem (Raab et al., 2007; Liu et al., 2010; W.-L. Ye et al., 2010).

Translocation towards reproductive organs by phloem loading of arsenic has also been documented, with specific transporters associated to the process. Inositol transporters (INTs) are involved in As(III) loading in the phloem of *Arabidopsis* (Duan et al., 2016).In rice, a putative peptide transporter belonging to the nitrate transporter 1/peptide transporter (NRT1/PTR) family, is associated with root-to-shoot translocation and grain accumulation of DMA (Tang et al., 2017). This effect seems to result from phloem-loading but needs additional investigation.

A few studies have shown that plants are able to evacuate arsenite into the external medium by an efflux process in their roots. This process has been observed in *Arabidopsis thaliana, Lycopersicon esculentum* and *Oryza sativa* plants provided with arsenate in their growth media (Liu et al., 2010; Vetterlein et al., 2007; X. Y. Xu et al., 2007; Zhao et al., 2010*a*). In rice, OsLsi1 was identified as partially accountable for the efflux of arsenite towards the growth medium (Zhao et al., 2010*a*). Such experiments support the principle of a cycle of arsenate uptake, reduction and arsenite efflux in plant roots which may be of great applied science interest. However, the understanding of this efflux process is far from being complete and deserves to be further studied.

1.2.4. Methods of phytoremediation

Phytoremediation is an innovative technology based on the use of plants (and sometimes their associated root microbiome) to clean up the environment from contaminants such as heavy metals/trace elements (Ali et al., 2013; Antoniadis et al., 2017), organic compounds (Trapp and Karlson, 2001), and radioactive isotopes (S. Dushenkov, 2003). It is a relatively

slow but highly cost-effective method of reducing the negative impact of industrial activity on soil and water quality compared to traditional approaches such as landfilling, chemical fixation or acid leaching for soils, and precipitation, ion exchange, reverse osmosis or filtration for water (Salt et al., 1995; Wan et al., 2016). This is notably due to the fact that biological processes involved in phytoremediation are basically self-sustaining, relying on solar energy, water uptake by plants and microbial metabolism.

Phytoremediation can be divided into six main technologies: rhizofiltration, phytostimulation, phytostabilization, phytoextraction, phytodegradation and phytovolatilization (Pilon-Smits, 2005), which can be exclusive or happen simultaneously in remediation trials. Rhizofiltration is the use of plants root systems to filter out contaminants from water effluents or groundwater (V. Dushenkov et al., 1995). Plant roots can also stabilize contaminants in soil by sequestration in the rhizosphere (phytostabilization), preventing runoff and reducing their bioavailability (Bolan et al., 2011). Phytostimulation relies on the stimulation of microbial activity by root exudates to degrade toxic organic compounds found in the rhizosphere (T. A. Anderson et al., 1993). Degradation of organic contaminants can be carried out directly inside the plants through enzymatic activities, which is the process called phytodegradation (Newman & Reynolds, 2004). Organic and inorganic contaminants can also be transformed to a gaseous form by rhizosphere activity or inside plant tissues, and then released in the atmosphere in a process called phytovolatilization (Limmer & Burken, 2016; Sakakibara et al., 2010). Finally, the most popular phytoremediation method for decontaminating soils of heavy metals and trace elements is phytoextraction, the process by which plants extract contaminants from the soil and accumulate them in aboveground parts (McGrath & Zhao, 2003), which requires high

root-to-shoot translocation ability. Phytoextraction relies largely on the bulk water movements through the soil-plant-atmosphere continuum, which solubilize contaminants and delivers them to the plant. Unlike phytostabilization, in which contaminants are contained belowground but remain in the environment, phytoextraction allows the permanent removal of inorganic contaminants. To complete this process, the aboveground biomass produced is harvested after a certain period of growth and heavy contaminant accumulation, and then either disposed of in landfills, recycled into biofuel or non-food materials, or in certain cases treated for recovery of the accumulated elements (Pilon-Smits, 2005). This process of extracting and recycling elements using plants is called phytomining (C. W. N. Anderson et al., 1999) and relies on hyperaccumulating and fast-growing species.

The original definition of an hyperaccumulator was based on nickel accumulation measurements and corresponded to plants capable of accumulating over 1000 μg/g nickel in their dry biomass regardless of soil concentration (R. R. Brooks et al., 1977). This definition was later adapted to the expanding list of heavy metals and trace elements found in plants, based on their specific phytotoxicity levels to most species. Hyperaccumulators can be characterized by their capacity of accumulating and tolerating exceptionally high amounts of inorganic contaminants in their tissues without showing symptoms of toxicity. Hyperaccumulation has been documented for a variety of elements, such as arsenic, nickel, zinc, cobalt, copper, lead, manganese, selenium, cadmium, chromium, thallium and gold (C. W. N. Anderson et al., 1999; Baker & R. R. Brooks, 1989; L. Q. Ma et al., 2001; Reeves et al., 1995). Thresholds of hyperaccumulation for a selection of trace elements have been summarized by Verbruggen et al. (2009). This capacity found in many plant taxa has stimulated a lot of interest among researchers, both for phytomining purposes and

decontamination of soils through phytoextraction. However, hyperaccumulators tend to have relatively low biomass production, which limits the storage capacity of heavy metals.

1.2.5. Use of willows for phytoextraction

Although not designed as hyperaccumulators, trees (and shrubs) show great potential for phytoremediation, mainly due to their high biomass production which translates into high storage capacity for contaminants such as heavy metals. In addition, trees tend to be resistant to metal toxicity, can grow on nutrient-poor soils and have large root systems which allow them to tap into a large volume of contaminated soil, making them well suited for phytoextraction purposes (Pulford and Watson, 2003).

Among tree species used in phytoremediation, members of the Salicaceae family, and especially the genus *Salix* (willows) have distinguished themselves for phytoextraction purposes (Pulford and Watson, 2003; Wani et al., 2020). Willows make up a large and taxonomically diverse genus of plants, comprising about 450 different species around the world (Argus, 1997). Their high genetic diversity and hybridization capacity allows for phenotypic selection and modifications through breeding programs (Adegbidi et al., 2001, Smart et al., 2005) involving the creation of more efficient hybrids for phytoextraction. Willows are early successional species, and therefore possess specific characteristics such as higher growth rate and higher light requirements compared to later succession species, as well as extensive root systems dominated by fine roots (Smart et al., 2005). Most willow species can also be propagated vegetatively by cuttings, and can easily re-sprout after coppicing, making the planting process easy under unfavorable field conditions (Ruttens et al., 2011). These characteristics, along with a general resilience in degraded environments, make them well-suited for cultivation on open contaminated areas with

compact soil like brownfield with no vegetation coverage in a phytoremediation perspective (Wani et al., 2020). Because of their rapid growth, relatively high accumulation capacity of many heavy metals, high root-to-shoot translocation, high biomass production and economic uses for the harvested wood (Greger & Landberg, 1999), willows are particularly well suited for phytoextraction. Willows are water-loving species, meaning they transpire large quantities of water (Grip et al., 1989). Although this means higher water needs for cultivation, it results in higher mobilization of the soil solution and subsequent flow of contaminants towards aerial tissues. This allows for the combination of biomass production with phytoextraction to maximize economic benefits. Wood harvested from willows is also used for the production of a vast range of by-products such as baskets, woodcrafts, convenience wood, and biofuels (Karp et al., 2010; Verwijst, 2001), which can constitute a desirable economical use for the harvested biomass. Another interesting characteristic of willows for phytoextraction uses is the possibility of conducting frequent harvests without killing the plant by coppicing, in short rotation coppice cultures (SRC). This allows for combining phytoextraction and biomass production to maximize economic benefits (Ruttens et al., 2011). Among phytoremediation experiments, willows have been shown to perform well for phytoextraction of heavy metals such as arsenic, cadmium, copper, lead and zinc to name a few (Greger & Landberg, 1999; Vysloužilová et al., 2003, Yanitch et al., 2020). Notably, Purdy & Smart (2008) demonstrated the capacity in a variety of willow clones to accumulate arsenic in a hydroponic experiment, supporting their use in phytoremediation targeted on this contaminant.

1.3. Water movements in vascular plants

Water (H_2O) is a universal solvent on Earth and a vital constituent of all forms of life. It is the main component of the cell's fluids, allowing the movement of molecules and participating in biochemical reactions essential to life (Ball, 2017). In plants, water is the main limiting abiotic factor for development and growth (Boyer, 1982; McIntyre, 2001). Water provides structural support to plants via turgor pressure, and is required in cell composition, in the long-distance transport in the xylem and phloem, in the photosynthesis process to reduce CO_2 molecules into organic compounds, and to replace water loss caused by transpiration. Since plants need to let CO_2 in for metabolic needs, they must open their stomata, which causes loss of water. However, the amount of water lost is disproportionately high relative to the small amount of CO_2 absorbed (Venturas et al., 2017). Transpiration accounts for over 95% of the water use by plants (Sperry, 2011). The rate of transpiration, although variable depending on multiple factors such as species, age and environmental conditions, is particularly high in trees. As reviewed by Wullschleger et al. (1998), 90% of observations made across 52 studies, covering 67 tree species, showed that between 10 and 200 liters of water were used per day in trees measuring 21 meters on average. At the upper end of the data treated in this review, some large canopy trees of the Amazonian rainforest were found to transpire upwards of 1180 liters of water per day (Jordan & Kline, 1977).

1.3.1. Water ascent in the xylem and through the leaves

In higher plants, the water column in the vascular system forms a continuous system between the soil (source) and the atmosphere (sink), called the soil-plant-air continuum (Steudle, 2001). Plants lack an active pump for the long-distance transport of fluids such

as the heart in animals, and instead rely on passive physical mechanisms. Over the course of evolution, some plants developed a specialized vascular tissue, called the xylem, which enables the transport of water (sap) over long distances to photosynthetic organs without the energy costs of an active system (Sperry, 2003; Sperry, 2011). Water in the xylem is transported in two different types of cells, tracheids and/or vessel elements. Both are characterized by their elongated shape, lignified cell walls, and programmed death of the protoplast (Sperry, 2003). Mature vessels and tracheids have thick lignified walls with small unlignified areas called pits that facilitate radial water uptake from xylem parenchyma cells (Venturas et al., 2017). Sap ascends through the plants' vascular system along a gradient of water potential (Hellkvist et al., 1974), as described by a well supported transpiration-driven process called the cohesion-tension theory (H. H. Dixon & Joly, 1895; Sperry, 2011; Steudle, 2001; Tyree, 1997). The chemical structure of the water molecule is at the basis of this behavior, along with water's inherent role in biological processes (Ball, 2017). Water molecules being dipolar, they can form hydrogen bonds between themselves (cohesion) and with other hydrophilic molecules (adhesion). Water absorbed by plants is sucked up by the xylem, which it fills by capillary action all the way to the mesophyll cells (internal leaf cells). Water molecules evaporate from the leaves due to lower water potential in the air, creating a negative pressure on the water meniscus in mesophyll cell wall. Evaporated water molecules are replaced by others in the sap, pulling on the water column like a rope under tension. This transpirational pull extends all the way from the leaf mesophyll cells through the xylem, into the soil water. The cohesive strength of water creates surface tension in the meniscus at the plant-air interface and combined

with the adhesion of water molecules to the xylem conduit keeps the integrity of the water column while it ascends through the vascular system (Venturas et al., 2017).

Although water uptake through leaves can occur under certain conditions (Laur & Hacke, 2014; Mayr et al., 2014; Roth-Nebelsick et al., 2023), a plant's foliage mainly acts as site of loss of water through transpiration. Stomata, apertures in the epidermis delimited by two guard cells, are key morphological features of the leaves and sometimes stems of vascular plants (Kirkham, 2014). These pore-like structures usually open during daytime and reduce their aperture at night under regulation of guard cells' turgidity, optimizing photosynthesis in the presence of light (Caird et al., 2007; Costa et al., 2015). Their activity controls gas exchanges in the leaf such as CO_2 intake and water diffusion to the atmosphere (Venturas et al., 2017). To prevent desiccation, guard cells react to the plants' water status under the action of signaling compounds like abscisic acid (ABA), adjusting their turgor pressure. By doing so, guard cells can control the aperture of the stomata, inducing lower or higher transpiration depending on the plant's water and CO_2 needs (Buckley, 2019). Stomatal activity is also regulated by light exposure (Doi et al., 2015) and atmospheric CO_2 (Franks & Britton-Harper, 2016) to facilitate gas exchange between the plant and the atmosphere. This response is essential for the biological activity of terrestrial plants, optimizing photosynthesis and allowing water uptake as explained by the cohesion-tension theory.

1.3.2. Water uptake by the roots

Plants acquire most of their water in the soil by absorption through their roots. Inside the root, water can move by axial transport, as explained by the cohesion-tension theory, or by radial transport through living cells, which is necessary to reach the xylem for long-distance

transport (Steudle & Peterson, 1998). There are three pathways of radial water (and solutes) flow in the roots (Steudle & Peterson, 1998). The apoplastic path consists of the space on the outside of the plasma membrane of cells. This includes cell walls, intercellular space, and the lumen of vascular tissue cells. The symplastic path corresponds to the continuum of cytoplasm of adjacent cells, connected by plasmodesmata, excluding the vacuole. Finally, the transcellular or vacuolar pathway goes across membranes (Steudle, 2000). As described later, this movement involves aquaporins. The symplastic and transcellular pathways are difficult to distinguish and are often treated as the undifferentiated "cell-to-cell" pathway (Steudle & Peterson, 1998). Across the radial path of uptake, water has to cross differentiated cell layers. These include – from the outside towards the center of the root – the epidermis, hypodermis (or exodermis in the presence of Casparian bands (Perumalla & Peterson, 1986)), cortex, endodermis, pericycle, xylem parenchyma cells and tracheary elements (Steudle & Peterson, 1998). Upon absorption, water enters the epidermis by both the apoplastic and cell-to-cell pathways and is pulled towards the xylem following the decreasing water potential gradient (Steudle & Peterson, 1998). The apoplast of the exodermis and endodermis is modified by the presence of Casparian bands, a deposit of lignin and suberin, and sometimes suberin lamellae in radial cell walls. These features form a hydrophobic apoplast barrier, forcing the flow of water (and ions in solution) to go throught the symplastic and transcellular paths at these two cell layers (Enstone et al., 2002). In the cortex, pericycle and xylem parenchyma cells however, no such barrier limits the flow of water, which can occur by all three pathways described previously through these root tissues. To reach the lumen of xylem conduits, water diffuses through the

"membrane" of the pits, which is actually a thin unlignified area made up of the primary cell wall and middle lamella of dead vessel cells (Venturas et al., 2017).

1.3.3. Hydraulic redistribution

At all times, water will follow the water potential gradient through the soil-plant-atmosphere continuum. By this process, plant roots have the capacity to redistribute water across soil patches, a process called hydraulic redistribution. This was first described by Richards & Caldwell (1987) under the term "hydraulic lift", although previous experiments had already hinted at the existence of such a process (Corak et al., 1987; Mooney et al., 1980; van Bavel & Baker; 1985). It can be summarized as follows: during the day, water is absorbed by roots in all layers of soil, transported through the plant and lost due to transpiration following the existing water potential gradient in the soil-plant-air continuum. When stomata close at night, transpiration is suppressed, lowering water loss through the leaves and raising water potential in the whole plant. During that time, the more negative soil water potential becomes the main driver of water movement. The plant's water is then released in the dryer patches of the soil, usually in the surface layers where water has evaporated during the day. This process often results in an upwards transport of water from deep moist soil to the upper dry soil horizon, hence the term "lift". It is no coincidence that hydraulic lift was discovered in the Great Basin of Utah, where there is often a gradient in soil water potential between wetter, deeper soil layers and drier, shallow soil layers. This water transport activity has been extensively studied and reviewed since its first description (Alagele et al., 2021; Caldwell et al., 1998; Liste & White, 2008; Prieto et al., 2012), giving insight on its multiple physiological, ecological and agricultural implications.

In nature, hydraulic redistribution has been documented in a wide variety of grasses, shrubs, and trees in environmental conditions ranging from arid to tropical (Armas et al., 2010; Dawson, 1993; Moreira et al., 2003; Oliveira et al., 2005; Warren et al., 2007; Mooney et al., 1980; Jiménez-Rodríguez et al., 2019; Espelata et al., 2004).

Over time, new studies have emerged showing a greater range of implications of hydraulic lift in soil water movements. It was discovered that water was redistributed according to the soil water potential gradient regardless of its direction, whether it be from surface to deeper layers of soil (Burgess et al., 1998; Schulze et al., 1998), or horizontally (Hafner et al., 2020; Hansen & Dickson, 1979). These discoveries led to the use of the more accurate term "hydraulic redistribution".

The role of hydraulic redistribution in water commensalism between plants has been demonstrated in various experiments (J. R. Brooks et al., 2006; Caldwell & Richards, 1989; Corak et al., 1987; Hirota et al., 2004; Pang et al., 2013; Sekiya et al., 2011; Sekiya & Yano, 2004). These studies have shown the benefits of co-planting shallow-rooted and deep-rooted plants in agricultural systems in terms of irrigation efficiency and crop health. Hydraulic redistribution homogenizes soil moisture by tapping into the deep soil unexposed to evaporation and bringing water towards the dry surface rooting zone. By this process, deep-rooting species can act as water donors for neighbouring shallow-rooted crops, positively impacting their water status in water-limited conditions.

Hydraulic redistribution has been observed in senesced trees and even in plants subjected to the removal of their aerial tissues, supporting the passive mechanism basis of this process (Leffler et al., 2005; Sekiya et al., 2011). According to these findings, harvesting plants while leaving behind intact root systems can allow water flow to continue

through the leftover roots, acting as water conduits, which can help maintain water storage and homogenous distribution in soil. Notable other ecophysiological implications of hydraulic redistribution include improved soil microbial activity, enhanced revegetation of degraded land, increased nutrient availability and root proliferation (Alagele et al., 2021; Lambers et al., 2006; Liste & White 2008; Prieto et al., 2012).

Based on the ecological impact of hydraulic redistribution, its applications in a phytoremediation context are implied, not only by promoting root survival in dry soil, but also by providing vital moisture to soil microbes involved in organic chemicals degradation and by enhancing the mobilization of contaminants (Liste & White 2008). Even though the process in question has been extensively studied in terms of plant ecophysiology, much remains to be learned about the underlying cellular and molecular activity, especially with regards to phytoremediation trials.

1.3.4. Role of aquaporins in plant water movements

Aquaporins are bidirectional water channel proteins of the Major Intrinsic Protein superfamily (MIP) responsible for the passive transport of water and small uncharged solutes like glycerol, urea, hydrogen peroxide, ammonia and metalloids (Maurel et al., 2015, Hacke & Laur, 2017). Aquaporins in vascular plants can be categorized in 5 subfamilies: plasma-membrane intrinsic proteins (PIPs), nodulin 26-like intrinsic proteins (NIPs), tonoplast intrinsic proteins (TIPs), small and basic intrinsic proteins (SIPs) and uncategorized (X) intrinsic proteins (XIPs) (Maurel et al., 2015). These subfamilies show specific tissue and subcellular localization patterns, which plays an important role in water and solute distribution, in and through the cells and plant organs. Three of these protein groups are found in the plasma membrane, controlling the water and solute exchange at

this transcellular barrier. These are the PIPs (Zelazny et al., 2007), NIPs (J. F. Ma et al., 2006; J. F. Ma et al., 2007; Takano et al., 2006) and XIPs (Bienert et al., 2011). TIPs are the only subfamily of aquaporins associated with the vacuole's membrane, the tonoplast (Wudick et al., 2009). The endoplasmic reticulum membrane also hosts some isoforms of the SIP (Ishikawa et al., 2005; Noronha et al., 2014) and NIP (Mizutani et al., 2006) subfamilies. While PIPs and TIPs are the most efficient water channels, all five groups show a certain degree of water transport activity (Maurel et al., 2015).

It is well documented that aquaporins play a major role in the hydraulic conductivity (membrane permeability) of plant cells (Maurel, 1997). Aquaporins are expressed in the roots, shoots, leaves and reproductive organs of plants, at various abundance levels depending on environmental factors and developmental stages (Hachez et al., 2006). Aquaporin transport inhibitors such as mercury (Hg) have been used in multiple experiments to show the contribution of these water channels to root water transport (Barrowclough et al., 2000; Maggio & Joly, 1995; Katsuhara et al., 2014; Martre et al., 2001; Sutka et al., 2011; Vandeleur et al., 2014; Wan & Zwiazek, 1999). Mercury chloride ($HgCl_2$) interacts with aquaporins by binding to cysteine residues in the pore region, causing an occlusion which prevents the transport activity of the protein (Preston et al., 1993). Although Hg-induced inhibition is not specific to aquaporin activity, these studies demonstrated high reduction of root water permeability when blocking aquaporin channels in a variety of plants, showing their importance in water uptake by roots. Under a different approach, over-expression of PIP aquaporins results in higher root hydraulic conductance (Lee et al., 2012; Lian et al., 2004; Perrone et al., 2012), supporting again the major role of aquaporins in plant-water relations. Plasma membrane aquaporins expressed

in aerial tissues, including xylem, phloem, stomata, mesophyll and epidermis, have the same role as in roots in terms of hydraulic conductivity (Fraysse et al., 2005; Hachez et al., 2008; Laur & Hacke, 2014; Secchi et al., 2017). Experiments using both the $HgCl_2$ approach (Pou et al., 2013) and genetic manipulations of PIPs (Postaire et al., 2010; Prado et al., 2013) in aerial parts support the function of aquaporins for membrane permeation in the whole plant, and not just in roots. Expression of aquaporins is strongly regulated in response to abiotic factors such as drought (Šurbanovski et al., 2013), flooding (Rodríguez-Gamir et al., 2011), temperature (Lee et al., 2012), salinity (Jia et al., 2020), transpiration demand (Laur & Hacke, 2013) and circadian light cycles (Lopez et al., 2003). These responses show how aquaporins induce cell membrane plasticity and allow plants to adapt to their environment.

1.4. Summary of the literature review

Arsenic is a highly toxic element with harmful effects on most forms of life. It is ubiquitously found in water, soil, air and living organisms around the world as a result of contamination from natural sources and anthropogenic activities. The chemical speciation and bioavailability of arsenic depends on the conditions of the environment it is found in such as pH, redox potential or microbial activity. Arsenates (AsO_4^{3-}), arsenites (AsO_3^{3-}) and methylated arsenic species are the main forms of arsenic found in the environment, with different molecular processes of toxicity and transport mechanisms in cells. Uptake and translocation of arsenic through plants occurs under the control of several membrane-associated transporters. Depending on the chemical structure of arsenic compounds and the localization in the plant, different transporters are involved in this process. Phosphate

transporters and aquaporins are particularly important, as they allow the uptake and transport of inorganic arsenate and arsenite respectively.

Phytoremediation by phytoextraction is a promising way to decontaminate soils containing hazardous levels of arsenic. This technology relies on the uptake mechanisms of plants to absorb and sequester contaminants in their aboveground biomass thereafter harvested for commercial applications. Willows (*Salix spp.*) are particularly well-suited for this because of their physiological traits, notably their fast growth, high transpiration rate, high biomass production and resprouting ability after coppicing. These characteristics make them good candidates for decontamination, provided we can demonstrate their capacity to pump contaminant-laden soil solution from all soil profiles. Plant-water relations play a critical role here, as they explain the movement of the soil solution and the mobilization of hydrophilic contaminants from bulk soil towards the plant body (Bruemmer et al., 1986; Cernusak et al., 2011; Rouphael et al., 2012). Trees being sessile organisms, they have developed an efficient system of water acquisition to fulfill their water needs – particularly to compensate for transpiration. To enter the plant, water must first be absorbed by roots and transported radially across different cell layers to the vascular system. Then, through a passive process explained by the cohesion-tension theory, trees can transport water across long distance in their vascular system, up to the stomata apertures where most of the water loss occurs by transpiration. Through passive root activity, some plants (including trees) are also capable of redistributing water from moist to dry soil patches in a process called hydraulic redistribution, usually from deep soil towards the surface exposed to evaporation. Aquaporins, which are also involved in arsenic transport, play an important role in water movements throughout the plant. Their

expression level controls the flow of water through membranes but has not been studied in the context of combined hydraulic redistribution conditions and soil contamination.

Increasing our knowledge in the field of phytoremediation and understanding the underlying mechanisms of decontamination are crucial to take informed decisions and to optimize this technology. Cultivation practices must consider the complex plant-water relations that could affect the movement of contaminants and their availability in the soil for successful and more efficient decontamination. For example, a better understanding of the role of membrane proteins involved in arsenic accumulation could be used in future experiments focusing on gene expression manipulation, or in breeding programs along with genotyping for the selection of cultivars. This could either be applied to phytoremediation by promoting contaminant uptake for decontamination, or in crops with limited uptake activity to avoid food hazards.

2. Research article

Title: Hydraulic Lift and Arsenic Redistribution Occur Independently in *Salix nigra*

Nicolas Perrault [1,2], Vanessa Grenier [2], Laurianne Bedard [2], Laurie Saint-Fleur [2], Margot Cardenas [2], Corinne Oligny [2], Martin Lefrancois [2], Uwe Hacke [1], Joan Laur [2*]

[1] Department of Renewable Resources, University of Alberta, Edmonton, AB, Canada

[2] Institut de Recherche en Biologie Végétale, University of Montreal and Montreal botanical Garden, Montréal, QC, Canada

*Corresponding author: Joan Laur

2.2. Summary

Arsenic is a highly toxic and ubiquitous element to which millions of people are exposed worldwide. Willow plantations can be particularly well suited to clean up soils containing hazardous levels of arsenic. For this purpose, their ecophysiological properties need to be properly understood. From a phytoextraction perspective, plant-water relations explain the mobilization of hydrophilic contaminants like arssenic from bulk soil towards the plant body. Both water and arsenic uptake, and their movement across cell membranes, are regulated by transport proteins. Through two greenhouse experiments using a double-compartment design, physiological activity monitoring and mRNA profiling, we investigated the movement of water and arsenic in the soil and within willows under experimental treatments of drought and soil contamination. Our results show that a process

of water redistribution towards dry surface soil occurs through *Salix nigra* root system, although aquaporins activity is repressed in surface roots exposed to drought. We highlight the intricate willows' root activity in response to both arsenic and drought at the gene expression level using mRNA profiling. The expression of aquaporins, phosphate transporters and ABC transporters in roots identifies key genes responsible for water and arsenic transport under stress conditions. Their expression level indicates the presence of a weak exclusion mechanism of arsenic in *Salix nigra*, allowing the easy uptake of the contaminant from deep soil. Simultaneously, the repression of aquaporin genes in surface roots blocks a possible efflux pathway, confining the arsenic inside the plant. Most importantly, adverse growth conditions caused by contamination exposure and an extended episode of drought in surface soil are more likely responsible for root decay, and induce the arsenic redistribution to surface soil layers. This process does not seem to result from the hydraulic redistribution observed in the short-term.

2.3. Introduction

Arsenic (As) is a toxic metalloid found ubiquitously in the environment. Its two most common forms are arsenate (As(V)) in oxidating conditions and arsenite (As(III)) in reducing conditions (IARC, 2012). It occurs naturally in over 200 mineral forms throughout the earth's surface, with the majority being found as arsenopyrite (Cullen & Reimer, 1989; Mandal & Suzuki, 2002). Human activity strongly influences its distribution in water, soil, and air, and consequently increases our exposure to contamination Such anthropogenic sources, past and present, include mining activity, metallurgy, agriculture, fossil fuel combustion, as well as its use in desiccants, wood preservatives, feed additives, drugs, and poisons (Mandal & Suzuki, 2002). It is estimated that between 94 and 230

million humans are at risk of poisoning by As-contaminated groundwater (above the 10-ppb concentration in water recommended by the World Health Organization), across 108 countries including Canada and the United-States (Podgorski & Berg, 2020; Shaji et al., 2021). In living organisms, arsenic exposure is generally known to induce the production of reactive oxygen species (ROS) causing oxidative stress in cells, damaging molecules and cell structures (Hartley-Whitaker et al., 2001). Among arsenic species, arsenite induces toxicity due to its high affinity for thiol/sulfhydryl groups and can prevent vital cellular processes from happening by inducing conformational changes in proteins (Ramadan et al., 2007, Scott et al., 1993). In turn, arsenate is a chemical analogue to phosphate (P_i), which it can replace in major biochemical reactions (H. B. F. Dixon, 1996), potentially disrupting ATP synthesis, glycolysis, phospholipid and nucleic acid metabolism or protein phosphorylation (Finnegan & Chen, 2012, Delnomdedieu et al., 1994) and causing DNA damage by oxidative stress (A. Lin et al., 2008). To limit the negative anthropogenic impact on the environment, it is imperative to develop efficient, cost-effective, and ecologically sound decontamination technologies like phytoremediation (Ali et al, 2013; Antoniadis et al, 2017).

Phytoextraction is a neat phytoremediation approach to decontaminate soils from heavy metal(loid)s such as arsenic (Ali et al., 2013; Antoniadis et al., 2017; Yanitch et al., 2020). It relies largely on movement of the soil solution and the mobilization of hydrophilic contaminants (and simultaneously nutrients) from bulk soil up to the plant body (Bruemmer et al., 1986; Rouphael et al., 2012). This flow allow the high root-to-shoot translocation necessary for the accumulation in aboveground plant tissue and successful decontamination (McGrath & Zhao, 2003). Among plants used in phytoextraction, willows

have shown great potential for successful removal of heavy metals like arsenic (Greger & Landberg, 1999; Navazas et al., 2019; Purdy & Smart, 2008; Vysloužilová et al., 2003; Yanitch et al., 2017). Some key characteristics can explain their advantageous use as phytoextractors, such as a relative tolerance to heavy metals, a high growth rate, extensive root systems dominated by fine roots, relatively high accumulation capacity of heavy metals, high root-to-shoot translocation, high biomass production and viable economic uses of the harvested wood (Greger & Landberg, 1999; Karp et al., 2010; Smart et al., 2005; Verwijst, 2001). Most willows – like *Salix nigra*, a riparian tree native to eastern North America – are water-loving species that transpire large quantities of water (Grip et al., 1989). This transpiration activity results in the strong mobilization of the soil solution and subsequent flow towards aerial plant tissues (Cernusak et al., 2011).

Widespread among vascular plants, the well-documented hydraulic redistribution process (Alagele et al., 2021; Liste & White, 2008; Prieto et al., 2012) results in the movement of soil water from moist to dry patches, usually from deep soil towards the surface exposed to evaporation (Corak et al., 1987; Mooney et al., 1980; Richards & Caldwell, 1987; van Bavel & Baker; 1985). This movement is intrinsic to plant-water relations. Concurrently, water acquisition and transport inside plants depend strongly on plasma membrane aquaporins of the NIP and PIP subfamilies (Barrowclough et al., 2000; Maggio & Joly, 1995; Katsuhara et al., 2014; Martre et al., 2001; Sutka et al., 2011; Vandeleur et al., 2009; Vandeleur et al., 2014; Wan & Zwiazek, 1999). The same aquaporins allow the permeation of cell membranes to As(III) and its cell-to-cell transport (Bienert et al., 2008; Chen et al., 2017; Isayenkov & Maathuis, 2008; Kamiya et al., 2009; Katsuhara et al., 2014; J. F. Ma et al., 2006; J. F. Ma et al., 2008; Mosa et al., 2012; S.-K.

Sun et al., 2018; W. Xu et al., 2015). Adding to the intrigue is the fact that some plants have been shown to evacuate arsenite from roots by an active efflux process (Liu et al., 2010; Vetterlein et al., 2007; X. Y. Xu et al., 2007; Zhao et al., 2010), as a possible detoxification mechanism. In the context of the application of phytoremediation, such processes raise questions, more so given the similarity of the proteins involved in the transport of water and contaminants like arsenic.

Keeping this in mind, this study had three main objectives. First, we aimed to further describe the processes of hydraulic redistribution in willow roots at a molecular level. Secondly, we wanted to study the involvement of hydraulic redistribution in the movement of arsenic through the soil profile. Lastly, we wanted to provide a better understanding of the stress response and transport mechanisms related to arsenic exposure in willows.

To this end, *S. nigra* plants were grown under controlled conditions in an experimental setup that separates the soil and root systems into two overlaid compartments. This allowed for the combinations of different watering and arsenic contamination regimes. Arsenic accumulation, hydraulic redistribution, and the interaction between these two processes in willows at both the physiological and gene expression levels were thus investigated.

2.4. Material and methods

2.4.1. Greenhouse growth and double-compartment system

To study the physiological processes involved in arsenic and water movements in black willow roots, experiments were carried out in the controlled conditions of a greenhouse located at the Montreal Botanical Garden (Montreal, Canada) with 18 h light/6 h dark photoperiod and temperatures kept between 18 – 25 ˚C. Watering was done every 2 days. The substrate used was Berger® BM6 potting mix.

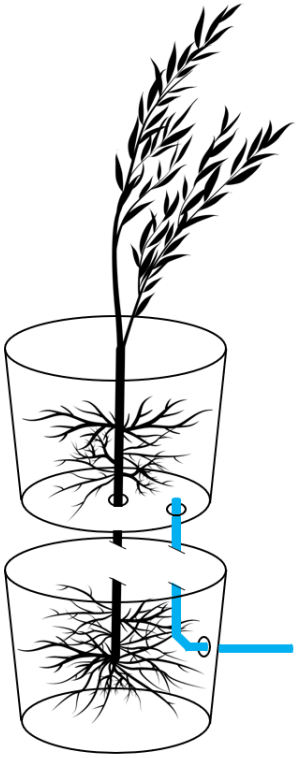

Figure 1. Representation of the double-compartment system. This model allows the clear separation of substrate and root systems in two distinct sections, with the willow plant being the only permeable connexion between bottom and top compartments.

A long-term experiment took place to investigate arsenic redistribution by willows over a whole growing season. Another experiment was also performed to investigate short-term processes. Both experiments were based on the same conceptual model: each experimental unit consisted of double-compartment pots that allow the physical separation of deep and shallow soil layers. The only connection between the two soil compartments being the plant taproot or cutting (Figure 1). Top pots were sealed using 100% silicone sealant to prevent leakage between compartments and a layer of perlite on the surface of the bottom compartment further prevented capillary water movement, evaporation, or exposition to light. Drainage was achieved with a tube for the top pot and holes in the bottom one to prevent possible rotting of the roots. With this method, root systems were also divided

distinctly in bottom and top sections, allowing us to study the individual response of root system sections to treatments, i.e., distinct water regimes and/or arsenic contamination.

2.4.2. Long-term experiment

In the long-term experiment, black willow cuttings (50 cm) were grown in systems made up of two 1-gallon nursery pots stacked vertically. After 5 weeks of growth to allow root establishment, all experimental units were treated with a solution of water and sodium heptahydrate arsenate ($HasNa_2O_4 \cdot 7H_2O$) to an element concentration of 100 mg As(V) / kg dry soil in the bottom pots. From a random selection, 5 systems were watered daily (8:00 AM) in both compartments, while 5 others were subjected to a prolonged drought stress in the top pots and watered normally in the bottom pots. Drought was characterized by a soil volumetric water content (VWC) of 0-10% in treated pots, while the watered condition corresponded to 30-40% VWC. After 16 weeks of treatment, each plant was harvested, and soil samples were collected from both compartments between 10 AM and noon just after watering.

2.4.3. Short-term experiment

For the short-term experiment, 20 black willows plants (3-year-old, previously grown in 1-gallon pots) of similar height were transferred into double-compartment systems made from two 4-gallon nursery pots. Before transfer, root systems were dipped in a 15% V/V solution of Pro-Mix® Root Booster® (5-15-5 + IBA + NAA). During the establishment phase, plants were watered daily (8:00 AM) and fertilized weekly with a 10% V/V solution of Golfgreen Organic™ Cricket Manure (7-4-3) to maximize root growth. After 4 weeks of root establishment, plants were randomly separated into four combinations of drought/watering and arsenic treatments (Figure 2). In addition, five experimental units

were setup with no plants and used as unplanted controls, treated with arsenic in the bottom compartment and drought in the top one to assess the double-pot system's permeability to water and arsenic movements. The watering regime was the same as in the previous experiment. After 2 weeks of treatment, each plant was harvested, and soil samples collected from both compartments between 10 AM and noon just after watering. Fresh root samples were also collected from all bottom and top compartments, placed in separate tubes, flash frozen in liquid nitrogen and stored at -80 ˚C until further analyses.

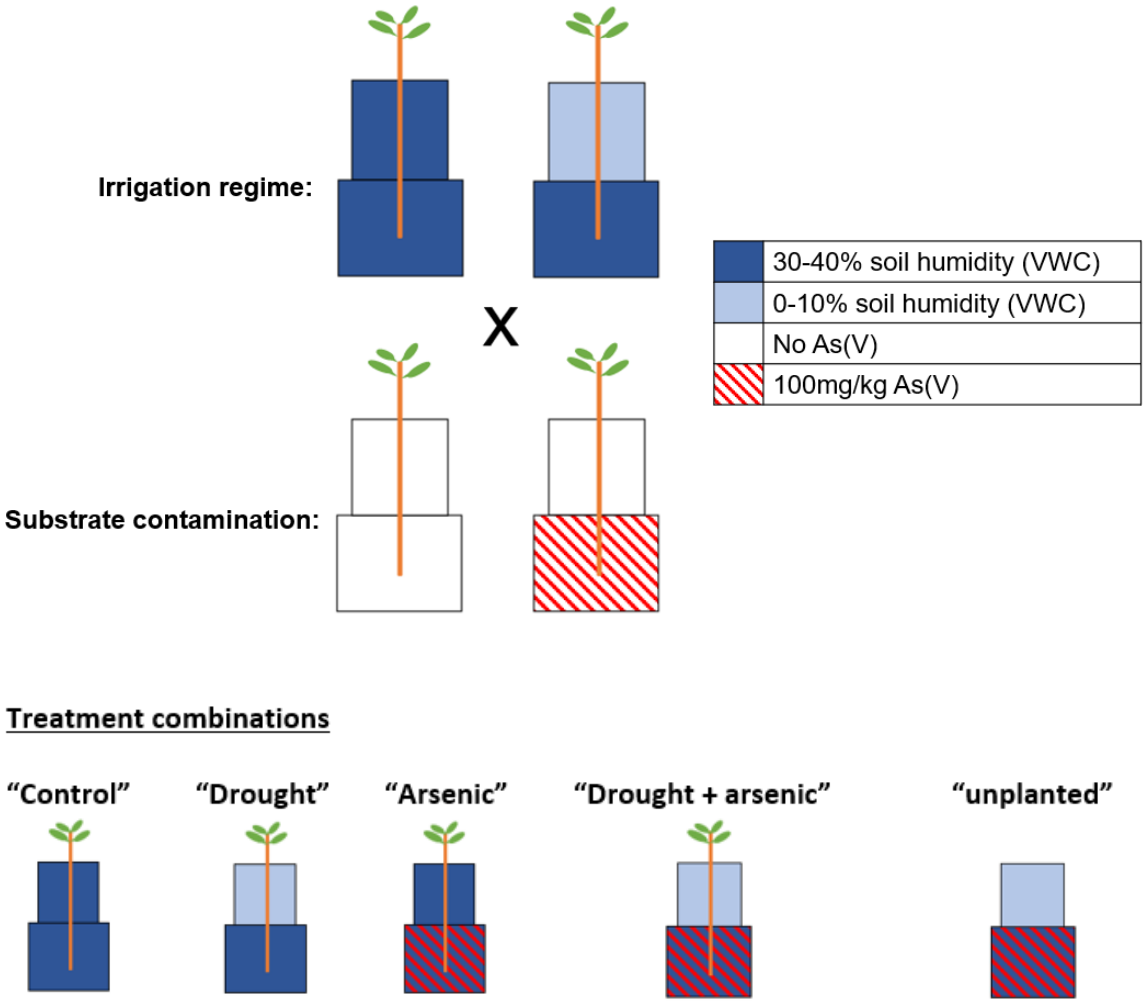

Figure 2. Schematic representation of the different treatments to which plants were exposed during the short-term experiment to assess the respective and combined effects of partial drought and localised arsenic contamination on 3-year-old willow trees. Dark blue boxes represent watered compartments and light blue boxes represent the drought-treated ones. White boxes represent compartment with no arsenic contamination, while red-dashed box represents the ones where a solution of arsenic was applied (100 mg As / kg dry soil). Hence, the four treatments combination were as followed: "control" (watered in both compartments and no contamination), "drought" (drought-treated in the top compartment and no contamination), "arsenic" (watered in both compartments and As(V) contamination in the bottom one), and "drought + arsenic" treatment (drought-treated in the top compartment and As(V) contamination in the bottom one). The additional unplanted group was similar to the "drought + arsenic" treatment, but with no willow plants. All treatment groups were replicated five time (n = 5).

2.4.4. Hydraulic redistribution measurements

One day before harvesting the short-term experiment, soil humidity was monitored over a 24-hour period using an ECH_20® EC-5 soil moisture sensor and a 2015 Decagon Devices ProCheck® handheld reader. Measurements were taken in the top compartment every two hours in triplicates for each experimental unit to determine the root systems' ability to influence the soil water content via hydraulic lift, as described by Richards and Caldwell (1987).

2.4.5. Biomass measurements and arsenic dosage

Once harvested, bottom roots, top roots, and aerial parts (stems and leaves combined) were oven-dried for 48 hours at 72 ˚C, weighed, ground and sieved to 2 mm. Soil samples were also sieved to 2 mm. Then, 200 mg of powder per plant section and soil samples were weighed and digested using 70% trace metal grade nitric acid (HNO_3). Digestates were diluted in ultrapure water (Milli-Q®) and sent to the CACEN (University of Montreal) for ICP-MS analysis.

2.4.6. RNA profiling analysis

2.4.6.1. RNA extraction and mRNA enrichment

Total RNA was extracted from ~100 mg of frozen root samples from the two compartments of each experimental unit. Extraction was performed with a modified CTAB protocol (Appendix – Protocol 1) derived from the ones described in Chang et al., 1993 and Mu et al., 2017. RNA quantification and purity was assessed with a spectrophotometer (NanoDrop™ 2000, Thermo Scientific™).

Following extraction, RNA samples were enriched in poly(A)-tailed mRNA using oligo(dT) magnetic beads (Oligo d(T)25 Magnetic Beads, New England BioLabs®) and a

6-tube magnetic separation rack (New England BioLabs®). The protocol used (Appendix – Protocol 2) was derived from an OpenWetWare protocol written by Kam D. Dahlquist (Dahlquist: RNA-seq Protocol, 2016). RNA quality was assessed by gel electrophoresis before further analysis.

2.4.6.2. RNA sequencing and profiling analysis

Non-degraded RNA samples were sent to the UQAM CERMO-FC genomics platform for preparation of 3' mRNA-seq libraries. In short, libraries were prepared using NEBNext® Ultra™ II Directional RNA Library Prep Kit for Illumina® (New England BioLabs®), following an optimized protocol to produce ~200 nucleotide inserts. Adapters used were NEBNext® Multiplex Oligos for Illumina® (96 Unique Dual Index Primer Pairs). Library quality was evaluated with the Agilent TapeStation system (Agilent Technologies) and by quantification of DNA contents with Qubit™ dsDNA HS (ThermoFisher). Paired-end 100bp sequencing was performed using an Illumina® NovaSeq™ 6000 s1 (version 1.5) system to a depth of >10 000 000 reads per sample.

Raw reads were quality checked using the fastQC software version 0.11.9 (Andrews, 2010) and low-quality reads were filtered out using fastp version 0.23.1 (S. Chen et al., 2018). Bowtie2 version 2.4.4 (Langmead & Salzberg, 2012) was used to build a reference genome index from the *Salix purpurea* 94006 v5.0 assembly (R. Zhou et al., 2020) accessible on the Phytozome version 13 website (Goodstein et al., 2012), and to map the processed reads to the reference genome of *Salix purpurea* was used as the reference genome since it is a fully sequenced organism taxonomically close to *S. nigra*. The BAM files output of Bowtie2 were converted to SAM files using SAMtools version 1.12 (H. Li et al., 2009). These were used as input for the featureCounts tool, a part of the Subread

version 2.0.3 software package (Liao et al., 2014) along with the reference genome's GFF3 gene annotation file from Phytozome version 13 to generate a read counts file. Read counts were then processed using the ExpressAnalyst 1.0 web-based tool for gene expression analysis derived from NetworkAnalyst 3.0 (G. Zhou et al., 2019). Data filtering and normalization were performed with default parameters (low abundance filter = 4, variance filter = 15) and the Trimmed Mean of M-values (TMM) method, respectively. Differential expression (DE) analysis was performed with the EdgeR method (Robinson et al., 2010) to conduct pairwise comparisons of differentially expressed genes (DEG) between treatment groups, in both bottom and top sections of root systems. Significance thresholds were set at: adjusted p-value = 0.05, log2-fold change = 1.0. Namely, 5 treatment comparisons were analyzed:

1) The "drought" and "control" groups were compared to evaluate the effect of drought imposed in the upper compartment on the transcriptome of stressed roots (upper compartment) and potentially its impact on those roots whose irrigation was still maintained (lower compartment)

2) In turn, the "arsenic" treatment was compared to "control" to identify the transcriptomic response of roots directly exposed to the contaminant (bottom compartment) and the consequences of such treatment on more distant roots (upper compartment).

3) Next, the root transcriptomes of the units that were exposed to both arsenic in the lower compartment and partial drought in the upper compartment ("drought + arsenic") were compared to those of the "control" systems.

4) In addition, the added potential impact of arsenic contamination on an already drought-stressed system was also verified by comparing the treatments “drought + arsenic” and “drought”.

5) A comparison was also performed between top and bottom roots in the “control” treatment to evaluate the existing differences between them due to the root system partitioning in the two compartments.

DeepVenn was used to illustrate DEG differences among those comparisons (Hulsen, 2022). The web-based program AgriGO (Tian et al., 2017) was used with default parameters and the plant GO Slim subset to perform a gene ontology (GO) Singular Enrichment Analysis (SEA). Finally, the Phytozome search tool (Goodstein et al, 2012; R. Zhou et al, 2020) was used to further look into *S. purpurea* annotations associated with targeted GO terms or specific IDs, and to curate manually all lists of DEG obtained from ExpressAnalyst.

2.4.7. Statistical analyses

In the greenhouse experiments, the layout of the experimental units was randomized to avoid blocking effects. Each treatment condition was replicated in 5 units for statistical purposes. All statistical analyses for biomass, arsenic dosage and soil water content data (treated as independent variables for statistical comparisons) were performed with the R programming environment (version 4.1.1). When necessary, logarithmic data transformation was performed. The soil arsenic content and biomass allocation over the long-term experiment were compared using a Student’s T-test, between the “arsenic” group and “drought + arsenic” group. For the physiological data obtained in the short-term experiment (biomass allocation, arsenic content, and relative soil humidity), differences

due to the treatments were analyzed by analysis of variance (ANOVA). One-way ANOVA was used for the comparison of relative soil humidity in top pots in response to a single factor (experimental condition). Two-way ANOVA was used for the comparison of biomass allocation and arsenic content data in response to the combinations of treatment. ANOVAs were followed by Tukey's HSD tests to account for multiple inferences and study the comparisons between treatment groups. Differential expression of genes was performed by the EdgeR tool of NetworkAnalyst 3.0 (G. Zhou et al., 2019), which performs statistical testing similarly to a Fisher's exact test (Robinson et al., 2010).

2.5. Results

2.5.1. Plant biomass is affected differently by drought and arsenic

All plants exposed to arsenic either during the short-term experiment or over the 16-weeks long treatment period survived. In both experiments, substantial signs of stress could be observed for plants exposed to partial drought with a significant impact on root system biomass. In the 16-weeks long experiment, bottom and top root biomass were respectively 34% and 80% lower in drought-treated pots than in control ($p < 0.01$ and $p < 0001$, Figure 3A-B) while aerial biomass was not impacted (Figure 3C).

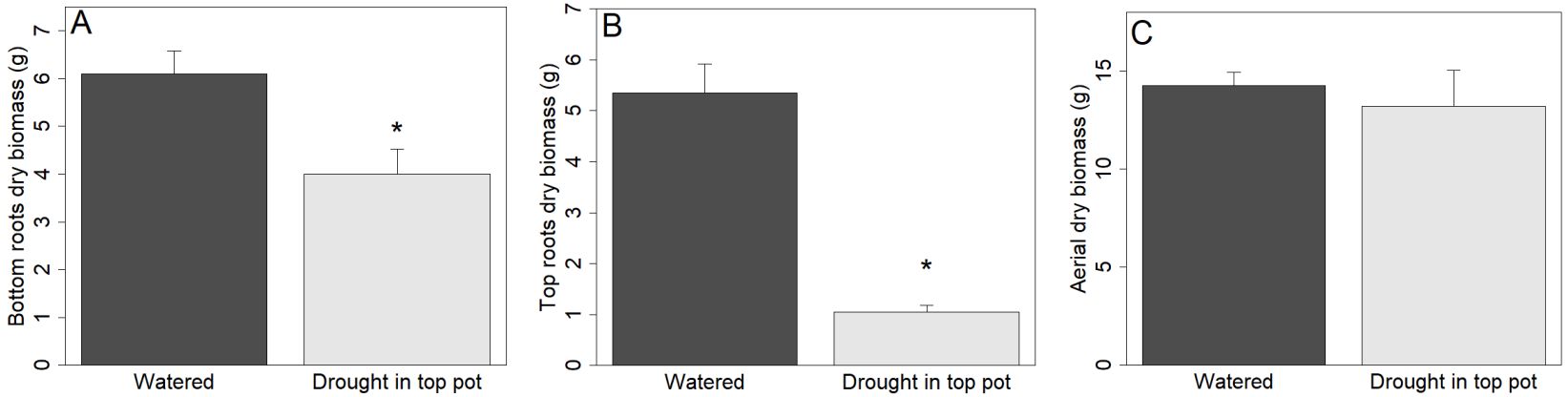

Figure 3. Bar plot of the dry biomass (g) of roots grown in the bottom (**A**), roots grown in the top pots (**B**) and aerial parts (**C**) from the long-term experiment. Data shown here comes from the experimental units treated with arsenic contamination in the bottom compartment and regular watering of both compartments ("arsenic" group; dark grey bars), and arsenic contamination in the bottom compartment with extended drought in the top compartment ("drought + arsenic" group; light grey bars). Asterisks show significant difference between drought and watered treatments ($p < 0.05$). Error bars show the upper limit of the standard error (SE).

Such a clear pattern was not observed in the shorter experiment (Figure 4). Only non-significant root biomass reductions because of drought were measured, both in bottom (Figure 4A) and top (Figure 4B) root sections. Up to a 19% decrease in the "drought + arsenic" group compared to "arsenic" were observed in bottom roots (Figure 4A) and 13% decrease in the "drought" group compared to "control" in top roots (Figure 4B). Furthermore, the results obtained in the short experiment showed that the arsenic treatment had a rapid effect on root biomass. Bottom roots biomass was significantly affected by the arsenic treatment factor ($p < 0.01$), with a 39% decrease in "drought + arsenic" compared to "control" (figure 4A).

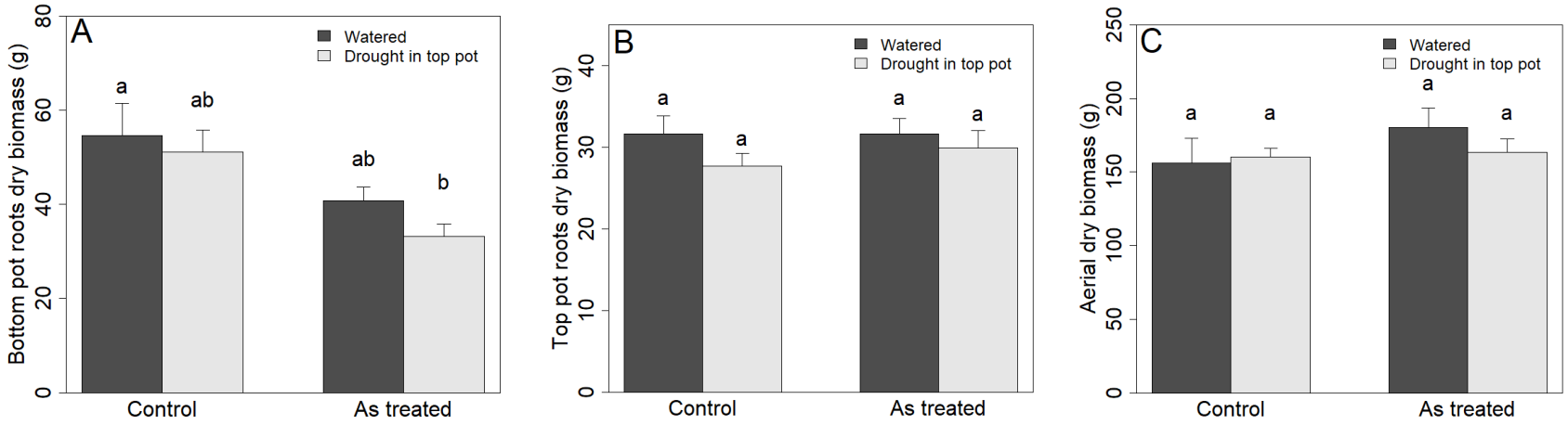

Figure 4. Bar plots of the dry biomass (g) of roots grown in the bottom pot (**A**), roots grown in the top pot (**B**) and aerial parts (**C**) from the short-term experiment, under a combination of watering/drought (watered or drought in top pot) and arsenic contamination (control or As treated). Each bar represents on of these treatments: "control" (watered in both compartments and no contamination), "drought" (drought treated top compartment and no contamination), "arsenic" treatment (watered in both compartments and As(V) contamination in the bottom one), and "drought + arsenic" treatment (drought treated top compartment and As(V) contamination in the bottom one). Different lowercase letters indicate significant difference between treatment groups ($p < 0.05$). Error bars show the upper limit of the standard error (SE).

2.5.2. Hydraulic redistribution by *S. nigra* occurs readily at night

Soil humidity was measured in top soil compartment over a 24h period at the end of the short-term experiment. It showed a pattern of fluctuation that clearly illustrates the "hydraulic lift" process towards top soil compartment when drought-treated, both with (Figure 5) and without arsenic contamination (Figure 6). After initial watering at 8:00 of both soil compartments in watered groups ("control" and "arsenic") or of the bottom compartment solely ("unplanted", "drought" and "drought + arsenic" groups), the variations of soil water contents coincided with changes in light exposure (Figure 5 and 6).

Soil humidity slowly decreased throughout the day as evaporation and plant transpiration drew out the moisture, until reaching its lowest point at the end of the light period (22:00). In the absence of drought (figure 5 and 6, dark blue line), humidity further decreased throughout the night and until the following morning, showing no clear sign of hydraulic redistribution by the willows. To the contrary, in "drought"-treated experimental units (figures 5 and 6, light blue lines), soil humidity slowly increased overnight until reaching a peak around the end of the dark period (6:00). Since the experiment took place under the controlled conditions of a greenhouse, water gain in the drought treated soil was not due to rain or any other source than hydraulic redistribution carried out by the willows. The absence of water gain in the top soil of the unplanted group (figures 5 and 6, black dotted lines) also proves that water moved due to the plants' root system connexion between bottom and top compartments, and not by simple capillary action of water in the substrate.

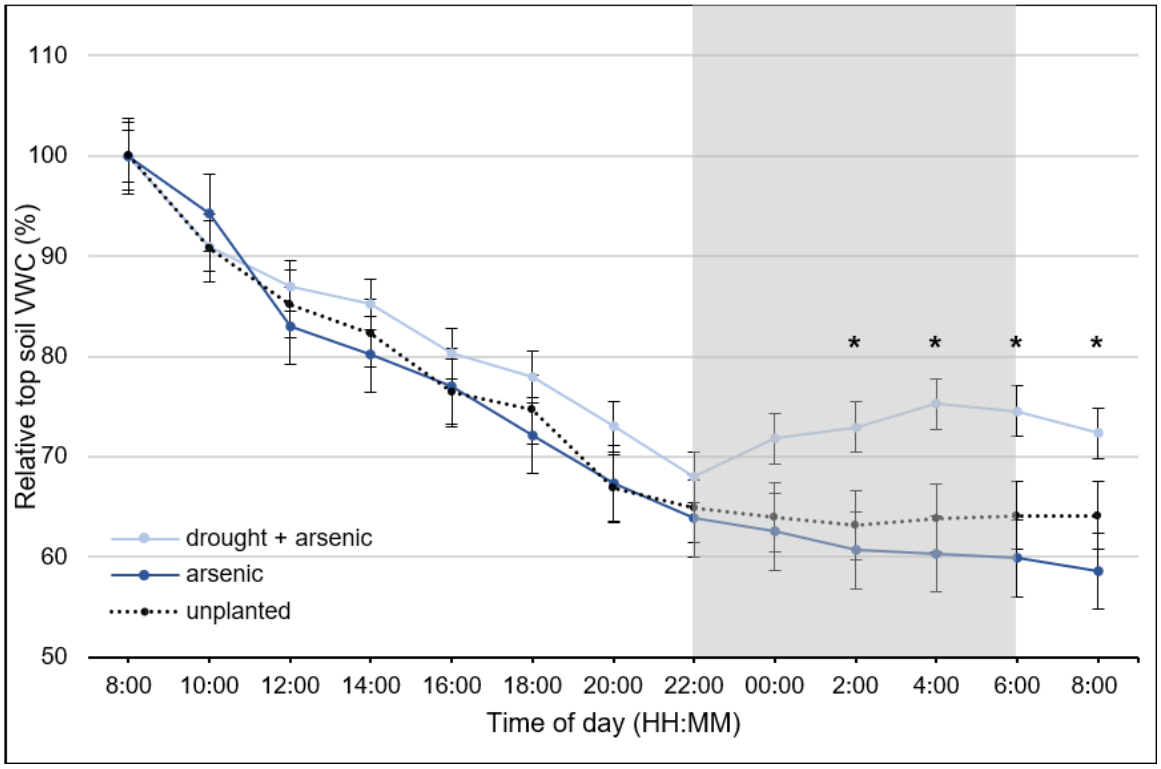

Figure 5. Soil volumetric water content (%) expressed as means relative to the maximum value measured in the top pots of *"*arsenic*"* (arsenic-treated units and watered top compartment; dark blue line), *"*drought + arsenic*"* (arsenic-treated units and drought-treated top compartment; light blue line) and *"*unplanted*"* groups (arsenic-treated units, drought-treated top compartment, and no willows; black dotted line). Dark period is represented by the shaded area. Error bars are ± standard error (SE).

At the 2:00, 4:00, 6:00 and the final 8:00am measurements, relative VWC in top soil compartment was significantly higher in "drought + arsenic" than in "arsenic" or "unplanted" experimental units ($p < 0.05$; Figure 5). Similar observations were made at 4:00, 6:00 and 8:00am for 'drought' experimental units ($p < 0.05$; Figure 6).

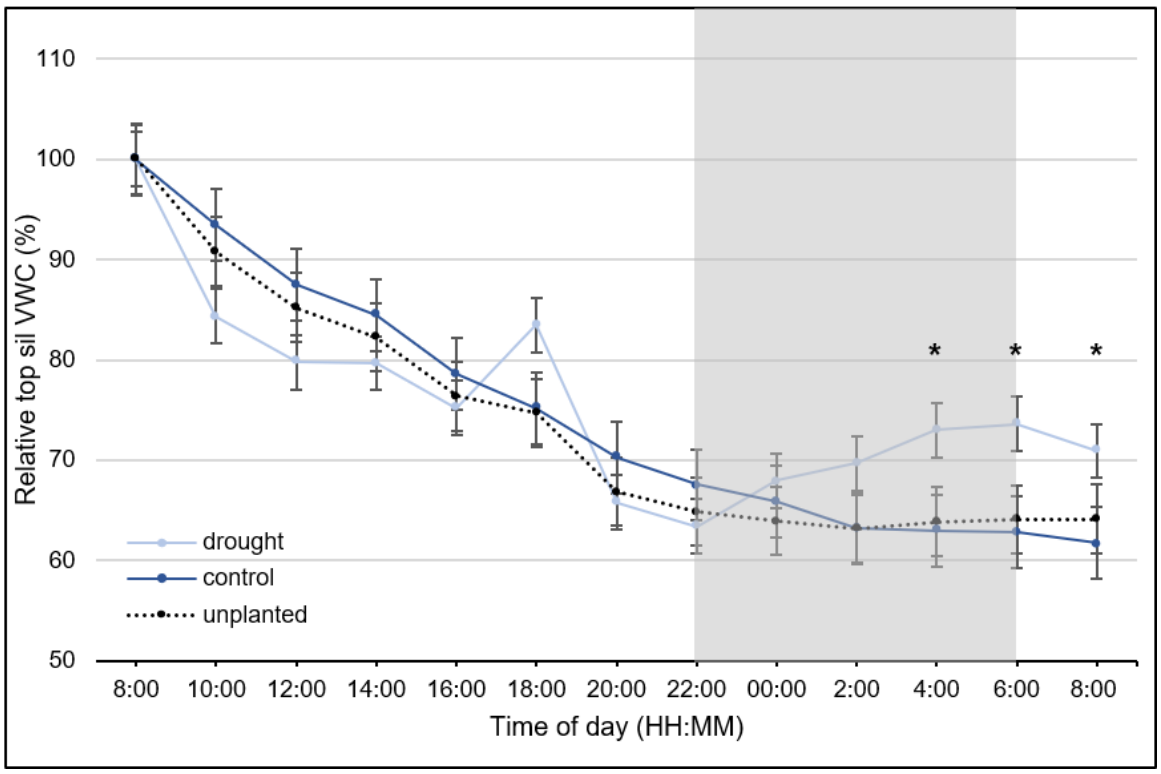

Figure 6. Soil volumetric water content (%) expressed as means relative to the maximum value measured in the top pots of "control" (watered top compartment; dark blue line), "drought" (drought-treated top compartment; light blue line) and "unplanted" (drought-treated top compartment and no willows; black dotted line) groups. Dark period is represented by the grey shaded area. Error bars are ± standard error (SE).

2.5.3. Arsenic uptake is fast, but its redistribution throughout soil layers is not

In order to analyze all the results in an integrated way, arsenic content was measured in plant biomass after the short-term experiment (Figure 7). Arsenic treatment had a significant effect on contaminant contents in all plant parts ($p < 0.001$). Although a tendency for greater accumulation was systematically observed when drought was applied to the upper soil compartment, it did not affect plant arsenic accumulation in such a short time (Figure 7). Specifically, bottom roots directly exposed to arsenic contamination accumulated over 50 mg/kg (Figure 7A), i.e., over ten-times more than the upper roots and aboveground biomass where the contaminant was only translocated (Figure 7B-C).

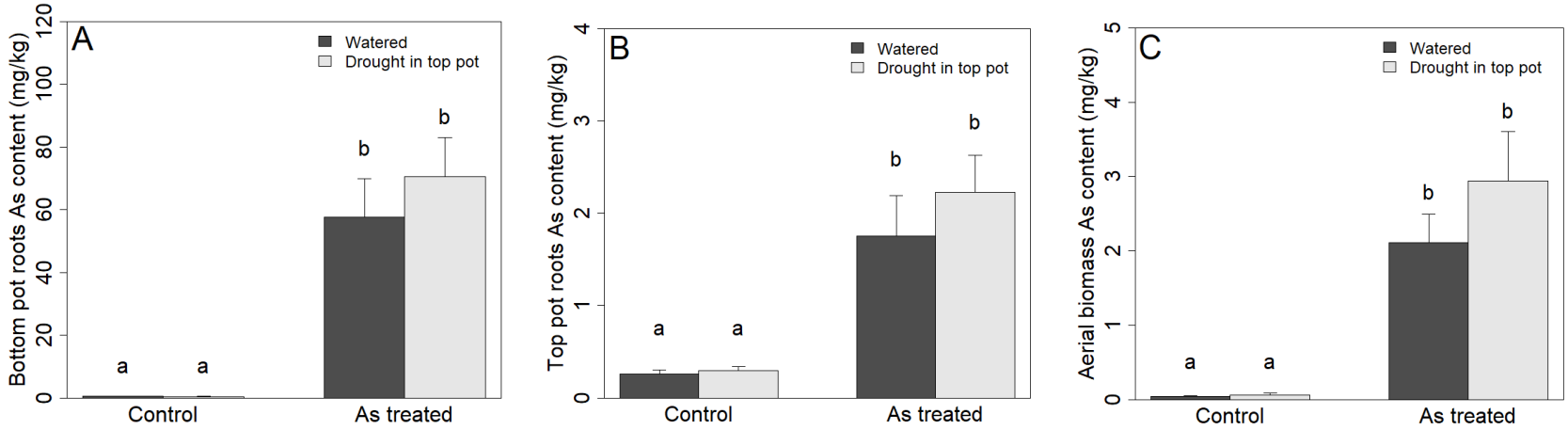

Figure 7. Bar plots of the arsenic contents (mg/kg) measured in roots grown in the bottom pot (**A**), roots grown in the top pot (**B**) and aerial parts (**C**) from the short-term experiment, under a combination of watering/drought (watered or drought in top pot) and arsenic contamination (control or As treated). Each bar represents one of these treatments: "control" (watered in both compartments and no contamination), "drought-control" (drought treated top compartment and no contamination), "arsenic" treatment (watered in both compartments and As(V) contamination in the bottom one), and "drought + arsenic" treatment (drought treated top compartment and As(V) contamination in the bottom one). Different lowercase letters indicate significant difference between treatment groups ($p < 0.05$). Error bars show the upper limit of the standard error (SE).

No arsenic redistribution was measured in soil top compartment at the end of the short-term experiment. Yet, at the end of the 16-weeks long experiment, the arsenic concentration in "drought + arsenic" experimental units was considerably higher than in the ones that were fully watered, approaching the conventional p-value of 0.05 ($p = 0.0568$). In average, arsenic contents increased by 30% in the "drought + arsenic" group compared to the "arsenic" group (Figure 8).

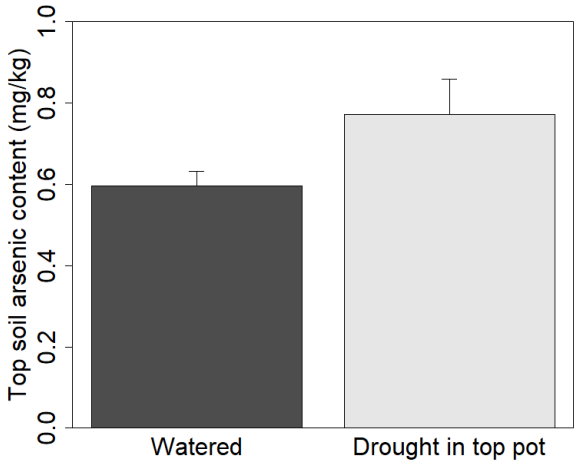

Figure 8. Bar plot of the soil arsenic contents (mg/kg) measured in the top compartment soil after the long-term experiment. Data shown here comes from the experimental units treated with arsenic contamination in the bottom compartment and regular watering of both compartments ("arsenic" treatment; dark grey bar), and arsenic contamination in the bottom compartment with extended drought in the top compartment ("drought + arsenic" treatment; light grey bar). Error bars show the upper limit of the standard error (SE).

2.5.4. Distinctive root transcriptomes in top and bottom soil compartments

Little differences were observed in the transcriptome of bottom compartment and top compartment of roots grown under "control" conditions. Of the 35,125 genes in *S. purpurea* genome, only 433 were differentially expressed (> 2-fold change, see Table

S1 for detailed expression data). Among them, 34 genes showed very high differential expression level (> 100-fold-change) between the two compartments. There were several genes involved in root photomorphogenesis, gravitropism, or lateral root development. As such, genes encoding ELIP proteins (early light-induced protein) were noticeably over-expressed in top roots while members of the FLA (fasciclin-like arabinogalactan) and PMEI (pectin methylesterase inhibitor) families were repressed (Table S1).

Despite this apparent similarity, the extent to which the two transcriptomes responded to the experimental conditions was remarkably different. Overall, the top compartmented roots showed significantly more responsiveness to the experimental conditions. Regardless of the comparison made, a combined total of 539 genes were differentially expressed in the lower compartment (i.e., < 2% of the coding genome), whereas 9,587 genes were differentially expressed in the upper root system (i.e., >27% of the coding genome).

2.5.5. Patchy drought does not regulate remotely the expression of transporter genes

The transcriptomic response of top roots directly exposed to drought was broad, as 3,651 genes were upregulated and 5,474 were downregulated compared to “control” conditions (>25% of the coding genome). In the bottom compartment (watered throughout the experiment), the root transcriptome was much less affected by the distant drought in the top compartment: 58 genes were upregulated and 127 were repressed. Interestingly, about 90% (53 genes) of upregulated genes in bottom roots were also induced in the top compartment but only 4% (5 genes) of downregulated genes in the bottom compartment follow the same pattern above (Figure 9; Table S1).

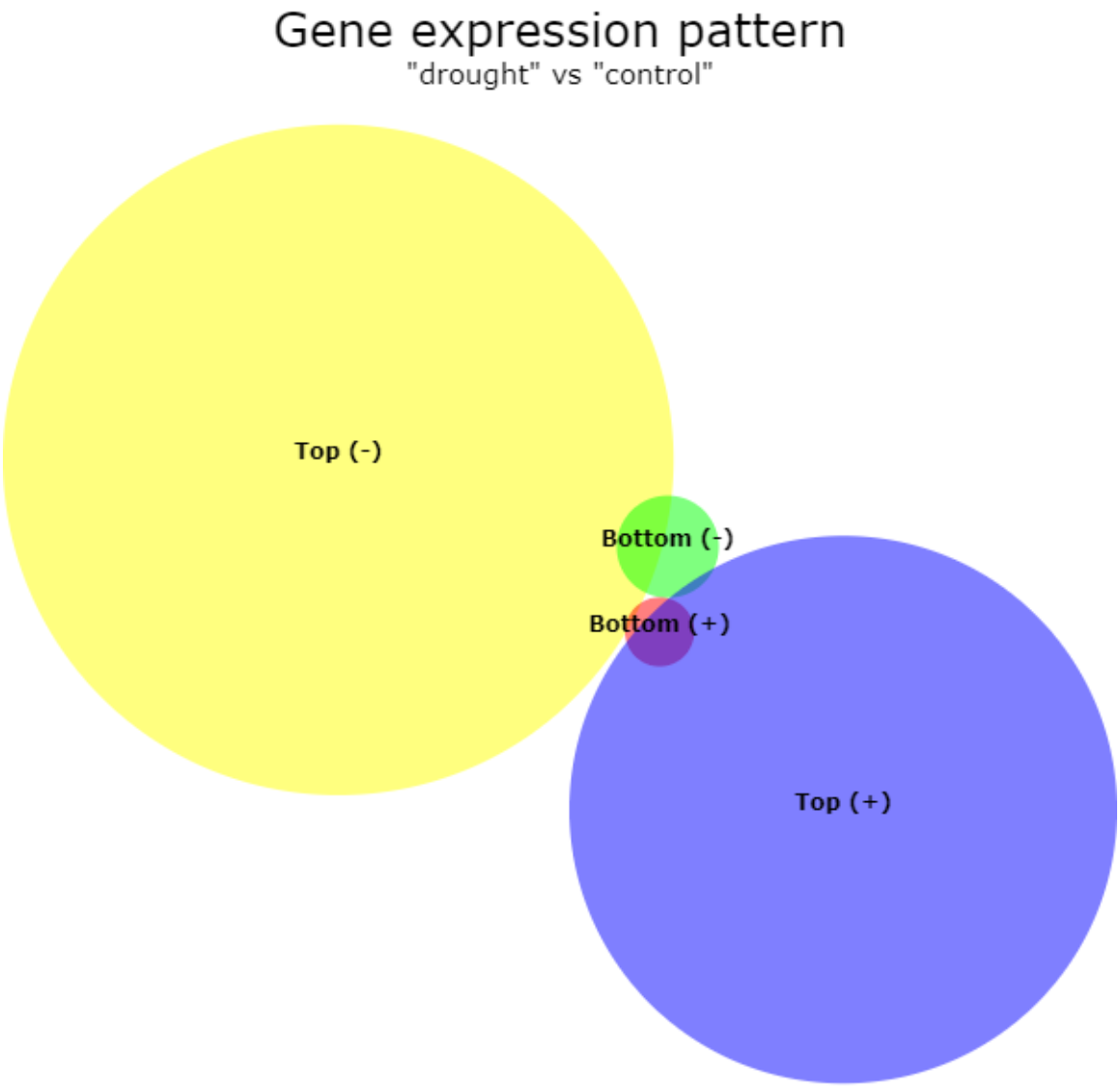

Figure 9. Proportional Venn diagram of the differentially expressed genes in response to drought, in bottom and top compartment roots. EdgeR comparisons were made between the root transcriptome of plants exposed to drought in the upper compartment ("drought" group) and the "control" group. Yellow and blue circles show downregulated (-) and upregulated (+) genes respectively, in top compartment roots. Green and red circles show downregulated (-) and upregulated (+) genes respectively, in bottom compartment roots.

When compared to "control" conditions, some similarities exist in the AgriGO enrichment analyses of the root transcriptomes in roots exposed to drought directly (top compartment) or indirectly (bottom compartment). GO terms such as "response to abiotic

stimulus" and "carbohydrate metabolic process" were commonly overrepresented. Genes coding for several drought responsive proteins like LEA (late embryogenesis abundant), Kunitz proteinases inhibitors as well as numerous enzymes involved in amino acid biosynthesis are for example similarly overexpressed in both transcriptomes (> 5-fold change). No such clear pattern was observed between the two sets of downregulated genes. Also of special interest, the "transport" GO term was overrepresented in top roots (for downregulated genes) but not in the bottom compartment. Out of the targeted transporter families in this study, only one gene encoding an ABC-G transporter was differentially expressed (downregulated) in the lower compartment (*Sapur.010G169700*, < 3-fold change). In top roots directly exposed to drought conditions, 3 phosphate transporters were upregulated under drought conditions and 5 were downregulated, while 16 ABC transporters were upregulated and 27 were downregulated. Finally, no less than 23 aquaporin-coding genes were differentially expressed in the transcriptome of the drought-stressed top roots (out of 35 genes in the family; Almeida-Rodriguez et al., 2016). The majority of these were significantly reduced under drought conditions (up to > 100-fold change for three PIP and a member of the NIP subfamily (Table 1 & S1)).

Table 1 – Subset of the ten most differentially expressed membrane transporters genes associated with arsenic transport in the comparison "drought" vs "control". This comparison highlights the effect of surface drought on both root transcriptomes directly (top compartment) or indirectly (bottom compartment) exposed to stress.

S. purpurea v5.1 transcript ID	Description	Bottom DE (fold change)	Top DE (fold change)	Abundance (log2 CPM)
Sapur.009G102100	Aquaporin, PIP1	none	down (452)	3.6835
Sapur.003G137300	Aquaporin, NIP6	none	down (309)	0.32461
Sapur.009G102000	Aquaporin, PIP1	none	down (254)	-0.14294
Sapur.009G102200	Aquaporin, PIP1	none	down (107)	2.6974
Sapur.010G102500	ABC-G transporter	none	up (24)	3.3127
Sapur.002G168500	ABC-G transporter	none	down (16)	2.0915
Sapur.010G178000	Aquaporin, PIP1	none	down (16)	9.0837
Sapur.016G253000	ABC-G transporter	none	down (15)	2.6943
Sapur.010G001900	ABC-C transporter	none	down (13)	6.0313
Sapur.008G038400	Aquaporin, TIP1	none	down (11)	8.4549

2.5.6. Transcriptomic response in roots to arsenic contamination

In the upper root compartment of the plants in the "arsenic" group, where roots were not directly exposed to arsenic, 658 genes were upregulated and 659 were downregulated compared to "control" conditions (<4% of the coding genome). In the bottom root compartment that was directly exposed to arsenic contamination, 208 genes were differentially expressed compared to the "control" group in total, with about 45% of them being upregulated. Only 48 of these genes were also differentially expressed in the top compartment (Figure 10; Table S1). AgriGO singular enrichment analyses of the differentially expressed genes revealed the overrepresentation of the similar GO terms, those related to "response to stress" being however more significantly overrepresented in the roots of the lower compartment, while those related to primary metabolism where more

significant in the upper compartment: “generation of precursor metabolite and energy”, “regulation of metabolic process” or “enzyme regulator activity”.

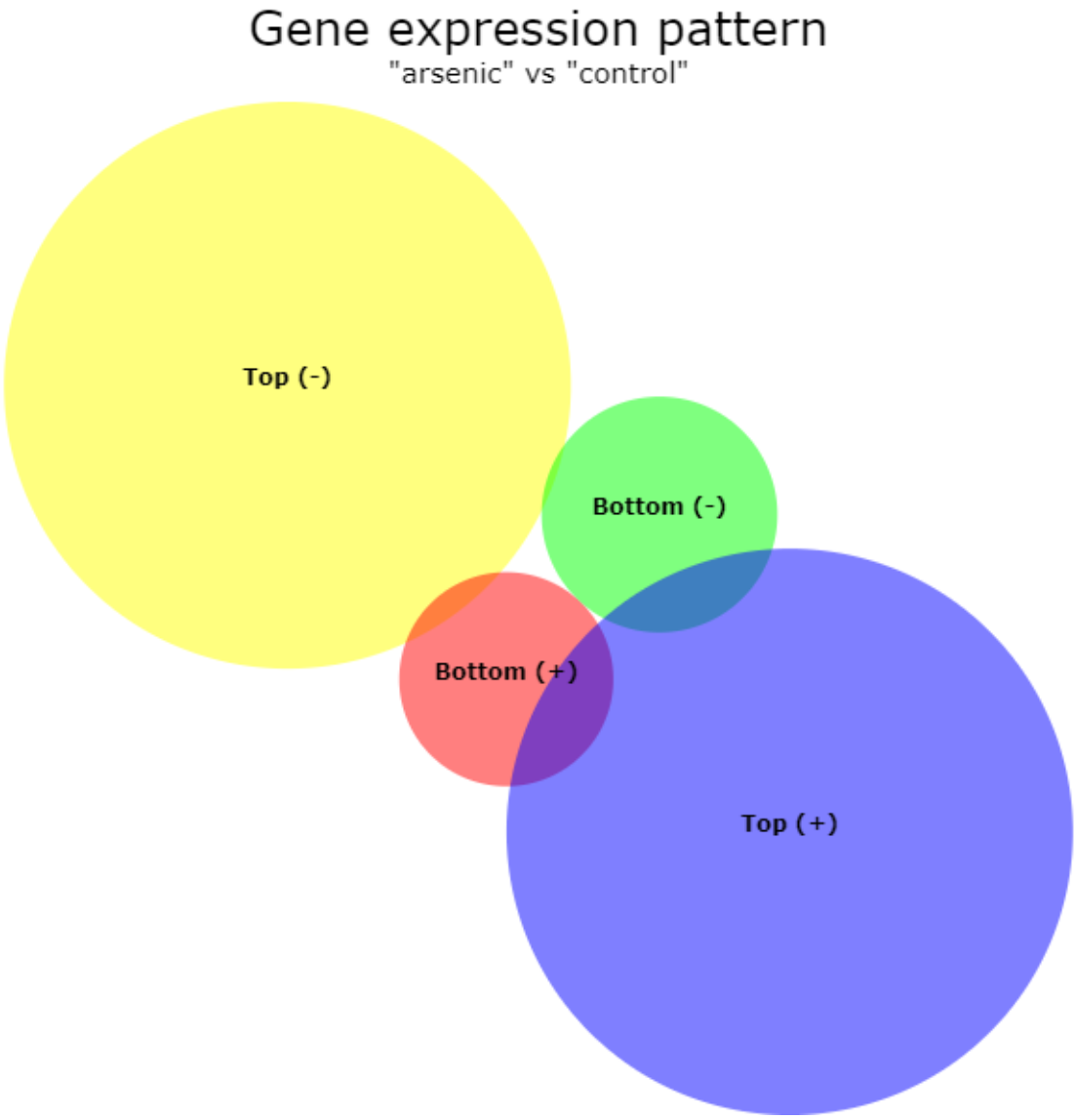

Figure 10. Proportional Venn diagram of the differentially expressed genes in response to arsenic, in bottom and top compartment roots. EdgeR comparisons were made between the root transcriptome of plants exposed to arsenic in the lower compartment (“arsenic” group) and the “control” group. Yellow and blue circles show downregulated (-) and upregulated (+) genes respectively, in top compartment roots. Green and red circles show downregulated (-) and upregulated (+) genes respectively, in bottom compartment roots.

The DEG mentioned here include genes involved in heavy metal and ROS-mediated stress response (feruloyl-CoA ortho-hydroxylase *Sapur.001G005700*; TBH oxidase *Sapur.016G299600*) and protease inhibitors such as *Sapur.019G021100*, *Sapur.010G037400*, *Sapur.010G037500*, *Sapur.010G037000*, *Sapur.006G173500*, *Sapur.016G187300*, *Sapur.010G037600*, which are particularly induced (>10-fold change) in the lower compartment, while genes involved in primary metabolic processes and development like some glutathione s-transferase (*Sapur.017G114200*), beta-glucosidase (*Sapur.011G002500*), auxin response factor 30 (*Sapur.009G083200*), cytochrome p450 (*Sapur.014G027000*; *Sapur.014G027300*) were also among the most DEG in the top compartment (Table S1).

The GO term “transport” was not overrepresented in the set of DEG in the “arsenic” vs “control” comparison, neither in bottom nor in top compartment. Considering that they could play a key role in arsenic uptake and transport throughout the whole plant body, a particular emphasis was still placed on the analysis of their regulation (Table 2). Among all the comparisons made in this study, 125 of the 403 transcripts associated with the GO terms "transport" (GO:0006810) and "transporter activity" (GO:0005215) were indeed differentially expressed (> 30% of the reference gene pool), showing their involvement in the willows’ response to arsenic.

In the bottom roots directly in contact with arsenic, the expression of a PIP1 aquaporin channel (*Sapur.006G080400*) was downregulated, while another PIP1, a PIP2 and a TIP1 (*Sapur.008G038400*, *Sapur.009G102000* and *Sapur.016G084700* respectively) were repressed in the top root system (Table 2). All of these transcripts were also differentially expressed in the top roots of the “drought” group. The *Sapur.006G080400*

transcript was upregulated, while the other ones were downregulated in response to drought. No other aquaporin genes were differentially expressed in “arsenic” compared to “control” conditions.

Among other transporter genes, 3 PHO1 and 11 ABC transporters were differentially expressed in the top compartment in the “arsenic” treatment compared to “control” (Table 2). Under drought stress, all of them but 4 were also differentially expressed in top roots (Table S1). Incidentally, ABC transporters *Sapur.15WG004700* and *Sapur.15ZG004600*, which were the most upregulated in “arsenic” conditions (Table 2), were repressed under “drought” conditions (Table S1).

Table 2 – Subset of the most differentially expressed membrane transporters genes associated with arsenic transport in the comparison "arsenic" vs "control". This comparison highlights the effect of surface drought on both root transcriptomes directly (bottom compartment) or indirectly (top compartment) exposed to stress.

S. purpurea v5.1 transcript ID	Description	Bottom DE (fold change)	Top DE (fold change)	Abundance (log2 CPM)
Sapur.006G080400	Aquaporin, PIP1	down (2)	no	3.7136
Sapur.009G102000	Aquaporin, PIP1	no	down (3)	-0.14294
Sapur.016G084700	Aquaporin, PIP2	no	down (4)	6.2089
Sapur.008G038400	Aquaporin, TIP1	no	down (2)	8.4549
Sapur.008G088600	PHO1 transporter	no	up (3)	1.4281
Sapur.008G071100	PHO1 transporter	no	down (4)	0.28179
Sapur.008G071300	PHO1 transporter	no	down (2)	0.7191
Sapur.15WG004700	ABC-G transporter	no	down (18)	2.8161
Sapur.15ZG004600	ABC-G transporter	no	down (17)	2.8944
Sapur.016G230700	ABC-C transporter	no	down (3)	2.862
Sapur.010G102500	ABC-G transporter	no	down (2)	3.3127
Sapur.017G057900	ABC-B transporter	no	down (2)	4.716
Sapur.003G018100	ABC-G transporter	no	down (2)	5.4737
Sapur.006G094300	ABC-G transporter	no	down (2)	5.2791
Sapur.005G180900	ABC-I transporter	no	up (6)	1.6097
Sapur.002G028100	ABC-I transporter	no	up (4)	4.8401
Sapur.006G163000	ABC-G transporter	no	up (3)	3.399
Sapur.006G070400	ABC-I transporter	no	up (2)	5.4877

2.5.7. An extended response of the root transcriptomes to combined stresses

In a subset of experimental units, the two stresses were combined in order to mimic the conditions of a phytoremediation field trial. In such conditions, soil contamination and drought may not be homogeneously distributed. For example, contaminated water tables or deep soil layers can occur at the same location as less-contaminated shallow soil profiles more exposed to drought. The transcriptomic response of roots in the upper compartment to the combination of the two stresses ("drought + arsenic"), direct drought and more

distant arsenic contamination, is essentially similar to that observed when only the local drought stress is applied (figure 11A). In total, 3497 genes were upregulated and 5333 were downregulated in the top compartment of the "drought + arsenic" group. Only about 3% were specific to the combination of the two stresses, 3% of the ones differentially expressed in the "arsenic" group were specific to that condition, and 8% of the ones differentially expressed in the "drought" group were specific to that condition. In contrast, the response of the root transcriptome of the lower compartment is of a much larger magnitude when the combination of the two stresses is applied to the system ("drought + arsenic") compared to the application of either "drought" or "arsenic". In the bottom roots under this treatment, more than 400 genes were differentially expressed, i.e., about twice as many as with a single stress (figure 11B). It is also interesting to note that this pool of DEG is only partially overlapping with the one of "drought" treatment, unlike in top roots. In summary, only 25% of the DEG pool in the "drought-control" group and 12% of the DEG pool in the "watered-arsenic" group are specific to the respective condition. In contrast, 235 genes (> 58% of the DEG pool) were specific to the "drought-arsenic" stress combination (Figure 11B).

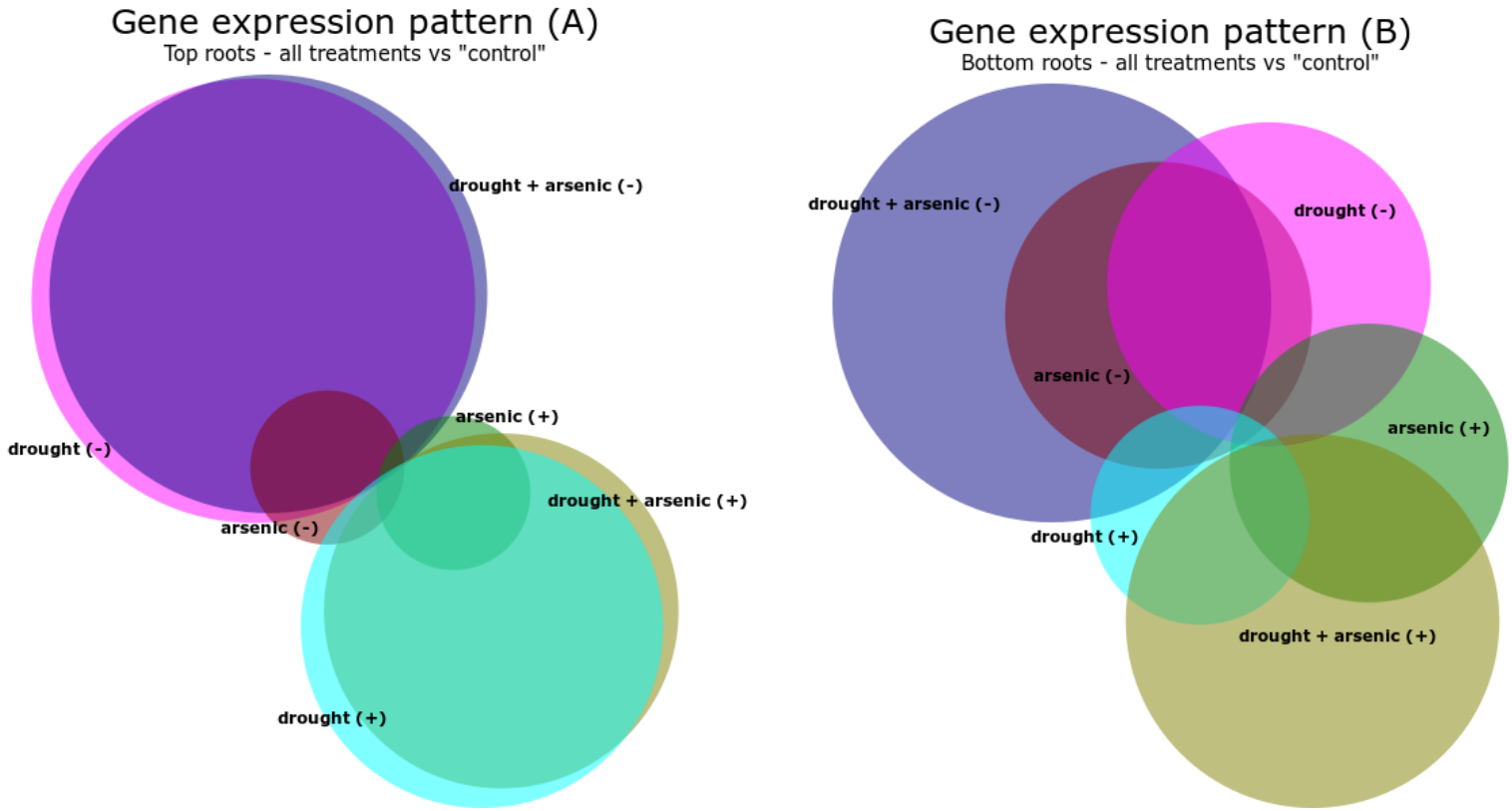

Figure 11. Proportional Venn diagram of the differentially expressed genes in response to the drought treatment ("drought"), arsenic treatment ("arsenic"), and the combined drought and arsenic treatments ("drought + arsenic"), from the EdgeR comparisons to the "control". The data is separated between top compartment roots (**A**) and bottom compartment roots **(B**). Pink, maroon, and navy circles show downregulated genes (-) in response to "drought", "arsenic" and "drought + arsenic" treatments respectively, while light-blue, green and brown circles show upregulated genes (+) in response to "drought", "arsenic" and "drought + arsenic" treatments respectively.

A high degree of similarity was observed in GO SEA analyses between the transcriptomic responses in “drought” and “drought + arsenic” versus “control” comparisons with respect to the upper compartment roots. No new GO terms were over-represented under the combination of stress conditions “drought + arsenic”, compared to the “drought” response. In the bottom compartment roots, numerous new transcripts were differentially expressed under the combined stress conditions (figure 11A), but the GO pattern still remained similar. In both compartments, “stress”- and “primary-metabolism”-related GO terms were the most significantly over-represented. No new transcriptome component (i.e., gene family, molecular process) appears as an active root response element to this combination of stresses, compared to the response to “drought”.

On the other hand, it should be noted that the magnitude of the response is accentuated under the “drought + arsenic” condition, both by the recruitment of additional transcripts in the lower part of the plant and by the extent of the differential expression level observed. This is notably the case for genes coding for membrane transporters (Table 3), whose expression was almost systematically more strongly regulated when two stresses were applied. For example, three transporters were specifically repressed in the lower compartment but not differentially expressed in the top compartment: a PIP1 aquaporin and two ABC-G transporters. Under the “drought” treatment, none of these showed differential expression in bottom roots (Table 3). In the top compartment, some aquaporins and PHO1 transporters were remarkably repressed (up to 1757-fold change for *Sapur.009G102200*; 31-fold change for *Sapur.008G071300*) while a member of the ABC-G transporter family (*Sapur.010G102500*) was significantly induced in “drought + arsenic”

compared to “control” conditions. These DEG showed much lower fold-changes when drought stress alone was applied.

Table 3 – Subset of ten differentially expressed genes representing the strongest differential expression of membrane transporters associated with arsenic transport in the comparison “drought + arsenic” vs “control”. This comparison highlights the combined effect of surface drought and deep soil contamination on the root transcriptome, in both bottom and top compartmented roots.

S. purpurea v5.1 transcript ID	Description	Bottom DE (fold change)	Top DE (fold change)	Abundance (log2 CPM)
Sapur.006G080400	Aquaporin, PIP1	down (3)	no	3.7136
Sapur.010G119700	ABC-G transporter	down (5)	no	4.7904
Sapur.017G057900	ABC-G transporter	down (5)	no	4.7529
Sapur.009G102200	Aquaporin, PIP1	no	down (1757)	2.6974
Sapur.009G102100	Aquaporin, PIP1	no	down (627)	3.6835
Sapur.009G102000	Aquaporin, PIP1	no	down (254)	-0.14294
Sapur.008G071300	PHO1 transporter	no	down (31)	0.7191
Sapur.008G071100	PHO1 transporter	no	down (22)	0.28179
Sapur.009G032500	Na^+-dependent P_i transporter	no	down (7)	3.1864
Sapur.010G102500	ABC-G transporter	no	up (65)	3.3127
Sapur.016G253000	ABC-G transporter	no	down (24)	2.6943
Sapur.001G039100	ABC-G transporter	no	down (11)	0.32145

2.6. Discussion

The increased implementation and more frequent use of nature-based solutions is now on the agenda of decision makers for landscaping and urban planning practices. It is in this favorable context for innovation that applied sciences such as phytotechnology are refined, through transdisciplinary approaches, and with benefits from the contributions of engineering, policy making, landscape ecology, agronomy, but most importantly from fundamental plant biology perspectives (Guidi Nissim et al., 2023; B. Lin & Anderson,

2023; Lipper et al., 2014). A more detailed understanding of the conductive system of plants is necessary for the success of the phytoextraction alternative to conventional soil remediation techniques. We present here the first comprehensive analysis of the phytoextraction process of arsenic-contaminated deep soil layers and highlight the physiological and transcriptomic mechanisms in black willows regulating the fate of arsenic underground.

2.6.1. Partial drought stress has positive effects for field applications

Phytoremediation, which aims to treat large areas of contaminated land such as post-industrial brownfields, is essentially presented as an improved version of short rotation coppice crop management that is both economically viable and ecologically sound. In this sense, current practices are those of conventional agriculture, based on the selection of high biomass yielding cultivars, even on contaminated sites (Labrecque & Teodorescu, 2005; Pajević et al., 2016). The black willow, *S. nigra,* is a species of choice for this purpose, showing high contaminant accumulation and tolerance (Grenier et al., 2015; Kuzovkina et al., 2004; Massenet et al., 2021), its harvestable biomass is only marginally affected in the experimental conditions applied (Figure 3 & 4) whether in the short (2 weeks) or much longer term (16 weeks). This tolerance is of great interest for practical phytoremediation ends, as the unaffected aerial biomass allows to effectively stock and manage contaminants (arsenic in this case). Of course, these trials were done under "pampered" controlled conditions and reflect only to a certain extent the field environment where plants are more exposed to the elements. They nonetheless reflect the ability of sessile plants to adjust to stressful conditions that root systems experience in the field because of heterogeneous soil drought (Bargués Tobella et al., 2017). It is easy to imagine here that a part of the well

irrigated root system can opportunistically compensate for partial and punctual water limitations. Partial root drying is even a favored method of irrigation in agricultural practices that increases plant resilience (Kang & Zhang, 2004). In addition to increasing water use efficiency and in some cases nutrient (N, P) uptake (Dos Santos, 2003; Y. Wang et al., 2017; Yactayo et al., 2013), it can induce the production of osmoprotectants and antioxidant compounds that help mitigate abiotic stresses (Raza et al., 2017). In our case, partial drought caused little yield change. Only the root compartment exposed to extended drought had its biomass altered (Figure 3A), but without noticeable decrease for the aboveground harvestable biomass.

Similarly, the arsenic contamination did not impact shoot productivity (Figure 3C & 4C). These results are in accordance with those of Purdy and Smart (2008), who observed no toxicity symptoms and no effect of arsenic contamination of several willow clones on aerial biomass, where the contaminant was translocated and therefore available for harvest (or to enter the ecosystem if not taken care of). On the contrary of these perceptible results, it should be noted that belowground, the double-compartment experiment demonstrates transient impact on the biomass of roots that were directly exposed to arsenic in the short-term experiment (bottom compartment; Figure 4A). The absence of such an observation in the longer experiment likely implies the role of profound and unique changes of plant root development and distribution to improve resistance to abiotic stresses in the long term (Karlova et al, 2021; V. Kumar et al, 2020). These changes can be observed through deeper rooting and reduced investment in lateral root branching in response to drought (Lynch, 2018; Zhan et al, 2015), and a better control of the soil solution uptake in response to arsenic (J. F. Ma et al, 2008; Navarro et al, 2021), which is of the greatest phytotechnology

interest. Beyond the plant itself, the impact we seek for phytoremediation is on the nearby soil environment, through the deep root system. During partial drought, the phenomenon of hydraulic redistribution is now considered as an element of agricultural irrigation strategy. In agroforestry ecosystems, Bayala and Prieto (2019) recently termed "bioirrigators" the plants capable of releasing water and nutrients from deep soil up to upper soil layers, emphasizing their importance in ecosystems. Plants have also been shown to uplift nutrients through soil layers, tapping into deep pools and redistributing them towards the soil surface (Jobbágy & Jackson, 2004). Considered as essentially a passive mechanism (Leffler et al., 2005; Sekiya et al., 2011), upward water redistribution was perfectly illustrated here by the soil humidity variations resulting from transport through *S. nigra* (Figure 5 & 6). This process takes place despite with the near-systematic repression of aquaporins in top roots due to the drought stress (Table 2 & 3), indicating the involvement of the apoplastic pathway in this water movement. Yet, it failed to be directly linked to arsenic movement within the plant body or soil column, adding more complexity to the dynamics at play in phytoremediation trials.

2.6.2. The fate of arsenic is explained by transcriptomics

The initial objective of the work presented here was to describe as best as possible the processes leading to contaminant movement within plants (Figure 7) and their environment, more particularly towards upper soil profiles like we observed (Figure 8). Far from being purely theoretical, increased concentrations of contaminants near the shallow roots of planted species in a phytoremediation context have been reported several times in the specialized literature for both organic compounds and trace elements (Fortin Faubert et al., 2021; Klassen et al., 2010; Watson et al., 2003). Regulated by the hydraulic control

exerted by the plant transport system on the movement of the soil solution, the process of "pumping" contaminants by an accumulating flow is mainly used in phytotechnologies to limit the diffusion of contaminants (Ferro et al., 2003). However, the implications of hydraulic redistribution on nutrient/contaminant uptake by plants may explain the variability in results obtained and the current limitations of phytoremediation techniques (Lambers et al., 2006; Liste and White, 2008). This is particularly the case in the context of the experiments conducted here, since arsenic could follow the same pathway as water and nutrients, up to its efflux at the root level (Liu et al., 2010; Vetterlein et al., 2007; X. Y. Xu et al., 2007; Zhao et al., 2010). But although arsenic was absorbed and accumulated throughout the plant tissues, it was not easily excreted in the experiment where we measured hydraulic lift. Only in the long term was a higher soil arsenic content measured in the top compartment. The mechanisms involved in water, nutrient and arsenic movement are indeed under the strong control of many genes coding for transporters. Those showed marked expression patterns during the transcriptomic analysis of the two root compartments (Figures 9-11; tables 1-3).

Here, the moderate dynamics of the deep root transcriptome confirms the passive character of the hydraulic lift process. Although clearly experienced as an abiotic stress at the upper root level, the partial drought had almost no distant effect at the gene expression level in the deeper root system (Figure 9). Only a very few genes encoding proteins of typical downstream stress-responsive families such as dehydrins and Kunitz proteinases inhibitors were differentially expressed in the whole root system, indistinctly of the compartment (Table S1; Pucholt et al., 2015). Still, water stress or high water demand tend to result in the variable regulation of transporter genes such as aquaporins, often without a

clear and uniform pattern of overexpression or repression due to their high specificity and individual function at the cell membrane (Hachez et al, 2006; Laur and Hacke, 2013; Meng et al, 2016; Patel and Mishra, 2021). Thus, while virtually none of the following elements were differentially regulated in well-irrigated bottom roots, many phosphate transporters and as many as 43 genes encoding ABC transporters, whose substrates may include arsenic compounds, abscisic acid, auxin, secondary metabolites or osmolytes (Gräfe & Schmitt, 2021; Song et al., 2010; Song et al., 2014; Tang et al., 2019, H. Zhang et al., 2014) were differentially regulated in drought-treated top roots (Table 1 & S1). Such variable patterns of expression are to be expected under drought conditions (Jarzyniak & Jasiński, 2014; C. Zhang et al, 2016). Nevertheless, of particular interest was the near-systemic repression of the aquaporin gene family measured in the drought-stressed compartment (Table 1), most likely as part of a localized water conservation mechanism by lowering cell membrane hydraulic conductance in the dry soil patch, while unchanged diurnal transpiration demand controls water uptake by the deeper root system (Smart et al, 2001; Porcel et al, 2004). This drought-resistance mechanism incidentally blocks a possible efflux path for arsenic in surface roots.

Similar to the effect of the partial drought treatment on top roots, the transcriptomic response of roots to arsenic contamination is proportionately more pronounced in the lower root section, when arsenic exposure is direct (Figure 11). However, similar responses to arsenic were observed from both compartments, including many genes already identified as arsenic response elements in willow roots from the hydroponic experiment of Yanitch et al. (2017). Contrary to the results obtained in the latter study, we observed little differential regulation of genes encoding transporters in response to arsenic contamination

(Table 2 & S1). No differential expression was observed for phosphate transporters in bottom pot roots directly exposed to the contaminant, and only one aquaporin gene was downregulated, which suggests a weak exclusion mechanism in *S. nigra*, at least at this level of exposure (Meharg and Macnair, 1992; Mosa et al., 2012). Therefore, arsenic can easily enter the plant through the roots and accumulate in the willows. The transcriptome of arsenic-contaminated roots suggests the implementation of intracellular detoxification processes by the plant at the hormonal level and through protease inhibitors, ROS protection or even phytochelatin activity (Table S1; Raab et al., 2005; Verbruggen et al., 2009), along with a high responsiveness of ABC transporters genes for vacuolar sequestration (Table 2 & 3), rather than an exclusion/exudation mean of alleviating arsenic stress as described for other plant species (Liu et al., 2010; Vetterlein et al., 2007; X. Y. Xu et al., 2007; Zhao et al., 2010). In support of this hypothesis is the notable repression of putative arsenic-transporting proteins in the upper compartment roots of *S. nigra*, as all but one of the differentially expressed phosphate transporter, and all aquaporin genes were repressed, limiting arsenic efflux into the root external environment (Table 3 & S1).

Most notable in this experiment is certainly the amplified reactivity observed at the transcriptome level upon co-occurrence of the two stresses, with the enlarged mobilization of the transcriptome (Figure 11, Table 3) compared to the responses observed with single stresses (either arsenic or drought). The chain of events is in this context much more pronounced, showing both new genes responsive to abiotic stress in the compartment in direct contact with the contaminated soil and a stronger amplitude of reaction in the compartment subjected to drought stress. Conceivably, a response of this magnitude induces a strong remodeling of the tissue functions, eventually of the root architecture as

observed after a severe and persistent drought at the end of the long-term experiment in which few roots were kept in the upper compartment (Figure 3A). The degradation of the existing roots would then justify the presence of arsenic in the soil compartment, as measured. Similar results have been reported in the case of lead (Pb) phytoextraction, where harvesting of willows was followed by a significant increase in soil Pb concentrations due to root degradation (Watson et al., 2003). From the perspective of the phytotechnology scientist, these processes could be easily manipulated.

The conditions leading to the hydraulic lift phenomenon and slow contaminant stabilization in shallow soil profiles are here identified. It is a simple process to either avoid this movement of contaminant, by maintaining root health through regular watering, or to design combined planting strategies that allow for the controlled and efficient harvesting of the contaminants at reach. Bioirrigators like *S. nigra* can be co-planted with shallow-rooted hyperaccumulator species such as the arsenic hyperaccumulating fern *Pteris vittata* (L. Q. Ma et al., 2001). Their hydraulic redistribution capacity can promote shallow roots survival in dry soil by irrigation in dry field plots. *S. nigra* could therefore support the hyperaccumulation activity of the fern while contributing at the same time to the phytoextraction in deeper soil layers with its own root system, optimizing the decontamination process.

3. Conclusion and outlook

In conclusion, our results show that in hydraulic lift occurs in *S. nigra* plants grown in dry surface soil with abundant water reserves in deeper soil. Arsenic does not move across soil layers due to these short-term water movements. However, these growth conditions induce drought stress to the shallow roots of the willows, which in the long-term could cause root degradation, leaving behind the sequestered arsenic in the surface soil. This process can explain the upwards movements of contaminants observed in some phytoextraction trials. Proper management of plant biomass and experimental design is therefore of crucial importance for efficient phytoextraction in the field. This process of stress-induced root degradation and subsequent arsenic accumulation in the soil can be easily avoided by proper irrigation in field trials, or by maintaining healthy soil humidity with mulch cover. This arsenic redistribution towards surface soil could also be exploited. Willows and arsenic hyperaccumulators like *Pteris vittata* (L. Q. Ma et al., 2001) could be used in rotational cultures for example to decontaminate the deep soil with the willow roots and then extract the remaining arsenic from the surface soil with the shallow-rooted fern. On the molecular scale, transport activity is strongly affected at the transcript level in response to drought, arsenic contamination, and their combination. Phosphate and ABC transports show complex and variable responses to these factors, which needs to be investigated with further testing to uncover their specific roles in arsenic transport. Aquaporins, on their part, show clearer expression patterns indicating their role in the willows' response to arsenic and drought exposition. For the three families of transporters studied here, the next logical step in the analysis of their activity would be to conduct tests targeted on individual transporters. Sub-cellular localization by immunostaining has shown to be an important

method of assessing direction of arsenic transport/diffusion. Heterologous expression systems are another method which can be used to study the transport activity of selected transporters. During the course of this master's degree, an experiment using *Pichia pastoris* as an expression system was undertaken to look at the water and/or arsenic transport activity of some aquaporin and phosphate transporters but could not be finished in time. The steps necessary for conducting such experiments was established and is presented in protocol 4 of the appendix. For this project, the first steps, up to the preparation of competent cells and their transformation, were achieved and led to the successful production of a few plasmids containing aquaporin and phosphate transporters genes of *S. nigra*. This experiment should be carried through to completion with the transport pathways identified in this thesis to provide further knowledge in the molecular processes of arsenic phytoextraction by willows. Knowledge on the underlying molecular activity in phytoextraction of arsenic will allow for the development of more efficient plants for decontamination purposes, through genetic engineering or genotypic selection of plants favoring contaminant transport.

References

Adegbidi, H. G., Volk, T. A., White, E. H., Abrahamson, L. P., Briggs, R. D., & Bickelhaupt, D. H. (2001). Biomass and nutrient removal by willow clones in experimental bioenergy plantations in New York State. Biomass and Bioenergy, 20(6), 399–411.

Alagele, S. M., Jose, S., Anderson, S. H., & Udawatta, R. P. (2021). Hydraulic lift: processes, methods, and practical implications for society. Agroforestry Systems, 95(4), 641–657.

Ali, H., Khan, E., & Sajad, M. A. (2013). Phytoremediation of heavy metals—Concepts and applications. Chemosphere, 91(7), 869–881.

Almeida-Rodríguez, A. M., Gómes, M. P., Loubert-Hudon, A., Joly, S., & Labrecque, M. (2016). Symbiotic association between Salix purpurea L. and Rhizophagus irregularis: Modulation of plant responses under copper stress. Tree Physiology, 36(4), 407–420.

Anderson, T. A., Guthrie, E. A., & Walton, B. T. (1993). Bioremediation in the rhizosphere. Environmental Science & Technology, 27(13), 2630–2636.

Anderson, C. W. N., Brooks, R. R., Chiarucci, A., LaCoste, C. J., Leblanc, M., Robinson, B. H., Simcock, R., & Stewart, R. B. (1999). Phytomining for nickel, thallium and gold. Journal of Geochemical Exploration, 67(1–3), 407–415.

Andrews, S. (2010). FastQC: A Quality Control Tool for High Throughput Sequence Data. Babraham Bioinformatics. http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Antoniadis, V., Levizou, E., Shaheen, S. M., Ok, Y. S., Sebastian, A., Baum, C., Prasad, M. N. V., Wenzel, W. W., & Rinklebe, J. (2017). Trace elements in the soil-plant interface: Phytoavailability, translocation, and phytoremediation–A review. Earth-Science Reviews, 171, 621–645.

Argus, G. W. (1997). Infrageneric Classification of Salix (Salicaceae) in the New World. Systematic Botany Monographs, 52, 1–121.

Armas, C., Padilla, F. M., Pugnaire, F. I., & Jackson, R. B. (2010). Hydraulic lift and tolerance to salinity of semiarid species: Consequences for species interactions. Oecologia, 162(1), 11–21.

Awasthi, S., Chauhan, R., Srivastava, S., & Tripathi, R. D. (2017). The Journey of Arsenic from Soil to Grain in Rice. Frontiers in Plant Science, 8, 1007.

Baker, A., & Brooks, R. R. (1989). Terrestrial Higher Plants Which Hyperaccumulate Metallic Elements, A Review of Their Distribution. Biorecovery, 1, 81–126.

Ball, P. (2017). Water is an active matrix of life for cell and molecular biology. Proceedings of the National Academy of Sciences, 114(51), 13327–13335.

Bargués Tobella, A., Hasselquist, N. j., Bazié, H. r., Nyberg, G., Laudon, H., Bayala, J., & Ilstedt, U. (2017). Strategies trees use to overcome seasonal water limitation in an agroforestry system in semiarid West Africa. Ecohydrology, 10(3), e1808.

Barrowclough, D. E., Peterson, C. A., & Steudle, E. (2000). Radial hydraulic conductivity along developing onion roots. Journal of Experimental Botany, 51(344), 547–557.

Bayala, J., & Prieto, I. (2020). Water acquisition, sharing and redistribution by roots: Applications to agroforestry systems. Plant and Soil, 453(1), 17–28.

Bienert, G. P., Bienert, M. D., Jahn, T. P., Boutry, M., & Chaumont, F. (2011). Solanaceae XIPs are plasma membrane aquaporins that facilitate the transport of many uncharged substrates. The Plant Journal, 66(2), 306–317.

Bienert, G. P., Thorsen, M., Schüssler, M. D., Nilsson, H. R., Wagner, A., Tamás, M. J., & Jahn, T. P. (2008). A subgroup of plant aquaporins facilitate the bi-directional diffusion of As(OH)3 and Sb(OH)3across membranes. BMC Biology, 6(1), 26.

Bolan, N. S., Park, J. H., Robinson, B., Naidu, R., & Huh, K. Y. (2011). Phytostabilization. Advances in Agronomy, 145–204.

Boyer, J. S. (1982). Plant Productivity and Environment. Science, 218(4571), 443–448.

Brooks, R. R., Lee, J., Reeves, R. D., & Jaffre, T. (1977). Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. Journal of Geochemical Exploration, 7, 49–57.

Brooks, J. R., Meinzer, F. C., Warren, J. M., Domec, J.-C., & Coulombe, R. (2006). Hydraulic redistribution in a Douglas-fir forest: Lessons from system manipulations. Plant, Cell & Environment, 29(1), 138–150.

Bruemmer, G. W., Gerth, J., & Herms, U. (1986). Heavy metal species, mobility and availability in soils. Zeitschrift Für Pflanzenernährung Und Bodenkunde, 149(4), 382–398.

Brunner, I., Brodbeck, S., BÜchler, U., & Sperisen, C. (2001). Molecular identification of fine roots of trees from the Alps: Reliable and fast DNA extraction and PCR–RFLP analyses of plastid DNA. Molecular Ecology, 10(8), 2079–2087.

Buckley, T. N. (2019). How do stomata respond to water status? New Phytologist, 224(1), 21–36.

Burgess, S. S. O., Adams, M. A., Turner, N. C., & Ong, C. K. (1998). The redistribution of soil water by tree root systems. Oecologia, 115(3), 306–311.

Caird, M. A., Richards, J. H., & Donovan, L. A. (2007). Nighttime Stomatal Conductance and Transpiration in C3 and C4 Plants. Plant Physiology, 143(1), 4–10.

Caldwell, M. M., Dawson, T. E., & Richards, J. H. (1998). Hydraulic lift: Consequences of water efflux from the roots of plants. Oecologia, 113(2), 151–161.

Caldwell, M. M., & Richards, J. H. (1989). Hydraulic lift: Water efflux from upper roots improves effectiveness of water uptake by deep roots. Oecologia, 79(1), 1–5.

Cao, G.-H., Li, Z.-D., Wang, X.-F., Zhang, X., Zhao, R.-H., Gu, W., Chen, D., Yu, J., & He, S. (2020). Phosphate transporters, PnPht1;1 and PnPht1;2 from Panax notoginseng enhance phosphate and arsenate acquisition. BMC Plant Biology, 20(1), 124.

Cao, Y., Sun, D., Ai, H., Mei, H., Liu, X., Sun, S., Xu, G., Liu, Y., Chen, Y., & Ma, L. Q. (2017). Knocking Out OsPT4 Gene Decreases Arsenate Uptake by Rice Plants and Inorganic Arsenic Accumulation in Rice Grains. Environmental Science & Technology, 51(21), 12131–12138.

Catarecha, P., Segura, M. D., Franco-Zorrilla, J. M., García-Ponce, B., Lanza, M., Solano, R., Paz-Ares, J., & Leyva, A. (2007). A Mutant of the Arabidopsis Phosphate Transporter PHT1;1 Displays Enhanced Arsenic Accumulation. The Plant Cell, 19(3), 1123–1133.

Cernusak, L. A., Winter, K., & Turner, B. L. (2011). Transpiration modulates phosphorus acquisition in tropical tree seedlings. Tree Physiology, 31(8), 878–885.

Chang, S., Puryear, J., & Cairney, J. (1993). A simple and efficient method for isolating RNA from pine trees. Plant Molecular Biology Reporter, 11(2), 113–116.

Chao, D.-Y., Chen, Y., Chen, J., Shi, S., Chen, Z., Wang, C., Danku, J. M., Zhao, F.-J., & Salt, D. E. (2014). Genome-wide association mapping identifies a new arsenate reductase enzyme critical for limiting arsenic accumulation in plants. PLoS Biology, 12(12), e1002009.

Chen, Y., Sun, S.-K., Tang, Z., Liu, G., Moore, K. L., Maathuis, F. J. M., Miller, A. J., McGrath, S. P., & Zhao, F.-J. (2017). The Nodulin 26-like intrinsic membrane protein OsNIP3;2 is involved in arsenite uptake by lateral roots in rice. Journal of Experimental Botany, 68(11), 3007–3016.

Chen, S., Zhou, Y., Chen, Y., & Gu, J. (2018). fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34(17), i884–i890.

Conger, R. M. (2003). Black willow (Salix nigra) use in phytoremediation techniques to remove the herbicide bentazon from shallow groundwater. Doctoral dissertation, Louisiana State University.

Costa, J. M., Monnet, F., Jannaud, D., Leonhardt, N., Ksas, B., Reiter, I. M., Pantin, F., & Genty, B. (2015). OPEN ALL NIGHT LONG: The Dark Side of Stomatal Control1. Plant Physiology, 167(2), 289–294.

Corak, S. J., Blevins, D. G., & Pallardy, S. G. (1987). Water Transfer in an Alfalfa/Maize Association: Survival of Maize during Drought. Plant Physiology, 84(3), 582–586.

Cullen, W. R., & Reimer, K. J. (1989). Arsenic speciation in the environment. Chemical Reviews, 89(4), 713–764.

Dahlquist, K. D. (2016, July 8). RNA-seq Protocol. OpenWetWare. https://openwetware.org/mediawiki/index.php?title=Dahlquist:RNA-seq_Protocol&oldid=944164

Dawson, T. E. (1993). Hydraulic lift and water use by plants: Implications for water balance, performance and plant-plant interactions. Oecologia, 95(4), 565–574.

Delnomdedieu, M., Basti, M. M., Styblo, M., Otvos, J. D., & Thomas, D. J. (1994). Complexation of Arsenic Species in Rabbit Erythrocytes. Chemical Research in Toxicology, 7(5), 621–627.

Dhankher, O. P., Li, Y., Rosen, B. P., Shi, J., Salt, D., Senecoff, J. F., Sashti, N. A., & Meagher, R. B. (2002). Engineering tolerance and hyperaccumulation of arsenic in plants by combining arsenate reductase and γ-glutamylcysteine synthetase expression. Nature Biotechnology, 20(11), 1140–1145.

Dhankher, O. P., Rosen, B. P., McKinney, E. C., & Meagher, R. B. (2006). Hyperaccumulation of arsenic in the shoots of Arabidopsis silenced for arsenate reductase (ACR2). Proceedings of the National Academy of Sciences, 103(14), 5413–5418.

DiTusa, S. F., Fontenot, E. B., Wallace, R. W., Silvers, M. A., Steele, T. N., Elnagar, A. H., Dearman, K. M., & Smith, A. P. (2016). A member of the Phosphate transporter 1 (Pht1) family from the arsenic-hyperaccumulating fern Pteris vittata is a high-affinity arsenate transporter. New Phytologist, 209(2), 762–772.

Dixon, H. B. F. (1996). The Biochemical Action of Arsonic Acids Especially As Phosphate Analogues. Advances in Inorganic Chemistry, 44, 191–227.

Dixon, H. H., & Joly, J. (1895). On the Ascent of Sap. Philosophical Transactions of the Royal Society of London. B, 186, 563–576.

Doi, M., Kitagawa, Y., & Shimazaki, K. (2015). Stomatal Blue Light Response Is Present in Early Vascular Plants. Plant Physiology, 169(2), 1205–1213.

Dos Santos, T. P., Lopes, C. M., Rodrigues, M. L., Souza, C. R. de, Maroco, J. P., Pereira, J. S., Silva, J. R., & Chaves, M. M. (2003). Partial rootzone drying: Effects on growth and fruit quality of field-grown grapevines (Vitis vinifera). Functional Plant Biology: FPB, 30(6), 663–671.

Duan, G.-L., Hu, Y., Schneider, S., McDermott, J., Chen, J., Sauer, N., Rosen, B. P., Daus, B., Liu, Z., & Zhu, Y.-G. (2015). Inositol transporters AtINT2 and AtINT4 regulate arsenic accumulation in Arabidopsis seeds. Nature Plants, 2(1), 1-6.

Dushenkov, S. (2003). Trends in phytoremediation of radionuclides. Plant and Soil, 249(1), 167–175.

Dushenkov, V., Kumar, P. B. A. N., Motto, H., & Raskin, I. (1995). Rhizofiltration: The Use of Plants to Remove Heavy Metals from Aqueous Streams. Environmental Science & Technology, 29(5), 1239–1245.

Enstone, D. E., Peterson, C. A., & Ma, F. (2002). Root Endodermis and Exodermis: Structure, Function, and Responses to the Environment. Journal of Plant Growth Regulation, 21(4), 335–351.

Espeleta, J. F., West, J. B., & Donovan, L. A. (2004). Species-specific patterns of hydraulic lift in co-occurring adult trees and grasses in a sandhill community. Oecologia, 138(3), 341–349.

Finnegan, P., & Chen, W. (2012). Arsenic Toxicity: The Effects on Plant Metabolism. Frontiers in Physiology, 3, 182.

Fortin Faubert, M., Desjardins, D., Hijri, M., & Labrecque, M. (2021). Willows Used for Phytoremediation Increased Organic Contaminant Concentrations in Soil Surface. Applied Sciences, 11(7), 2979.

Franks, P. J., & Britton-Harper, Z. J. (2016). No evidence of general CO2 insensitivity in ferns: One stomatal control mechanism for all land plants? New Phytologist, 211(3), 819–827.

Fraysse, L. C., Wells, B., McCann, M. C., & Kjellbom, P. (2005). Specific plasma membrane aquaporins of the PIP1 subfamily are expressed in sieve elements and guard cells. Biology of the Cell, 97(7), 519–534.

Goldberg, S. (2002). Competitive Adsorption of Arsenate and Arsenite on Oxides and Clay Minerals. Soil Science Society of America Journal, 66(2), 413–421.

Goodstein, D. M., Shu, S., Howson, R., Neupane, R., Hayes, R. D., Fazo, J., Mitros, T., Dirks, W., Hellsten, U., Putnam, N., & Rokhsar, D. S. (2012). Phytozome: A comparative platform for green plant genomics. Nucleic Acids Research, 40(Database issue), 1178-1186.

Gräfe, K., & Schmitt, L. (2021). The ABC transporter G subfamily in Arabidopsis thaliana. Journal of Experimental Botany, 72(1), 92–106.

Greger, M., & Landberg, T. (1999). Use of Willow in Phytoextraction. International Journal of Phytoremediation, 1(2), 115–123.

Grenier, V., Pitre, F. E., Guidi Nissim, W., & Labrecque, M. (2015). Genotypic differences explain most of the response of willow cultivars to petroleum-contaminated soil. Trees, 29(3), 871–881.

Grip, H., Halldin, S., & Lindroth, A. (1989). Water use by intensively cultivated willow using estimated stomatal parameter values. Hydrological Processes, 3(1), 51–63.

Guidi Nissim, W., Castiglione, S., Guarino, F., Pastore, M. C., & Labra, M. (2023). Beyond Cleansing: Ecosystem Services Related to Phytoremediation. Plants, 12(5), 1031.

Hachez, C., Heinen, R. B., Draye, X., & Chaumont, F. (2008). The expression pattern of plasma membrane aquaporins in maize leaf highlights their role in hydraulic regulation. Plant Molecular Biology, 68(4), 337–353.

Hachez, C., Zelazny, E., & Chaumont, F. (2006). Modulating the expression of aquaporin genes in planta: A key to understand their physiological functions? Biochimica et Biophysica Acta (BBA) - Biomembranes, 1758(8), 1142–1156.

Hacke, U. G., & Laur, J. (2016). Aquaporins: Channels for the Molecule of Life. eLS, 1–6.

Hafner, B. D., Hesse, B. D., Bauerle, T. L., & Grams, T. E. E. (2020). Water potential gradient, root conduit size and root xylem hydraulic conductivity determine the extent of hydraulic redistribution in temperate trees. Functional Ecology, 34(3), 561–574.

Hansen, E. A., & Dickson, R. E. (1979). Water and Mineral Nutrient Transfer Between Root Systems of Juvenile Populus. Forest Science, 25(2), 247–252.

Hartley-Whitaker, J., Ainsworth, G., & Meharg, A. A. (2001). Copper- and arsenate-induced oxidative stress in Holcus lanatus L. clones with differential sensitivity. Plant, Cell & Environment, 24(7), 713–722.

Heikrujam, J., Kishor, R., & Mazumder, P. B. (2020, May 19). The Chemistry Behind Plant DNA Isolation Protocols. IntechOpen. https://www.intechopen.com/chapters/72074

Hellkvist, J., Richards, G. P., & Jarvis, P. G. (1974). Vertical Gradients of Water Potential and Tissue Water Relations in Sitka Spruce Trees Measured with the Pressure Chamber. Journal of Applied Ecology, 11(2), 637–667.

Hirota, I., Sakuratani, T., Sato, T., Higuchi, H., & Nawata, E. (2004). A split-root apparatus for examining the effects of hydraulic lift by trees on the water status of neighbouring crops. Agroforestry Systems, 60(2), 181–187.

Huang, J.-H. (2014). Impact of Microorganisms on Arsenic Biogeochemistry: A Review. Water, Air, & Soil Pollution, 225(2), 1848.

Hughes, M. F. (2002). Arsenic toxicity and potential mechanisms of action. Toxicology Letters, 133(1), 1–16.

Hulsen, T. (2022). DeepVenn—A web application for the creation of area-proportional Venn diagrams using the deep learning framework Tensorflow.js. arXiv. https://arxiv.org/abs/2210.04597

IARC. (2012). Arsenic, metals, fibres, and dusts. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 100(Pt C), 11–465.

Isayenkov, S. V., & Maathuis, F. J. M. (2008). The Arabidopsis thaliana aquaglyceroporin AtNIP7;1 is a pathway for arsenite uptake. FEBS Letters, 582(11), 1625–1628.

Ishikawa, F., Suga, S., Uemura, T., Sato, M. H., & Maeshima, M. (2005). Novel type aquaporin SIPs are mainly localized to the ER membrane and show cell-specific expression inArabidopsis thaliana. FEBS Letters, 579(25), 5814–5820.

Jarzyniak, K. M., & Jasiński, M. (2014). Membrane transporters and drought resistance—A complex issue. Frontiers in Plant Science, 5, 687.

Jia, J., Liang, Y., Gou, T., Hu, Y., Zhu, Y., Huo, H., Guo, J., Gong, H. (2020). The expression response of plasma membrane aquaporins to salt stress in tomato plants. Environmental and Experimental Botany, 178, 104190.

Jiménez-Rodríguez, C. D., Coenders-Gerrits, M., Uhlenbrook, S., & Wenninger, J. (2019). What Do Plants Leave after Summer on the Ground?—The Effect of Afforested Plants in Arid Environments. Water, 11(12), 2559.

Jobbágy, E. G., & Jackson, R. B. (2004). The Uplift of Soil Nutrients by Plants: Biogeochemical Consequences Across Scales. Ecology, 85(9), 2380–2389.

Jordan, C. F., & Kline, J. R. (1977). Transpiration of Trees in a Tropical Rainforest. Journal of Applied Ecology, 14(3), 853–860.

Kamiya, T., Islam, R., Duan, G., Uraguchi, S., & Fujiwara, T. (2013). Phosphate deficiency signaling pathway is a target of arsenate and phosphate transporter OsPT1 is involved in As accumulation in shoots of rice. Soil Science and Plant Nutrition, 59(4), 580–590.

Kamiya, T., Tanaka, M., Mitani, N., Ma, J. F., Maeshima, M., & Fujiwara, T. (2009). NIP1;1, an Aquaporin Homolog, Determines the Arsenite Sensitivity of Arabidopsis thaliana. Journal of Biological Chemistry, 284(4), 2114–2120.

Kang, S., & Zhang, J. (2004). Controlled alternate partial root-zone irrigation: Its physiological consequences and impact on water use efficiency. Journal of Experimental Botany, 55(407), 2437–2446.

Karlova, R., Boer, D., Hayes, S., & Testerink, C. (2021). Root plasticity under abiotic stress. Plant Physiology, 187(3), 1057–1070.

Karp, A., Hanley, S. J., Trybush, S. O., Macalpine, W., Pei, M., & Shield, I. (2011). Genetic Improvement of Willow for Bioenergy and BiofuelsFree Access. Journal of Integrative Plant Biology, 53(2), 151–165.

Katsuhara, M., Sasano, S., Horie, T., Matsumoto, T., Rhee, J., & Shibasaka, M. (2014). Functional and molecular characteristics of rice and barley NIP aquaporins transporting water, hydrogen peroxide and arsenite. Plant Biotechnology, 31(3), 213–219.

Khan, S., Qureshi, M. I., Kamaluddin, Alam, T., & Abdin, M. Z. (2007). Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion. African Journal of Biotechnology, 6(3), 175–178.

Kirkham, M. B. (2014). Stomatal Anatomy and Stomatal Resistance. Principles of Soil and Plant Water Relations, 431–451.

Klassen, S. P., McLean, J. E., Grossl, P. R., & Sims, R. C. (2000). Fate and Behavior of Lead in Soils Planted with Metal-Resistant Species (River Birch and Smallwing Sedge). Journal of Environmental Quality, 29(6), 1826–1834.

Kumar, A., Basu, S., Rishu, A. K., & Kumar, G. (2022). Revisiting the mechanisms of arsenic uptake, transport and detoxification in plants. Environmental and Experimental Botany, 194, 104730.

Kumar, V., Vogelsang, L., Schmidt, R. R., Sharma, S. S., Seidel, T., & Dietz, K.-J. (2020). Remodeling of Root Growth Under Combined Arsenic and Hypoxia Stress Is Linked to Nutrient Deprivation. Frontiers in Plant Science, 11, 569687.

Kuzovkina, Y. A., Knee, M., & Quigley, M. F. (2004). Cadmium and Copper Uptake and Translocation in Five Willow (Salix L.) Species. International Journal of Phytoremediation, 6(3), 269–287.

Labrecque, M., & Teodorescu, T. I. (2005). Field performance and biomass production of 12 willow and poplar clones in short-rotation coppice in southern Quebec (Canada). Biomass and Bioenergy, 29(1), 1–9.

Lambers, H., Shane, M. W., Cramer, M. D., Pearse, S. J., & Veneklaas, E. J. (2006). Root Structure and Functioning for Efficient Acquisition of Phosphorus: Matching Morphological and Physiological Traits. Annals of Botany, 98(4), 693–713.

Langmead, B., & Salzberg, S. L. (2012). Fast gapped-read alignment with Bowtie 2. Nature Methods, 9(4), 357–359.

Laur, J., & Hacke, U. G. (2013). Transpirational demand affects aquaporin expression in poplar roots. Journal of Experimental Botany, 64(8), 2283–2293.

Laur, J., & Hacke, U. G. (2014). Exploring Picea glauca aquaporins in the context of needle water uptake and xylem refilling. New Phytologist, 203(2), 388–400.

LeBlanc, M. S., McKinney, E. C., Meagher, R. B., & Smith, A. P. (2013). Hijacking membrane transporters for arsenic phytoextraction. Journal of Biotechnology, 163(1), 1–9.

Lee, S. H., Chung, G. C., Jang, J. Y., Ahn, S. J., & Zwiazek, J. J. (2012). Overexpression of PIP2;5 Aquaporin Alleviates Effects of Low Root Temperature on Cell Hydraulic Conductivity and Growth in Arabidopsis. Plant Physiology, 159(1), 479–488.

Leffler, A. J., Peek, M. S., Ryel, R. J., Ivans, C. Y., & Caldwell, M. M. (2005). Hydraulic Redistribution Through the Root Systems of Senesced Plants. Ecology, 86(3), 633–642.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The Sequence Alignment/Map format and SAMtools. Bioinformatics, 25(16), 2078–2079.

Li, R.-Y., Ago, Y., Liu, W.-J., Mitani, N., Feldmann, J., McGrath, S. P., Ma, J. F., & Zhao, F.-J. (2009). The Rice Aquaporin Lsi1 Mediates Uptake of Methylated Arsenic Species. Plant Physiology, 150(4), 2071–2080.

Li, N., Wang, J., & Song, W.-Y. (2016). Arsenic Uptake and Translocation in Plants. Plant & Cell Physiology, 57(1), 4–13.

Lian, H.-L., Yu, X., Ye, Q., Ding, X.-S., Kitagawa, Y., Kwak, S.-S., Su, W.-A., & Tang, Z.-C. (2004). The Role of Aquaporin RWC3 in Drought Avoidance in Rice. Plant and Cell Physiology, 45(4), 481–489.

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics, 30(7), 923–930.

Limmer, M., & Burken, J. (2016). Phytovolatilization of Organic Contaminants. Environmental Science & Technology, 50(13), 6632–6643.

Lin, B. B., & Andersson, E. (2023). A Transdisciplinary Framework to Unlock the Potential Benefits of Green Spaces for Urban Communities Under Changing Contexts. BioScience, 73(3), 196–205.

Lin, A., Zhang, X., Zhu, Y.-G., & Zhao, F.-J. (2008). Arsenate-induced toxicity: Effects on antioxidative enzymes and DNA damage in Vicia faba. Environmental Toxicology and Chemistry, 27(2), 413–419.

Lipper, L., Thornton, P., Campbell, B. M., Baedeker, T., Braimoh, A., Bwalya, M., Caron, P., Cattaneo, A., Garrity, D., Henry, K., Hottle, R., Jackson, L., Jarvis, A., Kossam, F., Mann, W., McCarthy, N., Meybeck, A., Neufeldt, H., Remington, T., … Torquebiau, E. F. (2014). Climate-smart agriculture for food security. Nature Climate Change, 4(12), 1068–1072.

Liste, H.-H., & White, J. C. (2008). Plant hydraulic lift of soil water – implications for crop production and land restoration. Plant and Soil, 313(1), 1–17.

Liu, W.-J., Wood, B. A., Raab, A., McGrath, S. P., Zhao, F.-J., & Feldmann, J. (2010). Complexation of Arsenite with Phytochelatins Reduces Arsenite Efflux and Translocation from Roots to Shoots in Arabidopsis. Plant Physiology, 152(4), 2211–2221.

Lomax, C., Liu, W.-J., Wu, L., Xue, K., Xiong, J., Zhou, J., McGrath, S. P., Meharg, A. A., Miller, A. J., & Zhao, F.-J. (2012). Methylated arsenic species in plants originate from soil microorganisms. New Phytologist, 193(3), 665–672.

Lopez, F., Bousser, A., Sissoëff, I., Gaspar, M., Lachaise, B., Hoarau, J., & Mahé, A. (2003). Diurnal Regulation of Water Transport and Aquaporin Gene Expression in Maize Roots: Contribution of PIP2 Proteins. Plant and Cell Physiology, 44(12), 1384–1395.

Lynch, J. P. (2018). Rightsizing root phenotypes for drought resistance. Journal of Experimental Botany, 69(13), 3279–3292.

Ma, L. Q., Komar, K. M., Tu, C., Zhang, W., Cai, Y., & Kennelley, E. D. (2001). A fern that hyperaccumulates arsenic. Nature, 409(6820), 579–579.

Ma, J. F., Tamai, K., Yamaji, N., Mitani, N., Konishi, S., Katsuhara, M., Ishiguro, M., Murata, Y., & Yano, M. (2006). A silicon transporter in rice. Nature, 440(7084), 688–691.

Ma, J. F., Yamaji, N., Mitani, N., Tamai, K., Konishi, S., Fujiwara, T., Katsuhara, M., & Yano, M. (2007). An efflux transporter of silicon in rice. Nature, 448(7150), 209–212.

Ma, J. F., Yamaji, N., Mitani, N., Xu, X.-Y., Su, Y.-H., McGrath, S. P., & Zhao, F.-J. (2008). Transporters of arsenite in rice and their role in arsenic accumulation in rice grain. Proceedings of the National Academy of Sciences of the United States of America, 105(29), 9931–9935.

Maggio, A., & Joly, R. J. (1995). Effects of Mercuric Chloride on the Hydraulic Conductivity of Tomato Root Systems (Evidence for a Channel-Mediated Water Pathway). Plant Physiology, 109(1), 331–335.

Mandal, B. K., & Suzuki, K. T. (2002). Arsenic round the world: A review. Talanta, 58(1), 201–235.

Martre, P., North, G. B., & Nobel, P. S. (2001). Hydraulic Conductance and Mercury-Sensitive Water Transport for Roots of Opuntia acanthocarpa in Relation to Soil Drying and Rewetting. Plant Physiology, 126(1), 352–362.

Massenet, A., Bonet, A., Laur, J., & Labrecque, M. (2021). Co-planting Brassica napus and Salix nigra as a phytomanagement alternative for copper contaminated soil. Chemosphere, 279, 130517.

Maurel, C. (1997). Aquaporins and Water Permeability of Plant Membranes. Annual Review of Plant Physiology and Plant Molecular Biology, 48(1), 399–429.

Maurel, C., Boursiac, Y., Luu, D.-T., Santoni, V., Shahzad, Z., & Verdoucq, L. (2015). Aquaporins in Plants. Physiological Reviews, 95(4), 1321–1358.

Mayr, S., Schmid, P., Laur, J., Rosner, S., Charra-Vaskou, K., Dämon, B., & Hacke, U. G. (2014). Uptake of Water via Branches Helps Timberline Conifers Refill Embolized Xylem in Late Winter. Plant Physiology, 164(4), 1731–1740.

McGrath, S. P., & Zhao, F.-J. (2003). Phytoextraction of metals and metalloids from contaminated soils. Current Opinion in Biotechnology, 14(3), 277–282.

McIntyre, G. I. (2001). Control of plant development by limiting factors: A nutritional perspective. Physiologia Plantarum, 113(2), 165–175.

Meharg, A. A., & Hartley-Whitaker, J. (2002). Arsenic uptake and metabolism in arsenic resistant and nonresistant plant species. New Phytologist, 154(1), 29–43.

Meharg, A. A., & Macnair, M. R. (1990). An altered phosphate uptake system in arsenate-tolerant Holcus lanatus L. New Phytologist, 116(1), 29–35.

Meharg, A. A., & Macnair, M. R. (1992). Suppression of the High Affinity Phosphate Uptake System: A Mechanism of Arsenate Tolerance in Holcus lanatus L. Journal of Experimental Botany, 43(249), 519–524.

Meng, D., Walsh, M., & Fricke, W. (2016). Rapid changes in root hydraulic conductivity and aquaporin expression in rice (Oryza sativa L.) in response to shoot removal—Xylem tension as a possible signal. Annals of Botany, 118(4), 809–819.

Mishra, S., Mattusch, J., & Wennrich, R. (2017). Accumulation and transformation of inorganic and organic arsenic in rice and role of thiol-complexation to restrict their translocation to shoot. Scientific Reports, 7(1), 40522.

Mizutani, M., Watanabe, S., Nakagawa, T., & Maeshima, M. (2006). Aquaporin NIP2;1 is mainly localized to the ER membrane and shows root-specific accumulation in Arabidopsis thaliana. Plant & Cell Physiology, 47(10), 1420–1426.

Mooney, H. A., Gulmon, S. L., Rundel, P. W., & Ehleringer, J. (1980). Further observations on the water relations ofProsopis tamarugo of the northern Atacama desert. Oecologia, 44(2), 177–180.

Moreira, M. Z., Scholz, F. G., Bucci, S. J., Sternberg, L. S., Goldstein, G., Meinzer, F. C., & Franco, A. C. (2003). Hydraulic lift in a neotropical savanna. Functional Ecology, 17(5), 573–581.

Mosa, K. A., Kumar, K., Chhikara, S., Mcdermott, J., Liu, Z., Musante, C., White, J. C., & Dhankher, O. P. (2012). Members of rice plasma membrane intrinsic proteins subfamily are involved in arsenite permeability and tolerance in plants. Transgenic Research, 21(6), 1265–1277.

Mu, W., Wei, J., Yang, T., Fan, Y., Cheng, L., Yang, J., Mu, R., Liu, J., Zhao, J., Sun, W., Xu, X., Liu, X., Drmanac, R., & Liu, H. (2017, February 28). RNA extraction for plant samples using CTAB-pBIOZOL. Protocols.Io. https://www.protocols.io/view/rna-extraction-for-plant-samples-using-ctab-pbiozo-gsnbwde

Navarro, C., Mateo-Elizalde, C., Mohan, T. C., Sánchez-Bermejo, E., Urrutia, O., Fernández-Muñiz, M. N., García-Mina, J. M., Muñoz, R., Paz-Ares, J., Castrillo, G., & Leyva, A. (2021). Arsenite provides a selective signal that coordinates arsenate uptake and detoxification through the regulation of PHR1 stability in Arabidopsis. Molecular Plant, 14(9), 1489–1507.

Navazas, A., Hendrix, S., Cuypers, A., & González, A. (2019). Integrative response of arsenic uptake, speciation and detoxification by Salix atrocinerea. Science of The Total Environment, 689, 422–433.

Newman, L. A., & Reynolds, C. M. (2004). Phytodegradation of organic compounds. Current Opinion in Biotechnology, 15(3), 225–230.

Nordstrom, D. K. (2002). Worldwide Occurrences of Arsenic in Ground Water. Science, 296(5576), 2143–2145.

Noronha, H., Agasse, A., Martins, A. P., Berny, M. C., Gomes, D., Zarrouk, O., Thiebaud, P., Delrot, S., Soveral, G., Chaumont, F., & Gerós, H. (2014). The grape aquaporin VvSIP1 transports water across the ER membrane. Journal of Experimental Botany, 65(4), 981–993.

Oliveira, R. S., Dawson, T. E., Burgess, S. S. O., & Nepstad, D. C. (2005). Hydraulic redistribution in three Amazonian trees. Oecologia, 145(3), 354–363.

Pajević, S., Borišev, M., Nikolić, N., Arsenov, D. D., Orlović, S., & Župunski, M. (2016). Phytoextraction of Heavy Metals by Fast-Growing Trees: A Review. In A. A. Ansari, S. S. Gill, R. Gill, G. R. Lanza, & L. Newman (Eds.), Phytoremediation: Management of Environmental Contaminants, Volume 3 (29–64). Springer International Publishing.

Pang, J., Wang, Y., Lambers, H., Tibbett, M., Siddique, K. H. M., & Ryan, M. H. (2013). Commensalism in an agroecosystem: Hydraulic redistribution by deep-rooted legumes improves survival of a droughted shallow-rooted legume companion. Physiologia Plantarum, 149(1), 79–90.

Patel, J., & Mishra, A. (2021). Plant aquaporins alleviate drought tolerance in plants by modulating cellular biochemistry, root-architecture, and photosynthesis. Physiologia Plantarum, 172(2), 1030–1044.

Perrone, I., Gambino, G., Chitarra, W., Vitali, M., Pagliarani, C., Riccomagno, N., Balestrini, R., Kaldenhoff, R., Uehlein, N., Gribaudo, I., Schubert, A., & Lovisolo, C. (2012). The Grapevine Root-Specific Aquaporin VvPIP2;4N Controls Root Hydraulic Conductance and Leaf Gas Exchange under Well-Watered Conditions But Not under Water Stress. Plant Physiology, 160(2), 965–977.

Perumalla, C. J., & Peterson, C. A. (1986). Deposition of Casparian bands and suberin lamellae in the exodermis and endodermis of young corn and onion roots. Canadian Journal of Botany, 64(9), 1873–1878.

Pickering, I. J., Prince, R. C., George, M. J., Smith, R. D., George, G. N., & Salt, D. E. (2000). Reduction and Coordination of Arsenic in Indian Mustard. Plant Physiology, 122(4), 1171–1178.

Pilon-Smits, E. (2005). Phytoremediation. Annual Review of Plant Biology, 56(1), 15–39.

Podgorski, J., & Berg, M. (2020). Global threat of arsenic in groundwater. Science, 368(6493), 845–850.

Porcel, R., Aroca, R., Azcón, R., & Ruiz-Lozano, J. M. (2006). PIP aquaporin gene expression in arbuscular mycorrhizal Glycine max and Lactuca sativa plants in relation to drought stress tolerance. Plant Molecular Biology, 60(3), 389–404.

Postaire, O., Tournaire-Roux, C., Grondin, A., Boursiac, Y., Morillon, R., Schäffner, A. R., & Maurel, C. (2010). A PIP1 Aquaporin Contributes to Hydrostatic Pressure-Induced Water Transport in Both the Root and Rosette of Arabidopsis. Plant Physiology, 152(3), 1418–1430.

Pou, A., Medrano, H., Flexas, J., & Tyerman, S. D. (2013). A putative role for TIP and PIP aquaporins in dynamics of leaf hydraulic and stomatal conductances in grapevine under water stress and re-watering. Plant, Cell & Environment, 36(4), 828–843.

Prado, K., Boursiac, Y., Tournaire-Roux, C., Monneuse, J.-M., Postaire, O., Da Ines, O., Schäffner, A. R., Hem, S., Santoni, V., & Maurel, C. (2013). Regulation of Arabidopsis Leaf Hydraulics Involves Light-Dependent Phosphorylation of Aquaporins in Veins. The Plant Cell, 25(3), 1029–1039.

Preston, G. M., Jung, J. S., Guggino, W. B., & Agre, P. (1993). The mercury-sensitive residue at cysteine 189 in the CHIP28 water channel. The Journal of Biological Chemistry, 268(1), 17–20.

Prieto, I., Armas, C., & Pugnaire, F. I. (2012). Water release through plant roots: New insights into its consequences at the plant and ecosystem level. New Phytologist, 193(4), 830–841.

Pucholt, P., Sjödin, P., Weih, M., Rönnberg-Wästljung, A. C., & Berlin, S. (2015). Genome-wide transcriptional and physiological responses to drought stress in leaves and roots of two willow genotypes. BMC Plant Biology, 15(1), 244.

Puckett, E. E., Serpiglia, M. J., DeLeon, A. M., Long, S., Minocha, R., & Smart, L. B. (2012). Differential expression of genes encoding phosphate transporters contributes to arsenic accumulation in shrub willow (Salix spp.). Environmental and Experimental Botany, 75, 248-257.

Pulford, I. D., & Watson, C. (2003). Phytoremediation of heavy metal-contaminated land by trees—A review. Environment International, 29(4), 529–540.

Purdy, J. J., & Smart, L. B. (2008). Hydroponic Screening of Shrub Willow (Salix Spp.) for Arsenic Tolerance and Uptake. International Journal of Phytoremediation, 10(6), 515–528.

Raab, A., Schat, H., Meharg, A. A., & Feldmann, J. (2005). Uptake, translocation and transformation of arsenate and arsenite in sunflower (Helianthus annuus): Formation of arsenic–phytochelatin complexes during exposure to high arsenic concentrations. New Phytologist, 168(3), 551–558.

Raab, A., Williams, P. N., Meharg, A. A., & Feldmann, J. (2007). Uptake and translocation of inorganic and methylated arsenic species by plants. Environmental Chemistry, 4(3), 197–203.

Rahman, M. A., Kadohashi, K., Maki, T., & Hasegawa, H. (2011). Transport of DMAA and MMAA into rice (Oryza sativa L.) roots. Environmental and Experimental Botany, 72(1), 41–46.

Ramadan, D., Cline, D. J., Bai, S., Thorpe, C., & Schneider, J. P. (2007). Effects of As(III) Binding on β-Hairpin Structure. Journal of the American Chemical Society, 129(10), 2981–2988.

Raza, M. A. S., Ahmad, S., Saleem, M. F., Khan, I. H., Iqbal, R., Zaheer, M. S., Haider, I., & Ali, M. (2017). Physiological and biochemical assisted screening of wheat varieties under partial rhizosphere drying. Plant Physiology and Biochemistry, 116, 150–166.

Reeves, R., Baker, A., & Brooks, R. R. (1995). Abnormal accumulation of trace metals by plants. Mining Environ. Manage, 3, 4–8.

Remy, E., Cabrito, T. R., Batista, R. A., Teixeira, M. C., Sá-Correia, I., & Duque, P. (2012). The Pht1;9 and Pht1;8 transporters mediate inorganic phosphate acquisition by the Arabidopsis thaliana root during phosphorus starvation. New Phytologist, 195(2), 356–371.

Richards, J. H., & Caldwell, M. M. (1987). Hydraulic lift: Substantial nocturnal water transport between soil layers by Artemisia tridentata roots. Oecologia, 73(4), 486–489.

Robinson, M. D., McCarthy, D. J., & Smyth, G. K. (2010). edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics, 26(1), 139–140.

Rodríguez-Gamir, J., Ancillo, G., González-Mas, M. C., Primo-Millo, E., Iglesias, D. J., & Forner-Giner, M. A. (2011). Root signalling and modulation of stomatal closure in flooded citrus seedlings. Plant Physiology and Biochemistry, 49(6), 636–645.

Roth-Nebelsick, A., Hacke, U. G., Voigt, D., Schreiber, S. G., & Krause, M. (2023). Foliar water uptake in Pinus species depends on needle age and stomatal wax structures. Annals of Botany, 131(2), 287–300.

Rouphael, Y., Cardarelli, M., Schwarz, D., Franken, P., & Colla, G. (2012). Effects of Drought on Nutrient Uptake and Assimilation in Vegetable Crops. In R. Aroca (Ed.), Plant Responses to Drought Stress: From Morphological to Molecular Features (171–195). Springer.

Ruttens, A., Boulet, J., Weyens, N., Smeets, K., Adriaensen, K., Meers, E., Van Slycken, S., Tack, F., Meiresonne, L., Thewys, T., Witters, N., Carleer, R., Dupae, J., & Vangronsveld, J. (2011). Short rotation coppice culture of willows and poplars as energy crops on metal contaminated agricultural soils. International Journal of Phytoremediation, 13 (Sup 1), 194–207.

Sakakibara, M., Watanabe, A., Inoue, M., Sano, S., & Kaise, T. (2010). Phytoextraction And Phytovolatilization Of Arsenic From As-Contaminated Soils By Pteris vittata. Proceedings of the Annual International Conference on Soils, Sediments, Water and Energy, 12(1), 26.

Sánchez-Bermejo, E., Castrillo, G., del Llano, B., Navarro, C., Zarco-Fernández, S., Martinez-Herrera, D. J., Leo-del Puerto, Y., Muñoz, R., Cámara, C., Paz-Ares, J., Alonso-Blanco, C., & Leyva, A. (2014). Natural variation in arsenate tolerance identifies an arsenate reductase in Arabidopsis thaliana. Nature Communications, 5(1), 4617.

Schulze, E. D. (1986). Carbon Dioxide and Water Vapor Exchange in Response to Drought in the Atmosphere and in the Soil. Annual Review of Plant Physiology, 37(1), 247–274.

Schulze, E. D., Caldwell, M. M., Canadell, J., Mooney, H. A., Jackson, R. B., Parson, D., Scholes, R., Sala, O. E., & Trimborn, P. (1998). Downward flux of water through roots (i.e. Inverse hydraulic lift) in dry Kalahari sands. Oecologia, 115(4), 460–462.

Scott, N., Hatlelid, K. M., MacKenzie, N. E., & Carter, D. E. (1993). Reactions of arsenic(III) and arsenic(V) species with glutathione. Chemical Research in Toxicology, 6(1), 102–106.

Secchi, F., Pagliarani, C., & Zwieniecki, M. A. (2017). The functional role of xylem parenchyma cells and aquaporins during recovery from severe water stress. Plant, Cell & Environment, 40(6), 858–871.

Sekiya, N., Araki, H., & Yano, K. (2011). Applying hydraulic lift in an agroecosystem: Forage plants with shoots removed supply water to neighboring vegetable crops. Plant and Soil, 341(1), 39–50.

Sekiya, N., & Yano, K. (2004). Do pigeon pea and sesbania supply groundwater to intercropped maize through hydraulic lift?—Hydrogen stable isotope investigation of xylem waters. Field Crops Research, 86(2), 167–173.

Shaji, E., Santosh, M., Sarath, K. V., Prakash, P., Deepchand, V., & Divya, B. V. (2021). Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. Geoscience Frontiers, 12(3), 101079.

Shi, S., Wang, T., Chen, Z., Tang, Z., Wu, Z., Salt, D. E., Chao, D.-Y., & Zhao, F.-J. (2016). OsHAC1;1 and OsHAC1;2 Function as Arsenate Reductases and Regulate Arsenic Accumulation1. Plant Physiology, 172(3), 1708–1719.

Shin, H., Shin, H.-S., Dewbre, G. R., & Harrison, M. J. (2004). Phosphate transport in Arabidopsis: Pht1;1 and Pht1;4 play a major role in phosphate acquisition from both low- and high-phosphate environments. The Plant Journal, 39(4), 629–642.

Smart, L. B., Moskal, W. A., Cameron, K. D., & Bennett, A. B. (2001). MIP genes are down-regulated under drought stress in Nicotiana glauca. Plant & Cell Physiology, 42(7), 686–693.

Smart, L., Volk, T., Lin, J., Kopp, R., Phillips, I., Cameron, K., White, E., & Abrahamson, L. (2005). Genetic improvement of shrub willow (Salix spp.) crops for bioenergy and environmental applications in the United States. Unasylva, 56.

Song, W.-Y., Park, J., Mendoza-Cózatl, D. G., Suter-Grotemeyer, M., Shim, D., Hörtensteiner, S., Geisler, M., Weder, B., Rea, P. A., Rentsch, D., Schroeder, J. I., Lee, Y., & Martinoia, E. (2010). Arsenic tolerance in Arabidopsis is mediated by two ABCC-type phytochelatin transporters. Proceedings of the National Academy of Sciences, 107(49), 21187–21192.

Song, W.-Y., Yamaki, T., Yamaji, N., Ko, D., Jung, K.-H., Fujii-Kashino, M., An, G., Martinoia, E., Lee, Y., & Ma, J. F. (2014). A rice ABC transporter, OsABCC1, reduces arsenic accumulation in the grain. Proceedings of the National Academy of Sciences, 111(44), 15699–15704.

Sperry, J. S. (2003). Evolution of Water Transport and Xylem Structure. International Journal of Plant Sciences, 164(S3), S115–S127.

Sperry, J. S. (2011). Hydraulics of Vascular Water Transport. In P. Wojtaszek (Ed.), Mechanical Integration of Plant Cells and Plants (pp. 303–327). Springer.

Steudle, E. (2000). Water uptake by plant roots: An integration of views. Plant and Soil, 226(1), 45–56.

Steudle, E. (2001). The Cohesion-Tension Mechanism and the Acquisition of Water by Plant Roots. Annual Review of Plant Physiology and Plant Molecular Biology, 52(1), 847–875.

Steudle, E., & Peterson, C. A. (1998). How does water get through roots? Journal of Experimental Botany, 49(322), 775–788.

Su, Y. H., McGrath, S. P., Zhu, Y. G., & Zhao, F.-J. (2008). Highly efficient xylem transport of arsenite in the arsenic hyperaccumulator Pteris vittata. New Phytologist, 180(2), 434–441.

Sun, S.-K., Chen, Y., Che, J., Konishi, N., Tang, Z., Miller, A. J., Ma, J. F., & Zhao, F.-J. (2018). Decreasing arsenic accumulation in rice by overexpressing OsNIP1;1 and OsNIP3;3 through disrupting arsenite radial transport in roots. New Phytologist, 219(2), 641–653.

Sun, D., Feng, H.-Y., Li, X.-Y., Ai, H., Sun, S., Chen, Y., Xu, G., Rathinasabapathi, B., Cao, Y., & Ma, L. Q. (2019). Expression of New Pteris vittata Phosphate Transporter PvPht1;4 Reduces Arsenic Translocation from the Roots to Shoots in Tobacco Plants. Environmental Science & Technology, 54, 1045–1053.

Šurbanovski, N., Sargent, D. J., Else, M. A., Simpson, D. W., Zhang, H., & Grant, O. M. (2013). Expression of Fragaria vesca PIP Aquaporins in Response to Drought Stress: PIP Down-Regulation Correlates with the Decline in Substrate Moisture Content. PLOS ONE, 8(9), e74945.

Sutka, M., Li, G., Boudet, J., Boursiac, Y., Doumas, P., & Maurel, C. (2011). Natural Variation of Root Hydraulics in Arabidopsis Grown in Normal and Salt-Stressed Conditions. Plant Physiology, 155(3), 1264–1276.

Takano, J., Wada, M., Ludewig, U., Schaaf, G., von Wirén, N., & Fujiwara, T. (2006). The Arabidopsis Major Intrinsic Protein NIP5;1 Is Essential for Efficient Boron Uptake and Plant Development under Boron Limitation. The Plant Cell, 18(6), 1498–1509.

Tang, Z., Chen, Y., Chen, F., Ji, Y., & Zhao, F.-J. (2017). OsPTR7 (OsNPF8.1), a Putative Peptide Transporter in Rice, is Involved in Dimethylarsenate Accumulation in Rice Grain. Plant and Cell Physiology, 58(5), 904–913.

Tang, Z., Chen, Y., Miller, A. J., & Zhao, F.-J. (2019). The C-type ATP-Binding Cassette Transporter OsABCC7 Is Involved in the Root-to-Shoot Translocation of Arsenic in Rice. Plant and Cell Physiology, 60(7), 1525–1535.

Tang, Z., & Zhao, F.-J. (2021). The roles of membrane transporters in arsenic uptake, translocation and detoxification in plants. Critical Reviews in Environmental Science and Technology, 51(21), 2449–2484.

Tian, T., Liu, Y., Yan, H., You, Q., Yi, X., Du, Z., Xu, W., & Su, Z. (2017). agriGO v2.0: A GO analysis toolkit for the agricultural community, 2017 update. Nucleic Acids Research, 45(W1), W122–W129.

Tiwari, M., Sharma, D., Dwivedi, S., Singh, M., Tripathi, R. D., & Trivedi, P. K. (2014). Expression in Arabidopsis and cellular localization reveal involvement of rice NRAMP, OsNRAMP1, in arsenic transport and tolerance. Plant, Cell & Environment, 37(1), 140–152.

Trapp, S., & Karlson, U. (2001). Aspects of phytoremediation of organic pollutants. Journal of Soils and Sediments, 1(1), 37–43.

Tyree, M. T. (1997). The Cohesion-Tension theory of sap ascent: Current controversies. Journal of Experimental Botany, 48(10), 1753–1765.

Van Bavel, C. H. M., & Baker, J. M. (1985). Water transfer by plant roots from wet to dry soil. Naturwissenschaften, 72(11), 606–607.

Vandeleur, R. K., Mayo, G., Shelden, M. C., Gilliham, M., Kaiser, B. N., & Tyerman, S. D. (2009). The Role of Plasma Membrane Intrinsic Protein Aquaporins in Water Transport through Roots: Diurnal and Drought Stress Responses Reveal Different Strategies between Isohydric and Anisohydric Cultivars of Grapevine. Plant Physiology, 149(1), 445–460.

Vandeleur, R. K., Sullivan, W., Athman, A., Jordans, C., Gilliham, M., Kaiser, B. N., & Tyerman, S. D. (2014). Rapid shoot-to-root signalling regulates root hydraulic conductance via aquaporins. Plant, Cell & Environment, 37(2), 520–538.

Venturas, M. D., Sperry, J. S., & Hacke, U. G. (2017). Plant xylem hydraulics: What we understand, current research, and future challenges. Journal of Integrative Plant Biology, 59(6), 356–389.

Verbruggen, N., Hermans, C., & Schat, H. (2009). Molecular mechanisms of metal hyperaccumulation in plants. New Phytologist, 181(4), 759–776.

Verwijst, T. (2001). Willows: An underestimated resource for environment and society. The Forestry Chronicle, 77(2), 281–285.

Vetterlein, D., Szegedi, K., Ackermann, J., Mattusch, J., Neue, H.-U., Tanneberg, H., & Jahn, R. (2007). Competitive mobilization of phosphate and arsenate associated with goethite by root activity. Journal of Environmental Quality, 36(6), 1811–1820.

Vysloužilová, M., Tlustoš, P., Száková, J., & Pavlíková, D. (2003). As, Cd, Pb and Zn uptake by Salix spp. Clones grown in soils enriched by high loads of these elements. Plant, Soil and Environment, 49, 191–196.

Wan, X., & Zwiazek, J. J. (1999). Mercuric Chloride Effects on Root Water Transport in Aspen Seedlings. Plant Physiology, 121(3), 939–946.

Wang, Y., Jensen, C. R., & Liu, F. (2017). Nutritional responses to soil drying and rewetting cycles under partial root-zone drying irrigation. Agricultural Water Management, 179, 254–259.

Wani, K. A., Sofi, Z. M., Malik, J. A., & Wani, J. A. (2020). Phytoremediation of Heavy Metals Using Salix (Willows). In R. A. Bhat, K. R. Hakeem, & M. A. Dervash (Eds.), Bioremediation and Biotechnology, Vol 2: Degradation of Pesticides and Heavy Metals (161–174). Springer International Publishing.

Wang, P., Zhang, W., Mao, C., Xu, G., & Zhao, F.-J. (2016). The role of OsPT8 in arsenate uptake and varietal difference in arsenate tolerance in rice. Journal of Experimental Botany, 67(21), 6051–6059.

Warren, J. M., Meinzer, F. C., Brooks, J. R., Domec, J.-C., & Coulombe, R. (2007). Hydraulic redistribution of soil water in two old-growth coniferous forests: Quantifying patterns and controls. New Phytologist, 173(4), 753–765.

Watson, C. (2002). The phytoremediation potential of Salix: studies of the interaction of heavy metals and willows. Doctoral dissertation, University of Glasgow.

Wu, Z., Ren, H., McGrath, S. P., Wu, P., & Zhao, F.-J. (2011). Investigating the Contribution of the Phosphate Transport Pathway to Arsenic Accumulation in Rice. Plant Physiology, 157(1), 498–508.

Wudick, M. M., Luu, D.-T., & Maurel, C. (2009). A look inside: Localization patterns and functions of intracellular plant aquaporins. New Phytologist, 184(2), 289–302.

Wullschleger, S. D., Meinzer, F. C., & Vertessy, R. A. (1998). A review of whole-plant water use studies in tree. Tree Physiology, 18(8), 499–512.

Xu, W., Dai, W., Yan, H., Li, S., Shen, H., Chen, Y., Xu, H., Sun, Y., He, Z., & Ma, M. (2015). Arabidopsis NIP3;1 Plays an Important Role in Arsenic Uptake and Root-to-Shoot Translocation under Arsenite Stress Conditions. Molecular Plant, 8(5), 722–733.

Xu, X. Y., McGrath, S. P., & Zhao, F.-J. (2007). Rapid reduction of arsenate in the medium mediated by plant roots. The New Phytologist, 176(3), 590–599.

Xu, J., Shi, S., Wang, L., Tang, Z., Lv, T., Zhu, X., Ding, X., Wang, Y., Zhao, F.-J., & Wu, Z. (2017). OsHAC4 is critical for arsenate tolerance and regulates arsenic accumulation in rice. New Phytologist, 215(3), 1090–1101.

Yactayo, W., Ramírez, D. A., Gutiérrez, R., Mares, V., Posadas, A., & Quiroz, R. (2013). Effect of partial root-zone drying irrigation timing on potato tuber yield and water use efficiency. Agricultural Water Management, 123, 65–70.

Yanitch, A., Brereton, N. J. B., Gonzalez, E., Labrecque, M., Joly, S., & Pitre, F. E. (2017). Transcriptomic Response of Purple Willow (Salix purpurea) to Arsenic Stress. Frontiers in Plant Science, 8, 1115.

Yanitch, A., Kadri, H., Frenette-Dussault, C., Joly, S., Pitre, F. E., & Labrecque, M. (2020). A four-year phytoremediation trial to decontaminate soil polluted by wood preservatives: Phytoextraction of arsenic, chromium, copper, dioxins and furans. International Journal of Phytoremediation, 22(14), 1505–1514.

Ye, Y., Li, P., Xu, T., Zeng, L., Cheng, D., Yang, M., Luo, J., & Lian, X. (2017). OsPT4 Contributes to Arsenate Uptake and Transport in Rice. Frontiers in Plant Science, 8, 2197.

Ye, W.-L., Wood, B. A., Stroud, J. L., Andralojc, P. J., Raab, A., McGrath, S. P., Feldmann, J., & Zhao, F.-J. (2010). Arsenic Speciation in Phloem and Xylem Exudates of Castor Bean. Plant Physiology, 154(3), 1505–1513.

Zelazny, E., Borst, J. W., Muylaert, M., Batoko, H., Hemminga, M. A., & Chaumont, F. (2007). FRET imaging in living maize cells reveals that plasma membrane aquaporins interact to regulate their subcellular localization. Proceedings of the National Academy of Sciences, 104(30), 12359–12364.

Zhan, A., Schneider, H., & Lynch, J. P. (2015). Reduced Lateral Root Branching Density Improves Drought Tolerance in Maize. Plant Physiology, 168(4), 1603–1615.

Zhang, C., Meng, S., Li, M., & Zhao, Z. (2016). Genomic Identification and Expression Analysis of the Phosphate Transporter Gene Family in Poplar. Frontiers in Plant Science, 7, 1398.

Zhang, H., Zhu, H., Pan, Y., Yu, Y., Luan, S., & Li, L. (2014). A DTX/MATE-Type Transporter Facilitates Abscisic Acid Efflux and Modulates ABA Sensitivity and Drought Tolerance in Arabidopsis. Molecular Plant, 7(10), 1522–1532.

Zhao, F.-J., Ago, Y., Mitani, N., Li, R.-Y., Su, Y.-H., Yamaji, N., McGrath, S. P., & Ma, J. F. (2010*a*). The role of the rice aquaporin Lsi1 in arsenite efflux from roots. The New Phytologist, 186(2), 392–399.

Zhao, F.-J., McGrath, S. P., & Meharg, A. A. (2010*b*). Arsenic as a Food Chain Contaminant: Mechanisms of Plant Uptake and Metabolism and Mitigation Strategies. Annual Review of Plant Biology, 61(1), 535–559.

Zhao, F.-J., Ma, J. F., Meharg, A. A., & McGrath, S. P. (2009). Arsenic uptake and metabolism in plants. The New Phytologist, 181(4), 777–794.

Zhou, G., Soufan, O., Ewald, J., Hancock, R. E. W., Basu, N., & Xia, J. (2019). NetworkAnalyst 3.0: A visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Research, 47(W1), W234–W241.

Zhou, R., Macaya-Sanz, D., Carlson, C. H., Schmutz, J., Jenkins, J. W., Kudrna, D., Sharma, A., Sandor, L., Shu, S., Barry, K., Tuskan, G. A., Ma, T., Liu, J., Olson, M., Smart, L. B., & DiFazio, S. P. (2020). A willow sex chromosome reveals convergent evolution of complex palindromic repeats. Genome Biology, 21(1), 38.

Appendix

Protocol 1 – plant tissue RNA extraction protocol

Equipment and reagents

- Sterile 1.5mL tubes
- Sterile micropipette tips (P10, P200 and P1000)
- P10, P200 and P1000 micropipettes
- Heating block for 1.5mL tubes
- Ceramic pestles and mortars
- Microspatula
- Refrigerated centrifuge
- Mini-centrifuge rotor
- Nanodrop spectrophotometer
- Vortex
- Lint-free wipes
- RNase Away® (or similar surface cleaner)
- Liquid nitrogen
- Ice
- LiCl 5M
- 24:1 chloroform:isoamylalcohol
- 2% CTAB (cetyltrimethylammonium bromide)
- β-mercaptoethanol
- 80% ethanol kept at -20˚C
- 3M sodium acetate (NaOAc)
- 100% ethanol
- Sterile nuclease-free water

Bench and material preparation

1. Autoclave 1.5 mL tubes and micropipette tips.
2. If necessary, prepare 2% CTAB solution (mix 20g CTAB powder, 20g PVP, 100mL Tris 1M pH 8.0, 50mL EDTA and 116.9g NaCl, and complete to 1000mL with sterile nuclease-free water).
3. If necessary, prepare 24:1 chloroform:isoamylalcohol solution and store in refrigerator (mix 96mL chloroform with 4mL isoamyl alcohol).
4. If necessary, prepare 5M LiCl solution (mix 21.197g LiCl in 100mL sterile H_2O).
5. Set water bath and heating block at 65˚C, and centrifuge at 4˚C.
6. Clean work area and equipment with RNase Away®.
7. Warm CTAB buffer at 65˚C in water bath (0.980mL per sample to have a suplus). For 24 samples batch, preheat 29.4mL of CTAB in 50mL conical tube.

8. *To be done right before the extraction step. * Mix β-mercaptoethanol with CTAB to a 2% concentration (20µL β-mercaptoethanol in 980µL CTAB per sample, or 0.6mL β-mercaptoethanol in 29.4mL CTAB for 24 samples).

Tissue grinding

1. Cool pestle and mortar with liquid nitrogen.
2. Add 50-100mg of plant sample to the mortar, cover with liquid nitrogen and grind until the nitrogen has almost fully evaporated.
3. Add a small amount of liquid nitrogen and repeat the grinding process until a fine powder is obtained.
4. Dip microspatula and 1.5mL tube in liquid nitrogen.
5. With the microspatula, scrape the sample powder and empty into the 1.5mL tube. It is important to act quickly and meticulously to avoid melting the powder while recovering all the sample. You should ideally have between 0.25mL and 0.5mL of powdered sample.
6. Put sample on ice.
7. Clean spatula with RNase Away® and repeat for every sample with a new pestle/mortar.

Extraction

1. In 1.5mL tubes containing ≤0.5 mL of ground tissue, add 650µL of CTAB/β-mercaptoethanol solution. Vortex samples quickly.
2. Incubate 1.5mL tubes in the heating block at 65˚C for 15min. Vortex samples every 3min.
3. Add 650µL chloroform:isoamylalcohol solution in each tube. Vortex quickly.
4. Centrifuge at 14 000rpm/15min/4˚C.
5. Recover supernatant with P200 micropipette and transfer to new 1.5mL tube for each sample.
6. Repeat steps 3 to 5.
7. Tare the balance with an empty tube, then weigh each sample. For each sample, add the equivalent mass in 5M LiCl solution.
8. Incubate 60min at -80 ˚C or overnight at -20˚C to precipitate RNA.
9. Centrifuge at 14 000rpm/15min/4˚C.
10. Empty the supernatant into a waste container and add 800µL of 80% ethanol (-20˚C) to the pellet to solubilize unwanted residue.
11. Centrifuge at 14 000 rpm/15min/4˚C.
12. Empty the supernatant into the waste container, quick-spin the tubes with the mini-centrifuge rotor and remove any remaining ethanol with P10 micropipette.
13. Open tubes in support and let ethanol evaporate completely.
14. Resuspend RNA with 20-40µL sterile nuclease-free water (depending on expected concentration) for 1min or until pellet is fully dissolved (up-and-down movements with the micropipette).

RNA quantification

1. Start nanodrop spectrophotometer and select settings for RNA quantification.
2. Clean aperture with sterile nuclease-free water and dry with a lint-free wipe.
3. Pipette 2µL sterile nuclease-free water on aperture and set reference blank. Dry aperture with lint-free wipe.
4. Repeat steps 2 and 3 with RNA samples.
5. Note A260/A280, A260/A230 ratios and RNA concentration.
6. Clean aperture with sterile nuclease-free water and close nanodrop.

Repurification (for low quality samples)

1. Add 1/10 volume NaOAc 3M and 2 volumes EtOH 100% to sample, quickly vortex.
2. Incubate 60min at -80 ˚C or overnight at -20˚C.
3. Centrifuge at 14 000 rpm/15min/4˚C.
4. Empty the supernatant into the waste container, quick-spin the tubes with the mini-centrifuge rotor and remove any remaining ethanol with a P10 micropipette.
5. Open tubes in support and let ethanol evaporate completely.
6. Resuspend RNA with 20-40µL sterile nuclease-free water (depending on expected concentration) for 1min or until pellet is fully dissolved (up-and-down movements with the micropipette).

Protocol 2 – mRNA enrichment protocol

Equipment and reagents

- Sterile 1.5mL tubes
- Sterile micropipette tips (P20 and P200)
- P20 and P200 micropipettes
- 2 water baths
- Magnetic stand
- Oligo(dT) magnetic beads
- Ice
- 7.5M LiCl
- 1M Tris-HCl pH 7.5
- 0.5M EDTA pH 8.0
- Sterile nuclease-free water

Bench and material preparation

1. Autoclave 1.5 mL tubes and micropipette tips.
2. Preheat 2 water baths: one at 65°C and the other at 80°C.
3. Clean work area and equipment with RNase Away®.
4. Prepare RNA binding buffer (20mM Tris-HCl pH 7.5, 1.0M LiCl and 2mM EDTA). For 50mL, mix 6667µL of 7.5M LiCl, 1000µL Tris-HCL pH 7.5 and 200µL of 0.5M EDTA pH 8.0 in ~25mL sterile nuclease-free water, then adjust volume to 50mL and autoclave.
5. Prepare RNA washing buffer (10mM Tris-HCl PH 7.5, 0.15M LiCl, 1mM EDTA). For 50mL, mix 1000µL of 7.5M LiCl, 500µL Tris-HCL pH 7.5 and 100µL of 0.5M EDTA pH 8.0 in ~25mL sterile nuclease-free water, then adjust volume to 50mL and autoclave.
6. Prepare 10mM Tris-HCL.

Enrichment

1. Mix the bead solution with vortex.
2. Aliquot 10 µL of oligo(dT) beads into 1.5 mL tubes.
3. In magnetic rack, wash the beads twice with 100µL of binding buffer, and remove the supernatant.
4. Resuspend the beads in 50 µl of binding buffer (pipette up and down 3X to mix).
5. In a separate 1.5mL tube, bring RNA sample to 50 µL with sterile nuclease-free water.
6. Heat tubes to 65°C in a water bath during 2 min, cool on ice 5 min, then add to the washed beads.
7. Incubate at room temperature for 5 min, mixing at 5, 2.5 and 0 min.
8. Collect beads using the magnetic stand, discard supernatant.
9. Wash the beads twice with 100 µL of washing buffer and remove the supernatant.

10. Add 15 µL of 10mM Tris-HCl and heat the beads at 80°C for exactly 3 minutes in a water bath to elute mRNA. Add each tube at 30s intervals.
11. Remove in the right order each tube from the water bath and save the supernatant in a fresh 0.5 mL tube, using the magnetic stand to separate the supernatant from the beads (keep the beads in a 2mL tube as they are reusable!). This step should not take longer than 30s per tube, so pre-set micropipette to 15uL to save time.
12. Freeze at -20°C (short term) or -80°C (long term).

Protocol 3 – DNA extraction protocol

Equipment and reagents

- Sterile 1.5mL tubes
- Sterile micropipette tips (P10, P200 and P1000)
- P10, P200 and P1000 micropipettes
- Heating block for 1.5mL tubes
- Ceramic pestles and mortars
- Microspatula
- Refrigerated centrifuge
- Mini-centrifuge rotor
- Nanodrop spectrophotometer
- Vortex
- Lint-free wipes
- 70% ethanol
- Liquid nitrogen
- Ice
- 25:24:1 phenol:chloroform:isoamylalcohol
- 3M sodium acetate (NaOAc)
- Molecular grade isopropanol
- β-mercaptoethanol
- 2% CTAB (cetyltrimethylammonium bromide)
- 80% ethanol kept at -20˚C
- Sterile nuclease-free water

Bench and material preparation

1. Autoclave 1.5 mL tubes and micropipette tips.
2. If necessary, prepare 2% CTAB solution (mix 20g CTAB powder (2%), 20g PVP (2%), 100mL Tris 1M pH 8.0, 50mL EDTA and 116.9g NaCl, and complete to 1000mL with sterile nuclease-free water).
3. Set water bath and heating block at 65˚C.
4. Clean work area and equipment with 70% ethanol.
5. Warm CTAB buffer at 65˚C in water bath (0.980mL per sample to have a suplus). For 24 samples batch, preheat 29.4mL of CTAB in 50mL conical tube.
6. *To be done right before the extraction step. * Mix β-mercaptoethanol with CTAB to a 2% concentration (20µL β-mercaptoethanol in 980µL CTAB per sample, or 0.6mL β-mercaptoethanol in 29.4mL CTAB for 24 samples).

Tissue grinding

1. Cool pestle and mortar with liquid nitrogen.
2. Add 50-100mg of plant sample to the mortar, cover with liquid nitrogen and grind until the nitrogen has almost fully evaporated.
3. Add a small amount of liquid nitrogen and repeat the grinding process until a fine powder is obtained.
4. Dip microspatula and 1.5mL tube in liquid nitrogen.
5. With the microspatula, scrape the sample powder and empty into the 1.5mL tube. It is important to act quickly and meticulously to avoid melting the powder while recovering all the sample. You should ideally have between 0.25mL and 0.5mL of powdered sample.
6. Put sample on ice.
7. Clean spatula with 70% ethanol and repeat for every sample with a new pestle/mortar.

Extraction

1. In 1.5mL tubes containing ≤0.5 mL of ground tissue, add 650µL of CTAB/β-mercaptoethanol solution. Vortex samples quickly.
2. Incubate 1.5mL tubes in the heating block at 65˚C for 15min. Vortex samples every 3min.
3. Add 650µL phenol:chloroform:isoamylalcohol solution in each tube. Vortex quickly.
4. Centrifuge at 10 000rpm/10min/22˚C.
5. Recover supernatant with P200 micropipette and transfer to new 1.5mL tube for each sample.
6. Repeat steps 3 to 5 at least once, until a clear aqueous phase without residue is obtained.
7. For each sample, add 1/10 volume of NaOAc and 1 volume of isopropanol. Vortex quickly.
8. Incubate 60min at -80 ˚C or overnight at -20˚C to precipitate DNA.
9. Centrifuge at 14 000rpm/15min/4˚C.
10. Empty the supernatant into a waste container and add 800µL of 80% ethanol (-20˚C) to the pellet to solubilize unwanted residue.
11. Centrifuge at 14 000 rpm/15min/4˚C.
12. Empty the supernatant into the waste container, quick-spin the tubes with the mini-centrifuge rotor and remove any remaining ethanol with a P10 micropipette.
13. Open tubes in support and let ethanol evaporate completely.
14. Resuspend DNA with 100-200µL sterile nuclease-free water (depending on expected concentration) for 1min or until pellet is fully dissolved (up-and-down movements with the micropipette).

DNA quantification

1. Start nanodrop spectrophotometer and select settings for double-stranded DNA (dsDNA) quantification.
2. Clean aperture with sterile nuclease-free water and dry with a lint-free wipe.
3. Pipette 2µL sterile nuclease-free water on aperture and set reference blank. Dry aperture with lint-free wipe.
4. Repeat steps 2 and 3 with RNA samples.
5. Note A260/A280, A260/A230 ratios and DNA concentration.
6. Clean aperture with sterile nuclease-free water and close nanodrop.

Repurification (for low quality samples)

1. Add 1/10 volume NaOAc 3M and 2 volumes EtOH 100% to sample, quickly vortex.
2. Incubate 60min at -80 ˚C or overnight at -20˚C.
3. Centrifuge at 14 000 rpm/15min/4˚C.
4. Empty the supernatant into the waste container, quick-spin the tubes with the mini-centrifuge rotor and remove any remaining ethanol with a P10 micropipette.
5. Open tubes in support and let ethanol evaporate completely.
6. Resuspend DNA with 50µL sterile nuclease-free water (depending on expected concentration) for 1min or until pellet is fully dissolved (up-and-down movements with the micropipette).

Protocol 4 – heterologous expression in yeast walkthrough

This "protocol" only gives the major steps necessary to conduct a successful heterologous expression experiment and serves as a rough map/outline for this type of experiment. For each step, different protocols can be used depending on the available equipment and are easily found in the literature. However, the description of the whole process involved in heterologous expression is harder to find or understand, in particular for people getting started in molecular biology research and with little to no background experience. Even though this walkthrough should simplify the process, each step still requires some researching/studying to be conducted successfully and ideally should be done with the assistance or explanations from an experienced molecular biologist.

1. Primer design for specific genes expressed as mRNA in the studied organism. These primers must allow the expression of the whole gene and contain restriction sites that allow its ligation in a plasmid (expression vector) for the transformation steps. While designing the primers, it is important to make sure the restriction sites are not found in the gene coding sequence, and that the restriction reaction will keep the gene in the right reading frame once inserted in the plasmid.

2. Growth of bacteria (competent cells) and yeast (expression system) from stock to make sure they are alive, on LB and YPD media respectively.

3. Glycerol stock preparation from live cultures and store at -80˚C for later use (theses stocks are viable for months to years later).

4. RNA extraction from studied organism.

5. Reverse transcription of RNA to produce cDNA.

6. PCR of selected genes, using cDNA as matrix and primers designed for specific genes of interest.

7. Electrophoresis gel migration to assess the amplification of the genes.

8. Gel extraction and purification of amplicons.

9. Repeat steps 6 to 8 to make sure the right genes have been amplified and extracted.

10. Amplicons restriction with the appropriate restriction enzymes.

11. Ligation of amplicons in the expression plasmid. Each ligation reaction is done in a separate tube to obtain different plasmid for each gene of interest.

12. Linearization of the plasmid with a different restriction enzyme, avoiding to cut the gene.

13. Growth of bacteria on non-selective LB medium.

14. Preparation of competent cells (chemical treatment or electroporation of bacterial cultures).

15. Transformation of competent cells with the plasmid.

16. Selection of bacteria transformants with resistance to antibiotic on selective medium. This can be done thanks to the presence of a resistance gene for a specific antibiotic in the plasmid. By growing the cells on a selective medium containing the antibiotic, only the cells which integrated the plasmid (the transformants) will survive and grow into colonies.

17. Colony PCR using primers specific for the amplification of the whole plasmid and electrophoresis gel to check integration and length of plasmid (indicator of the successful integration of the gene of interest).

18. Growth of the integrant colonies on liquid selective medium to produce a large quantity of plasmids.

19. Miniprep extraction of the plasmid from liquid culture.

20. Linearization of the plasmid with the appropriate restriction enzyme.

21. Growth of yeast on non-selective YPD medium.

22. Transformation of yeasts.

23. Selection of yeast transformants with resistance to antibiotic on selective medium.

24. Colony PCR using primers specific for the amplification of the whole plasmid and electrophoresis gel to check integration and length of plasmid (indicator of the successful integration of the gene of interest).

25. Growth of yeasts on characterization media to assess the cellular role of the newly expressed gene.