Effects of selenium uptake on plant proteins for phytoremediation

Divya Vinod

University of Technology Sydney

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Statement of authorship

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Divya Vinod, declare that this thesis is submitted in fulfillment of the requirements for the award of Ph.D. in the School of life sciences in the science faculty at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise referenced or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This document has not been submitted for qualifications at any other academic institution.

This research is supported by the Australian Government Research Training Program.

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Abstract

Selenium (Se) is a naturally occurring element first identified as a contaminant in the 1970s in agricultural drain water acting as a breeding ground for wetland wildlife. In addition to coal ash slurry waste, Se released from fossil fuel combustion, garbage, paper, and tire incineration, forest fires, soil erosion, volcanism, agriculture, coal combustion, insecticide production, oil refining, photovoltaic, and glass manufacturing among others, and can easily evolve into a significant environmental concern, despite also being essential for animal and plant tissue function. Se bioavailability, uptake, distribution, metabolism, and volatilization within living organisms are influenced by the type of vegetation, the chemical form of Se in soil, pH, moisture content, and soil composition.

Australia is well known for its rich coal exports, but coal is also consumed domestically for electricity generation by power plants around the country. One of the by-products of coal use for electricity production is a coal ash slurry. Coal ash slurry is dumped in unlined ponds near power plants and leaches into the surrounding soils and eventually into waterways. While there are several methods of seepage prevention, power plant companies are not obliged by government regulations to line historical coal ash slurry dump sites. They are also not required to remediate the land before or after power plant closure leaving behind a legacy of coal ash slurry waste contamination in soil. The resulting impact of the coal ash slurry seepage is an elevated level of carcinogenic and toxic compounds in the ground, including cadmium, arsenic, mercury, and selenium (Se). There have been numerous studies to show how plants absorb these contaminants from soil. Still, there are no studies to date demonstrating the phytoremediation of Se from the earth using plants as a potential solution in Australia.

The plants selected for this study were *Brassica* 'vitamin green', *Helianthus annuus*, *Neptunia amplexicaulis,* and *Solanum tuberosum* species used for phytoremediation on contaminated sites through tissue engineering. The specific aims of the current research project were:

1. To determine sublethal selenite and selenate concentrations in the selected plants
2. To measure the effects of sublethal selenite and selenate concentrations on reactive oxygen species enzyme activity under Se stress
3. To establish and compare patterns of Se uptake on a whole plant and proteomic scale
4. To study the effects of Se stress on plant protein regulation

The experiments described in this thesis attempt to quantify the uptake patterns in the leaves, stems, and roots of three plants, *Brassica species, Helianthus annuus and Neptunia amplexicaulis*. These plants are known for varying levels of selenium absorption. Understanding the location of Se accumulation in the plants will determine their suitability *in situ* phytoremediation, where the efficacy of currently used species is constrained by slow growth rates and low biomass production. Se is also an essential micronutrient for human function so an added benefit of using edible crops for phytoremediation is that if plants are storing Se in the edible parts of the plant such as the leaves or seeds, there is the potential for adding these plants to livestock feedstock. The addition of the Se enriched plants to livestock feedback may then be used to supplement to the human diet.

Sublethal Se concentrations were determined by subjecting the test plants to increasing concentrations of two selenium salts over 21 days, with sublethal concentrations determined by measurements of the shoot and root length, fresh and dry weights, and the number of leaves present as the selenium concentrations increased. Sublethal concentrations ranged between 15 µM for *Solanum tuberosum* to 500 µM for *Neptunia amplexicaulis*.

The reactive oxygen species were measured in the leaves, stems and roots based on the above exposure concentrations. The results indicated that exposure to elevated levels of selenite and selenate in plants results in higher reactive oxygen species (ROS) enzyme activity than seen in the controls, confirming that plants exposed to selenite and selenate were experiencing oxidative stress.

A radioactive isotope of selenium (Se^{75}) was used to quantify the Se concentration in each plant and translocation of Se in each plant part. The study indicated higher uptake in the shoots than the roots for selenite and the opposite for selenate uptake in the *Brassica* species and *Helianthus* annuus. At the same time, *Neptunia amplexicaulis* showed no significant differences in Se translocation between the roots and shoots.

Given that the results from the autoradiographic study were more reliable in *Helianthus annuus* and *Neptunia amplexicualis,* these two plants were the focus for subsequent proteomic analysis to identify whether selenoproteins could be detected in selenium-exposed plants. If selenoproteins were not detected then what effect selenite and selenate exposure have on protein expression in the selected plants. The final component of the research project indicated that proteins associated with reactive oxygen species were elevated in the selenium-treated plants compared to controls; however, proteomic methods did not conclusively identify specific selenoproteins.

This research identified the need to further optimize the use of radioisotopes for plant autoradiography and proteomic techniques for plant studies. It also highlighted the potential of using *Neptunia amplexicualis* as a tool for the phytoremediation of Se contaminated soils. However, further research is needed.

Chapter 1. General introduction

1.1 Australian coal production

Australia is amongst the world's largest producers and exporters of coal (Saunders, 2015). Since the 1990s, Australia's total domestic coal production has doubled, and exports have steadily grown (Figure 1). However, global market demand has been changing in recent years, influencing the longer-term view for coal exports where demand is expected to grow unobtrusively. This is dependent on the transition toward renewable energy sources, changes in steel production technology, and global economic growth (Cunningham et al., 2019)

1.2 Impacts of coal production

Coal production has been linked to air pollution and climate change through greenhouse gas generation; however, another waste product from coal-fired power stations and mines is coal ash (Agency, 2016). Coal ash is a toxic ash, post-combustion by-product from electricity production using coal (Jones et al., 2012). Coal naturally contains trace amounts of toxic chemicals which become concentrated when the coal is burned, forming coal ash (Bednar et al., 2013). The concentrated toxic chemicals in coal ash are known carcinogens, neurotoxins, and poisons which include but are not limited to selenium (Se), cadmium, lead, radium, mercury, and arsenic (Bednar et al., 2013). The coal ash is blended with saline wastewater directed into dump sites creating a slurry of toxic chemicals that leak into aquifers and soil and eventually into rivers and lakes where humans and animals can be exposed to it (EPA, 2015). The toxins from coal ash have been linked to asthma, heart disease, cancers, respiratory conditions, and stroke (Block and Calderón-Garcidueñas, 2009).

Increasingly the impact of coal ash on air pollution has become known. Still, minimal research has been done in Australia to demonstrate the impact of coal ash chemicals in water and soil (Jones et al., 2012). Communities near power plants are particularly susceptible to the dust from dried uncontained coal ash and exposure to water bodies contaminated with leaked coal ash slurry (Gao et al., 2013, Kish et al., 2013, Orazzo et al., 2009). Regardless of this, regulators permit coal ash ponds to be built and operate in a manner that does not intercept surface and groundwater contamination, allowing leaks and community exposure to the contaminating chemicals in coal ash. Even with best practices, coal ash cannot be disposed of safely. There is still a contamination risk to people and the environment, highlighting the need for coal ash dump sites to be strictly monitored, managed, and rehabilitated to minimize the risk posed to human and environmental health (B and Dikshit, 2012, Bednar et al., 2013Bhatt et al., 2019).

1.3 Regulation of Coal ash waste management

Currently, regulation of coal ash dumping in Australia is very inadequate with a lack of reporting information. Additionally, there is a lack of requirements for clean maintenance of toxic coal ash dumps and a lack of best-practice rehabilitation. There are no clear closure plans for current coal dump sites, and nor is there a plan for future coal ash dump contamination risk mitigation (Kok et al., 2019, Suthar and Aggarwal, 2016, Whiteside and Herndon, 2019). Wet disposal is the most economical method of coal ash disposal despite the contamination risk, with very few power plants utilizing the coal ash dry and cover method (B and Dikshit, 2012). While preventing coal ash slurry seepage is the best form of management, it does not adequately mitigate environmental contamination.

A report by environmental injustice Australia demonstrates that coal ash dumps are already contaminating water, polluting aquatic ecosystems, and dispersing toxic coal ash over communities who live near them (Bhatt et al., 2019). The report identified that cleaning up existing coal ash dump sites is vital to minimizing water contamination, air pollution, and planning for future land use (Bhatt et al., 2019).

1.4 An introduction to Se

Selenium (Se) was first discovered by a Swedish chemist Jons Jakobin Berzelius in 1817. It was named after the Greek moon goddess ‘Selene’. Se belongs to the periodic table Group VIA, which is the same group as Sulfur (S). The first encounter with Se, thought to be by Marco Polo in 1295, travelled through Asia Minor from Venice to China. During this journey, his horses endured necrotic hoof disease. Initially, it was thought to be due to consuming what was thought to be a poisonous plant. Instead, it was confirmed to be due to Se toxicity due to excessive consumption of Se- accumulator plants (Birringer et al., 2002).

Se was first used commercially in a television system in 1884 and using Se photocell technology for imaging (Chasteen and Bentley, 2003). The usage of Se subsequently became popular in areas such as xerography, the electrical, electronic, and semiconductor industries, agriculture, paint, pigment production, vulcanization, oil refining, glass manufacturing, electricity generation using coal, metallurgy, and now increasingly in medicine (Lemly, 2004).

Se is an essential element for human and animal function (White and Brown, 2010). The role of Se in plant function remains controversial; Se stimulates growth and increases tolerance to environmental factors and provides resistance to pathogens. However, it can also lead to oxidative stress in plants in excessive concentrations (El Mehdawi and Pilon-Smits, 2012, Feng et al., 2013, Pilon-Smits et al., 2009b, Quinn et al., 2007b, White and Brown, 2010).

1.5 Naturally occurring sources of Se

The dispersal of concentrated forms of Se in natural soils is determined primarily by geology (Dhillon and Dhillon, 2003, Fordyce, 2012). Certain geological features enhance the Se concentrations in natural soils where the Se range can fall between 0·01–2·0 mg Se kg^{-1}, up to 1200 mg Se kg^{-1}(Barker and Pilbeam, 2016, Dhillon and Dhillon, 2003, Fordyce et al., 2000).

These selenium concentrations in natural soils can support uniquely adapted life but can be detrimentally toxic to fauna and flora (Brown and Shrift, 1982, Rosenfeld and Beath, 1964).

Seleniferous soils are defined as soils with naturally occurring high levels of Se. These soils to date have been identified in the Great Plains of the USA, Canada, South America, Australia, India, China, and Russia (Bujdoš et al., 2005, Dhillon and Dhillon, 2003, Foyer and Halliwell, 1976).

Se is an unevenly distributed naturally occurring element throughout the earth's crust (Lakin, 1972). While Se does not occur as an ore, it is in various rocks, minerals, lunar and volcanic material, fossil fuels, plant materials, and waters as a significant component of 40 minerals. Sedimentary rocks such as shales, sandstones, limestones, and phosphorate rocks contain concentrated sources of Se. Selenite minerals are also located in areas of high concentrations of sulphur, given their similar chemistry (Elkin, 1968.). Chalcopyrite, bornite, and pyrite are also Se-rich minerals (Cooper et al., 1970).

The sources of Se contamination vary depending on geological distribution, natural weathering occurrences, and anthropogenic activities (Anderson et al., 1961). As described above, whilst areas of the earth's surface have concentrated deposits of Se, it remains a rare element with an abundance of about 0.09 ppm (Lakin, 1972).

Anderson et al. (1961) estimated that 58% of sedimentary shales are naturally Se rich, some of which have led to the formation of Se-toxic soils in Ireland, Australia, and several other countries (Nicholas, 2012) Se rich rocks which have weathered form seleniferous soil (Moxon et al., 1950, Ohlendorf, 1989)

Limestone is estimated to have low levels of Se, and it has been challenging to estimate the Se content in sandstone, with values ranging from 0–112 ppm {Lakin, 1967). Phosphate fields in the U.S. over parts of Wyoming, Utah, Nevada, Idaho, and Montana contained 1.4 to 178 ppm Se (Robbins and Carter, 1970)

Atmospheric Se is partially caused by volcanic eruptions and in seleniferous sulfur and sulfur-containing inorganic salts. However, not all volcanic sulfur contains high concentrations of Se. Hawaiian and Japanese volcanic sulfur ranges from 67 ppm to 206 ppm and 1026 to 2000 ppm, respectively (Lakin and Davidson, 1967).

High Se concentrations in sedimentary deposits are either due to Se precipitation from the rain from volcanic eruptions, deposition of erosion products from volcanic sulfur seleniferous tuffs and sulfide deposits, or precipitation of Se from streams or groundwater carrying unusual quantities of Se from older seleniferous sediments (Ellis et al., 2004).

1.6 Se as a contaminant

Se concentrations can have detrimental impacts on the environment when they exceed optimal levels. Elemental Se liberates from rock and soil mixtures and combines with water and air, converts to one of two bioavailable high-water soluble forms of Se, selenate, and selenite ions. The high-water solubility of both selenite and selenate allows the ions to be easily absorbed by living organisms, including plants increasing the chances of Se toxicity within these organisms.

Se concentrations in plants are directly related to Se phyto availability in the soil. Se phyto availability is demonstrated by four observations where Se concentrations were higher in:

(1) Plants thriving in soils with greater Se phyto availability. (Brown and Shrift, 1982, Rosenfeld and Beath, 1964, Rosenfeld and Beath, 2013, Thomassen et al., 1994)
(2) Plants growing in contaminated soil with Se that was anthropogenically introduced (Wu, 2004)
(3) Crops cultivated in soils having greater Se phyto availability (Broadley et al., 2006a, Joy et al., 2015, Lee et al., 2011, Williams et al., 2009)
(4) Crops on which Se fertilizers (soil or foliar) have been applied (Alfthan et al., 2015, Broadley et al., 2006a, Chilimba, et al., 2012, White and Broadley, 2009)

1.7 Impact of Se soil contamination on humans

As mentioned above, Se is essential for healthy human bodily functions. The dietary intake of Se in the human population reflects the naturally occurring Se in the soil in which food crops are cultivated (Yang et al., 1983). In many countries, Se occurs naturally in soil. For example, in the Northwest of China along the geographic belt from Heilongjiang Province to Yunnan Province in the south-west, there are Se deficient soils (Tan et al., 1989). In the Enshi District, Hubei Province and Ziyang County, Shanxi Province in China, high Se levels lead to Se toxicity among the human population (Yang et al., 1983).

1.8 Phytoremediation as a solution for Se soil contamination

While studies remain inconclusive on the importance of Se to flowering plants (angiosperms), it has been recognized as a growth stimulant, can facilitate tolerance to environmental factors during oxidative stress, which allows plants to develop resistance to pathogens and herbivory (El Mehdawi and Pilon-Smits, 2012, Feng et al., 2013, Pilon-Smits et al., 2009b, Quinn et al., 2007b, White and Broadley, 2009). The angiosperms have been categorized into three main groups regarding their ability to accumulate Se into their tissues: non-accumulator, Se-indicator, and Se-accumulator species. Se concentrations of 10–100 µg Se g^{-1} dry matter (DM) cannot be tolerated by non- accumulator species (Beath et al., 1940, Rodriguez et al., 2005). Se-indicators can tolerate tissue Se concentrations of up to 1 mg Se g^{-1} DM and thrive in non-seleniferous and seleniferous soils (Beath et al., 1940, Rodriguez et al., 2005). Baker (1981) found that the Se phyto-availability in the soil was proportionate to the Se concentration in Se-indicator plants, allowing them to act as an "indicator" of soil Se concentrations. Se-accumulator species generally only survive and thrive on seleniferous soils, and their leaf Se concentrations can exceed 1 mg Se g^{-1} dry matter (DM) (Beath et al., 1940, Brown and Shrift, 1982). Of the 185 angiosperms that have been tested, most are non-accumulators (White et al., 2007, White et al., 2004b). A few plants that have been identified as Se indicator and Se accumulators have been outlined in Table 1.

Table 1. Angiosperm species studied for Se-(hyper) accumulation have demonstrated shoot Se concentrations >1000 mg Se kg–1 dry matter. Modified from (White, 2015)

Species	**Location**	**Se concentration (mg Se kg–1 DM)**	**Reference**
Stanleya pinnata	Pine Ridge, Fort Collins, CO, USA	>4000	Galeas *et al.* (2007)
Astragalus canadensis	Las Vegas, NE, USA	1110	(Byers, 1936)
Acacia can	NW Queensland, Australia	1121	(McCray and Hurwood, 1963)
Coelospermum decipiens	Cape York Peninsula, Queensland, Australia	1141	(Knott and McCray, 1959)
Gutierrezia microcephala	Thompson, UT, USA	1287	(Beath, 1943)
Astragalus bisulcatus	Pine Ridge, Fort Collins, CO, USA	13 685	(Sura-de Jong et al., 2015)

Astragalus flavus var. *candicans*	Thompson, UT, USA	1322	(Beath, 1943)
Astragalus flavus	Aztec, NM, USA	1361	(Beath et al., 1941)
Symphyotrichum ericoides	Pine Ridge, Fort Collins, CO, USA	1378	(El Mehdawi et al., 2015)
Astragalus racemosus	WY, USA	14 920	Knight and Beath (1937)
Dieteria canescens	Midwest USA	1600	Beath *et al.* (1939*a*)
Astragalus eastwoodiae	Utah, USA	1664	Beath (1943)
Atriplex confertifolia	Thompson, UT, USA	1734	Beath (1943)
Xylorhiza glabriuscula	Huerfano County, CO, USA	1750	(Byers, 1938))
Symphyotrichum lateriflorum	SD, USA	1800	(Moxton et al., 1939)
Cardamine hupingshanensis	Yutangba, Enshi, China	1965	(Yuan et al., 2013)
Astragalus crotalariae	Truckhaven, CA, USA	2175	(Beath et al., 1941)
Astragalus sabulosus	Thompson, UT, USA	2210	(Beath et al., 1941)
Astragalus bisulcatus var. *haydeni anus*	Cuba, NM, USA	2377	(Beath et al., 1941)
Stanleya bipinnata	Laramie, WY, USA	2490	(Beath et al., 1941)
Astragalus osterhoutii	Krem mLing, CO, USA	2678	(Beath et al., 1940)Beath *et al.* (1940)
Astragalus beathii	Cameron, AZ, USA	3135	(Beath et al., 1940)Beath *et al.* (1940)
Castilleja angustifolia var. *dubia*	Lysite, WY, USA	3460	(Beath et al., 1941)
Xylorhiza venusta	Midwest USA	3486	(Rosenfeld and Beath, 1964)
Oonopsis foliosa	Lascar, CO, USA	3630	Beath *et al.* (1939*b*)
Astragalus preussii	Thompson, UT, USA	4188	Beath (1943)

Neptunia amplexicaulis	Richmond, Queensland, Australia	4334	(Knott and McCray, 1959)Knott and McCray (1959)
Astragalus grayi	Carbon County, WY, USA	4450	(Byers, 1936)
Symphyotrichum ascendens	Soda Springs, ID, USA	4455	(Pfister and Zeeman, 2016)
Astragalus praelongus	Leupp, AZ, USA	4835	(Beath et al., 1941)
Astragalus pectinatus	Teton County, MT, USA	5170	(Byers, 1938, Draize and Beath, 1935)
Astragalus albulus	La Ventana, NM, USA	530	(Draize and Beath, 1935)
Xylorhiza parryi	Albany County, WY, USA	5390	(Draize and Beath, 1935)
Astragalus flavus var. *argillosus*	Greenriver, UT, USA	631	(Beath et al., 1941)
Astragalus praelongus var. ellisiae	Valmont, NM, USA.	656	(Beath et al., 1941)
Astragalus rafaelensis	Jensen, TX, USAA	716	(Beath et al., 1941)
Astragalus pattersonii	Thompson, UT, USA	8512	(Beath, 1943)
Oonopsis wardii	Albany County, WY, USA	9120	Byers (1935)
Grindelia squarrosa	Lower Brule Reservation, SD, USA	930	Lakin and Byers (1941)
Atriplex nuttallii	WY, USA	930	Beath *et al.* (1937)
Astragalus beckwithii var. *purpureus*	Clark County, NE, USA	970	Lakin and Byers (1941)
Stanleya pinnata var. *integrifolia*	Vernal, UT, USA	977	Beath *et al.* (1941)
Astragalus toanus	ID, USA	990	Lakin and Byers (1948)

1.9 Research gaps

Coal mining is just one of the several sources of potentially toxic elements (PTE's) which can alter the natural environment and disrupt the processes of the environments and the organisms in the soil (Ghori et al., 2016). Traditional land reclamation includes surface stabilization to prevent water erosion, prevent toxic pollutant runoff into the surrounding environment, and restore the natural landscape (Xie and van Zyl, 2020) . In situ physical chemicals: soil isolation and containment, solidification and stabilization, vitrification, soil flushing, and electrokinetic reclamation techniques are used on abandoned mine sites (AMS) (Dada et al., 2015). However, they are expensive, time-consuming, and complex to implement on a large scale, and the public perception of these techniques has been poor(Mahar et al., 2016).

Increasingly in the last 20 years, plants have been used to extract, stabilize, volatilize, and remediate the soil. Wood ash, manure, sawdust, compost, sewage sludge, biosolids, wood chips, and inorganic methods, including phosphate-derived products, liming materials, alkaline sludge, and sugar foam, are also used for soil remediation and are cheap to implement (Rodríguez et al., 2016). The disadvantage of these methods is that they do not remove all PTE's from the soil(Grobelak, 2016). In addition to the remediation of the soil, recreating the biodiversity in fauna and flora is also essential. Therefore, native flora that can thrive in the ecosystem must be planted during or soon after phytoremediation(Venkateswarlu et al., 2016).

The most critical stage in developing a phytoremediation plan is selecting the hyperaccumulator plant species(Suman et al., 2018). Hyperaccumulators are plants that store at least 1 g kg−1 in their aerial plants without plant death(Suman et al., 2018). Research to date has focused on the phytoextraction of As, Co, Cr, Cu, Ni, Pb, Sb, Se, and Tl(Tchounwou et al., 2012). Aside from hyperaccumulating PTE's in aerial parts of the plant, plants for phytoremediation also need to produce high biomass, rapid growth rates, easy crop management, genetic stability, and cannot be a food source for animals(Ghori et al., 2016).

While an increasing number of plants can be used for phytoremediation, there are still complexities as the ability of the plants to absorb PTE's is impacted by climate and soil pH(Xie and van Zyl, 2020). Despite these challenges, there are real-world examples of where phytoremediation has been used to remediate contaminated soils. In Spain, following a toxic mining site accident in 1998, *Lupinus albus* plants demonstrated an increase in acid soil pH, a decrease in soluble As and Cd and high concentrations of As and Cd levels in roots(Madejón et al., 2018). These finds, however, were made after several failed trials using *Helianthus* *annuus* and *Brassica juncea* (Madejón et al., 2003). Another study carried out in Castelo Branco in Portugal where soil contaminated with As, Sb and W was remediated with *Pinus pinaster* for As and W, *Cistus ladanifer* and *Cistus vulgaris* for Sb and W, *Digitalis purpurea* and *Erica umbellata* for Sb, and *Quercus ilex, Chamaespartium tridentatum*, and *Eucalyptus globulus* for W(Pratas et al., 2005).

The PTE's studied so far are hugely toxic to humans. However, Se unlike As, Sb and W is essential for human function discussed below. It is proposed that by understanding how plants absorb and metabolise Se, it can be determined how suitable it is, for Se to be given to livestock to continue to be metabolized by humans.

The detection of effective Se accumulator species is an area with vast benefits to animal and human health. Very little research has been done to address the physiological impacts of selenite and selenate on the selected plants. There is an opportunity to address the gaps in knowledge in this area. To date, there is ample research to demonstrate the importance of Se to healthy human function.

There have also been studies to demonstrate the importance of Se in microorganisms such as *Escherichia coli* (Deng et al., 2018, Eswayah et al., 2016); after this discovery, *Salmonella*, *Clostridium*, *Methanococcus, and* Methanococcus, *Desulfovibrio desulfuricans* were identified containing selenoproteins (Heider and Böck, 1993, Johansson et al., 2005, Oremland et al., 1989).

Studies have also shown, albeit to a comparatively lesser extent on studies the importance of Se in humans , that Se in low concentrations offers protection for plants to exposure to abiotic stresses including cold stress (Oremland et al., 1989), drought (Chu et al., 2010), desiccation (Kumar et al., 2012) and metal stress (Pukacka et al., 2011). Still, Se has rarely been studied as a soil contaminant using-sublethal concentrations to date.

1.10 Research aims

As discussed above, there is ample research into the naturally occurring Se in soil, and its importance to human health has been established. Still, very little research has been performed to study the physiological effects of Se on plants at sublethal concentrations as a contaminant.

The secondary goal of this research is to explore the early stages of the potential use of these plants to supplement human dietary Se requirements.

The current research aimed to address these gaps in knowledge by:

1. Identifying sublethal concentrations of selenite and selenate exposure in Se accumulator and non-accumulator plant
2. Identifying patterns of ROS related enzyme activity in a Se accumulator and non-accumulator plant
3. Quantifying Se in Se accumulator and non-accumulator plants through creating an autoradiographic image
4. Identifying relationships between seed storage proteins and selenium uptake as a precursor to proteomic studies
5. Identify up and downregulating proteins on exposure to sublethal concentration of Se

The overall objective of this study is to use the theoretical knowledge gained to inform plant selection for use for field trials on Se contaminated soils.

Chapter 2. Sublethal selenium concentration selection

Abstract

To date, limited research has been conducted to determine how the patterns of Se uptake and accumulation determine the suitability of plants for phytoremediation of mine sites. The plants, *Brassica rapa* ‘Vitamin Greens’, *Helianthus annuus* ‘Dwarf *Helianthus annuus*’ (secondary Se accumulators) and *Neptunia amplexicaulis* ‘. All plants were grown in tissue culture and harvested after 21 days of exposure to a range of increasing concentrations of selenite and selenate salts, with a range of widely accepted growth measurement variables, including fresh and dry weight, shoot and root length and water content used to assess plant health.

Each plant was able to withstand various concentrations of sodium selenite and sodium selenate salts. The results indicated that the sublethal concentration of selenite and selenate for *Brassica rapa* was 20µM, *Solanum tuberosum* was 10 µM, *Helianthus annuus* 25µM, and *Neptunia amplexicaulis* 500µM. Sublethal exposure concentrations were used for further experimental studies in enzymatic, autoradiography, and proteomics

2.1 Introduction

2.1.1 Plant selection for potential phytoremediation use

Selenium (Se) is an increasingly essential micronutrient for the health of humans and animals, and its importance in flowering plants remains an area of interest (Pilon-Smits et al., 2009). A greater understanding of Se accumulation in flowering plants offers a more economically feasible solution to phytoremediation with the added possibility of using these Se accumulating plants to supplement the shortage of dietary Se in humans and livestock.

Se accumulator plants need to meet several essential criteria before being nominated for their potential use in soil remediation. A plant must develop to maturity in a relatively short amount of time with a mature root system, have the nitrogen-fixing capacity, be adaptable to local climates, withstand a wide range of physicochemical conditions, grow in a mixture of soil compositions and be able to cycle nutrients in organic matter to improve substrate fertility to be suitable for use in phytoremediation, (Maiti, 2012). Based on the criteria as mentioned above, a few plants have been identified from a range of genera, some of which have been successfully used in eco-restoration (Bogdanović et al., 2008, Dragović et al., 2008, Gajić et al., 2018, Kostić et al., 2019, Maiti, 2012). Singh et al. (2016) previously found that grass and legume species, which include the genus of *Festuca, Lolium, Agropyron, Poa, Medicago,* and *Vicia,* are the most suitable grass and legume species for application on contaminated sites in a temperate climate. Additionally, *Populus, Robinia, Salix, Alnus, Betula*, and *Acer* are suitable phytostabiltizing trees among many other varieties of angiosperms and herbaceous plants (Garau et al., 2019).

In the past, some studies have dealt with Se accumulating plants for phytoremediation of Se in contaminated sites (Bañuelos and Dhillon, 2011, Wu et al., 2015). However, more recently, attention has been drawn to addressing Se deficiency in the human diet. For this reason, more excellent value has been placed on crops, vegetables, and edible mushrooms for enriching with Se (Zhao et al., 2005, Maseko et al., 2014, Dogan et al., 2016). Studies by Li et al. (2018) performed on *Brassica rapa var. rapa* (turnip), where results indicated that selenate was absorbed in greater concentrations than selenite. *Asteraceae*, *Brassicaceae*, and *Fabaceae* studied from America, Australia and China have accumulated Se in their leaf trichomes and epidermal cells (Freeman et al., 2006, Freeman et al., 2010, Yuan et al., 2013, White, 2015). Additionally, *Lecythidaceae* is said to accumulate Se in its fruits and seeds (Dernovics et al., 2007, Hammel et al., 1996, Chang et al., 1995).

2.1.2 Selenium accumulating plant groups

To date, plants have been categorised into three groups according to their capability to accumulate Se. Most plants are non-accumulators (Beath et al., 1940). The main groups of plants are Se accumulators, Se indicators, and non-Se accumulating plants.

Non Se accumulating plants are those that do not absorb significant amounts of Se under any circumstances. Se indicator plants/secondary accumulators absorb Se depending on how much Se is present in the soil. Secondary Se accumulators typically contain 5–30 mg Se kg−1 dry weight. Researchers Huang and Wu (1991) and White et al. (2004) have put plants such *as Aster, Atriplex*, *Brassica juncea,* and *Brassica napus* (canola), species of *Comondra*, *Grayia, Gutierrezia, Siderenthus,* and *Castilleja* into the secondary Se category. Se accumulator's plants accumulate Se into their tissues in concentrations in the range of 70–300 mg Se kg−1 dry weight. (Marschner and Rengel, 2012) and White et al. (2004) grouped *Astragalus*, *Stanleya pinnata*, *Melilotus officinalis*, *Grindelia squarrosa*, *Neptunia amplexicaulis*, *Bertholletia excelsa*, and species of *Lecythis*, *Morinda*, *Happlopappus,* and *Machaerantha* as Se accumulator plants. Some of the plants belonging to this group have been previously measured for Se concentrations in their natural environments. However, to date, not all plants have been studied to the same extent or level of detail, and Se absorption potential can vary significantly within species, highlighting the need to understand individual varieties (Li et al., 2018).

2.1.3 *Helianthus annuus* for potential Se phytoremediation

The *Helianthus* genera include *Helianthus annuus*, a significant source of edible oils that has also been mentioned for its phytoremediation ability, with the added advantage of the sturdy stems and plant material (Fan and Eskin, 2015). Numerous studies have demonstrated the ability of *Helianthus annuus* to absorb essential nutrients under stressed conditions with nitrate or ammonium as a nitrogen source (Kurvits and Kirkby, 1980). Forte and Mutiti (2017) demonstrated that *Helianthus annuus* accumulated cadmium and lead in significant concentrations. The only study at the time of writing was (Farzaneh et al., 2018), where *Helianthus annuus* absorbed higher levels of Se compared to maize. However, there are no studies to date that demonstrate the ability of *Helianthus annuus* to accumulate Se when exposure is at sublethal concentrations in tissue culture.

2.1.4 *Brassica rapa* for potential Se phytoremediation

Numerous studies have demonstrated the potential of Se accumulation by species from the *Brassica*ceae (Hladun et al., 2011, Seppänen et al., 2010, Suarez et al., 2003). Given that a large range of *Brassica*ceae varieties such as pakchoi, cabbage, broccoli, mustard, radish, and turnip are already grown on a commercial scale around the world, they are readily available plants for the Se biofortification as a means of improving dietary intake to address the inadequate levels of Se intake in humans. Given a large number of plant varieties within the *Brassica*ceae (Couvreur et al., 2010), each variety requires an individual assessment of its ability to absorb Se, to classify these plants as non-accumulators or Se hyperaccumulators (Seppänen et al., 2010, Suarez et al., 2003, Yuan et al., 2013).

Research has identified *Brassica* juncea (Indian mustard), popular in Japan as a Se accumulator, along with *Brassica rapa* var. *hakabura (Nozawana)* and *Brassica rapa* var. *peruviridis (komatsuna*) as suitable candidates for use in Se phytoremediation. Of these three plants, *Nozawana* accumulated the greatest concentration of Se (Harris et al., 2014). Minerals such as iron, copper, and zinc could be extracted easily from the plant (Kumar et al., 2020). The Se accumulation ability of *Nozawana* combined with the ease of extracting other minerals place it as a potential phytoremediation crop where Se soil contamination is present (Flors and Nonell, 2006). While there are many more varieties of *Brassicae* that have been identified as having the ability to absorb Se to varying ranges, there are no studies to indicate that *Brassica rapa* ‘Vitamin greens’ can also absorb Se let alone other minerals and if so, the concentration of selenite and selenate that can be tolerated.

2.1.5 *Neptunia amplexicaulis* for potential Se phytoremediation

A range of species such as *Stanleya pinnata* and *Astragalus* sp. have been well studied in areas where there are naturally occurring Se soils. On the other hand, some plants have received less attention, such as *Neptunia amplexicaulis,* which was first documented by Knott and McCray (1959). Since the discovery of Se accumulation in plants, there have only been two other studies on the ability of *Neptunia amplexicaulis* to absorb Se in Australian soils (Burnell, 1981, Peterson and Butler, 1967). This lack of research into this naturally occurring Australian Se accumulator justifies the selection of this plant for a detailed investigation. No research on *Neptunia amplexicaulis* has been performed to understand its Se uptake when Se is present as a contaminant. *Neptunia amplexicaulis* has been studied to a limited extent as a Se hyperaccumulator for its ability to absorb naturally occurring Se from its surrounding soils; however, the effect of the soil composition on Se uptake ability was not considered in this study (Peterson and Butler, 1967). Therefore, any beneficial effect of other compounds is not known to date.

2.1.6 Aim

This experiment aimed to determine the sublethal concentrations of sodium selenite and sodium selenate in *Solanum tuberosum*, *Helianthus annuus*, *Brassica rapa. var* vitamin greens and *Neptunia amplexicualis.* Growth variables on exposure to increasing concentrations of sodium selenite and sodium selenate in tissue culture were measured. The findings of this chapter will then be used as the basis for further investigations into the ROS enzymes and metabolites, uptake, quantification of Se within the plants, and trends in the upregulation and downregulation of proteins.

Methods

2.1.7 Se solution stock preparation

Stocks of 0.1M solution of sodium selenite, Na_2SeO_3 (CAS: 10102-18-8) with a MW: 172.94g/mol and 0.1M sodium selenate Na_2SeO_4 (CAS: 13410-01-0) MW: 188.94 g/mol were prepared in 100 mL deionized sterile H_20 for addition to culture media. Sodium selenite and sodium salt were purchased from Sigma Aldrich.

2.1.8 Tissue culture preparation

Murashige and Skoog (MS) 1/4 strength media solution was prepared with 1.0825g MS salts 7.5g sucrose and stirred over a hot plate to ensure that all components were dissolved for 1 hour. 600 mL was then placed into a 1L stock bottle with 0.8g of agar to solidify media for tissue culture.

All bottles of tissue culture media were placed in an autoclave in a-setting of 30 min at 121°C, 104 kPa, and left to set at room temperature. Culture media was used the same day as preparation in every instance. Controls did not contain selenium in any form.

2.1.9 Seed preparation

Seeds of *Brassica rapa cv.* Vitamin greens and *Helianthus annuus* were sourced from Rangeview seeds, Derby, Tasmania. *Neptunia amplexicaulis* seeds were sourced from Richmond, QLD by Dr Ashwa Nanjappa Central Queensland University, Australia, and identified by the Sydney Royal Botanical Gardens. Since plants utilize the hard seed coats to protect from the environmental conditions until suitable germination conditions arise, the seed coats do not form a seed storage compartment, and therefore where applicable, hard seed coats were removed by hand without damaging seeds inside (Erbaş et al., 2016). Once seed shells were-removed, all seeds were surface sterilized with 70 % (v/v) ethanol for 3 min, followed by autoclaved MQ water rinses five times. Seeds were then washed in 2% sodium hypochlorite solution for 5 min, after which they were rinsed five times again with autoclaved MQ water. All seeds were used immediately after sterilization. *Neptunia amplexicaulis* seeds were nicked with a nail clipper to break open the seed coat to ensure penetration of nutrients and initiate the first stage of the triphasic germination, i.e., rapid initial uptake of water (Bewley, 1997, Manz et al., 2005, Schopfer and Plachy, 1984). This was not necessary for the softer seed coatings. Plants were grown for 21 days at 24°C, relative humidity of 85-95 %, and a day/night cycle of 12 hours/12 hours. A data logger was used to ensure that humidity and temperature were consistent throughout the experiments.

2.1.10 Data collection

The variables on which sublethal concentrations were assessed are fresh weights (g), dry weights (g), shoot length (mM), root length (mM), the number of leaves excluding cotyledons, and leaf grading where 1= healthy green leaves and 5= dead leaves.

All plant samples were collected after 21 days. Culture media was washed off the root system with deionized water three times to ensure the removal of all culture media. Fresh weight (FW) was then measured on an analytical balance. Plants were laid flat, straightened and measured using a ruler from shoot tip to root tip. Plants were then placed in 6 mL kimble tubes at 80°C for 8 hours and weighed again for dry weights, by which time plants had achieved a constant weight.

2.1.11 Statistical analyses

$P < 0.05$ is indicated by an * to show significant differences between control and treatments. Two-way ANOVA was used to determine the difference between groups and followed by Tukey's *post hoc* pairwise test. Results are graphed as means ± standard error.

2.2 Results

Figures 2,3,4 and 5 illustrate the effects of selenium salts, selenite, and selenate on four plants, *Brassica rapa, Helianthus annuus, Neptunia amplexicaulis, and Solanum tuberosum.* Graphs a, b, and c in each figure represents the effect of selenite on fresh weight, dry weight, water content, total shoot length, root length, shoot to root ratio, the number of leaves and leaf grading. Graphs d, e and f, represent the effects of selenate on the same variables mentioned above. Data is shown as n=6.

2.2.5 Selenite and selenate sublethal concentrations in *Brassica rapa*

Figure 2 shows details of all the growth variables for *Brassica napa sp vitamin greens* to determine the sublethal concentrations of sodium selenite and sodium selenate for further research. Note that the results are in line with typically increased toxicity effects resulting from increasing concentrations of Se.

For sodium selenite (Figs. 2a, 2b and 2c), a concentration of 20 µM and greater significantly reduced fresh weight, dry weight, shoot length and root length compared to controls, whilst the effects of lower concentrations of selenite were inconsistent. At 20 µM and greater concentrations, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Importantly the number of leaves and poorer health grading of plants were only significant at 20 µM compared to controls; lower concentrations were inconsistent.

For sodium selenate (Figs. 2d, 2e and 2f) at concentrations of over 20-25 µM, there was a significant reduction in fresh weight, dry weight, shoot length and root length compared to controls whilst lower concentrations of selenate again had inconsistent effects. At >20-25 µM, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Importantly the number of leaves and poorer health grading of plants were significant at >20-25 µM compared to controls, whilst lower concentrations were inconsistent.

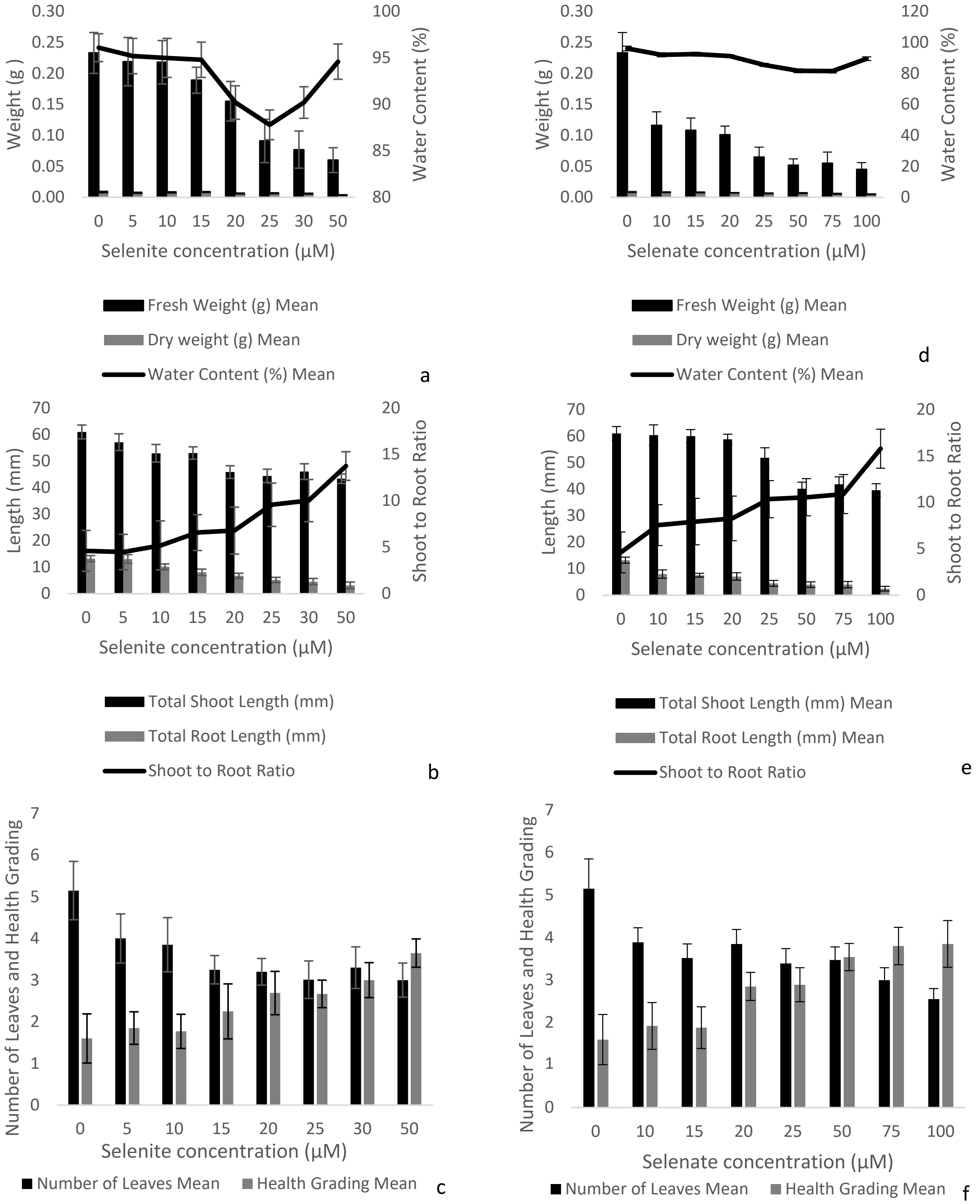

Figure 2. Brassica. rapa growth variable measurements on exposure to sodium selenite and sodium selenate. a and d) fresh weight, dry weight and and water content (%) b and e) total length of shots, roots and shoot to root ratio and (c and f) number of leaves grading where (1-healthy and 5-dead). All values are expressed as means±se, n=6.

2.2 6 Selenite and selenate sublethal concentrations in *Helianthus anus*

Note that the results are in line with typically increased toxicity due to increasing concentrations of Se.

For sodium selenite (Figs. 3a, 3b, and 3c), a concentration of >25 µM significantly reduced fresh weight, dry weight, shoot length, and root length compared to controls and lower concentrations of selenite were inconsistent. At 25 µM, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Similarly, taking into consideration the number of leaves and poorer health grading, plants were only significantly affected at >25 µM compared to controls; lower concentrations were inconsistent.

For sodium selenate (Figs. 3d, 3e, and 3f) at concentrations of >25 µM, there was a significant reduction of fresh weight, dry weight, shoot length, and root length compared to controls and lower concentrations of selenate were inconsistent. At >25 µM, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Importantly the number of leaves and poorer health grading of plants were significantly affected at >25 µM compared to controls; lower concentrations were inconsistent.

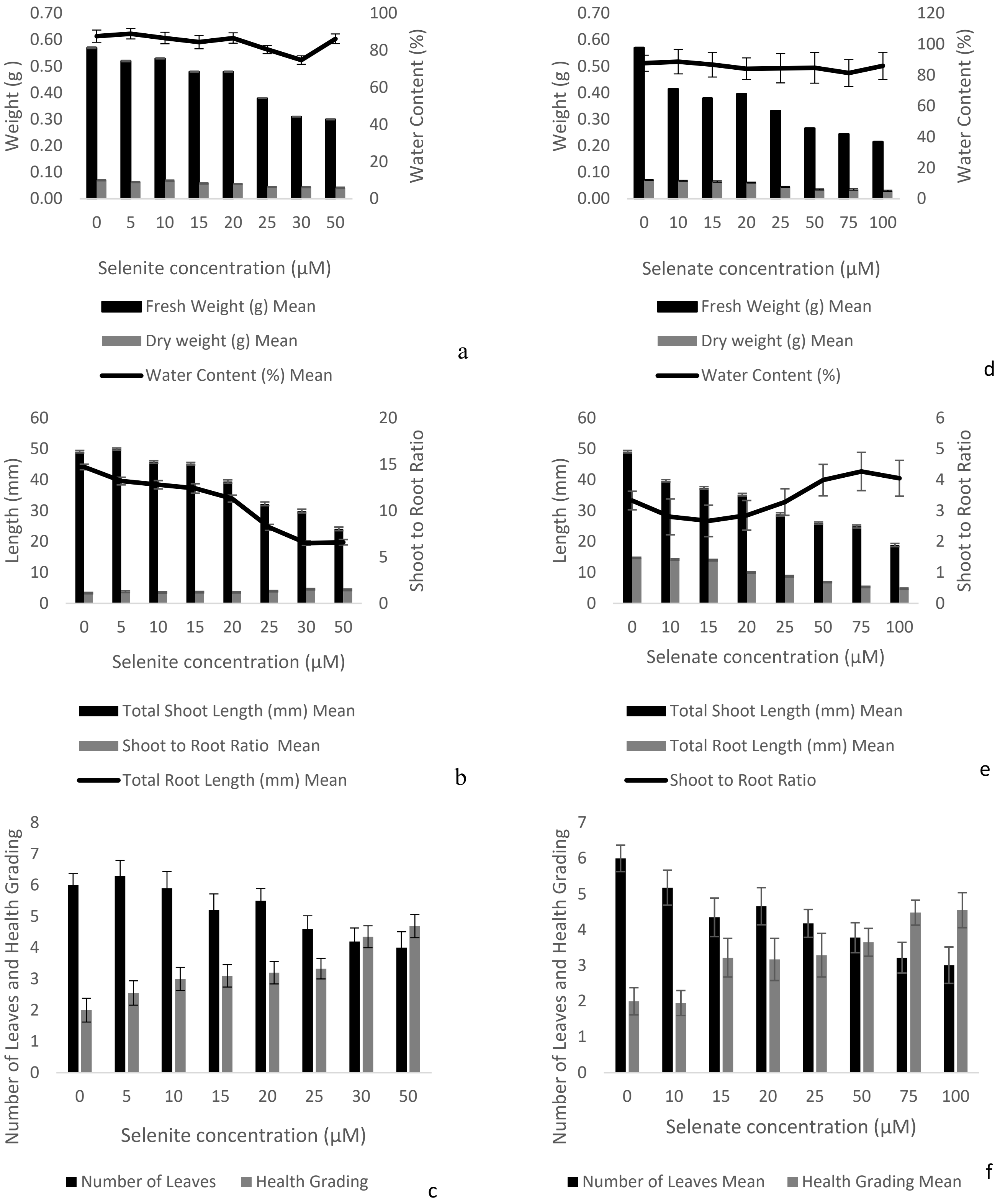

Figure 3 Helianthus annuus growth variable measurements on exposure to sodium selenite and sodium selenate. a and d) fresh weight, dry weight and water content (%) b and e) total length of shots, roots and shoot to root ratio and (c and f) number of leaves grading where (1-healthy and 5-dead). All values are expressed as means±se, n=6

2.2.7 Selenite and selenate sublethal concentrations in *Neptunia amplexicaulis*

Figure 4 shows details of all the growth variables for *Neptunia amplexicaulis* to determine the sublethal concentrations of sodium selenite and sodium selenate selected for further research.

For sodium selenite (Figures 4a, 4b, and 4c), a concentration of >500 µM significantly reduced fresh weight, dry weight, shoot length, and root length compared to controls, and lower concentrations of selenite were inconsistent. At >500 µM, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Importantly the number of leaves and poorer health grading of plants were only significantly affected at >500 µM compared to controls; lower concentrations were inconsistent.

For sodium selenate (Figs. 4d, 4e, and 4f) at concentrations of >500 µM, there was a significant reduction of fresh weight, dry weight, shoot length, and root length compared to controls and lower concentrations of selenate were inconsistent. At >500 µM, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Importantly the number of leaves and poorer health grading of plants were significantly affected at >500 µM compared to controls; lower concentrations were inconsistent.

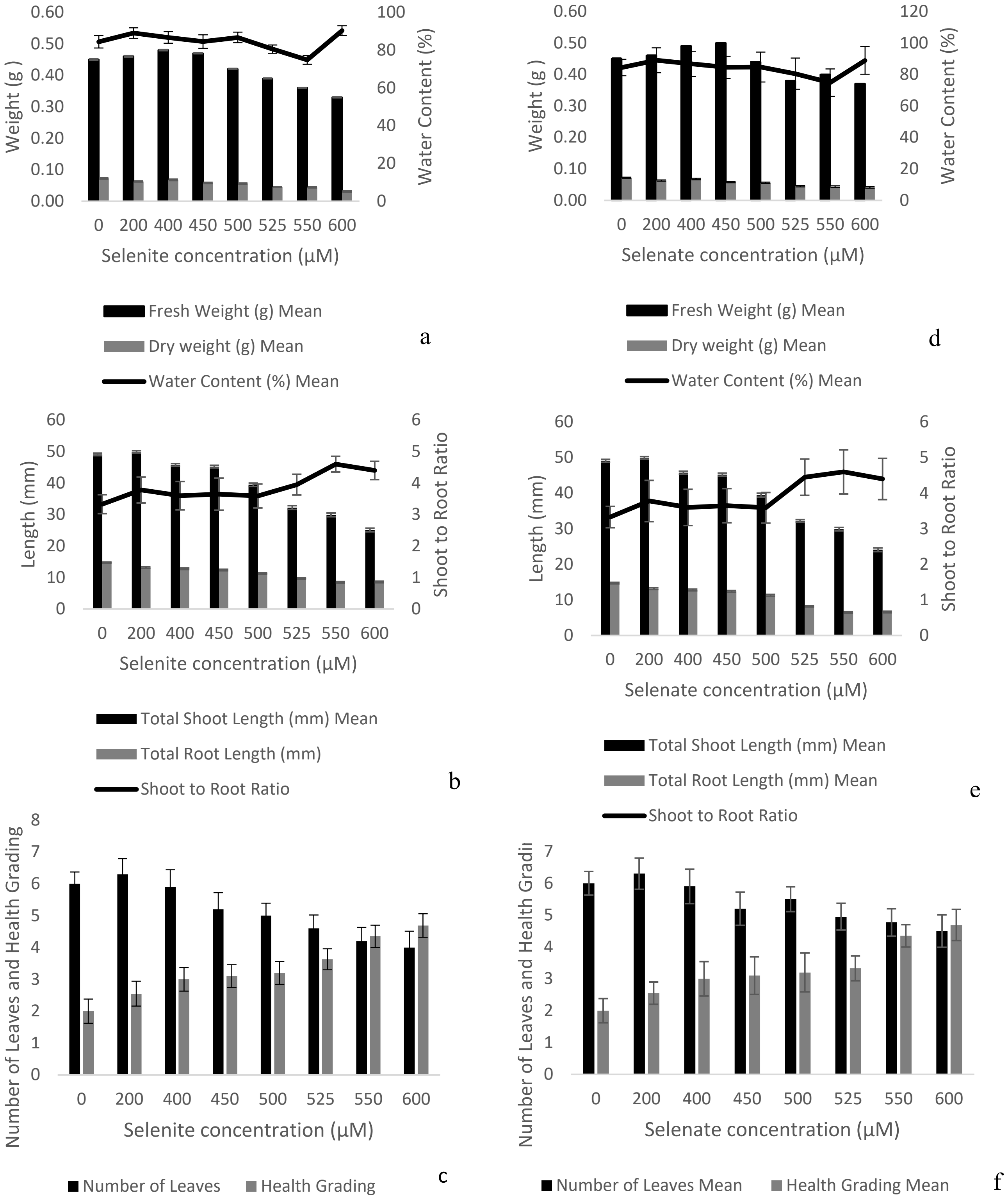

Figure 4. Neptunia amplexicaulis growth variable measurements on exposure to sodium selenite and sodium selenate. a and d) fresh weight, dry weight and water content (%) b and e) total length of shots, roots and shoot to root ratio and (c and f) number of leaves grading where (1-healthy and 5-dead). All values are expressed as means ± se, n=6.

2.2.8 Selenite and selenate sublethal concentrations in *Solanum tuberosum*

Figure 5 shows details of all the growth variables for *Solanum tuberosum* to determine the sublethal concentrations of sodium selenite and sodium selenate selected for further research.

For sodium selenite (Figs. 5a, 5b, and 5c), a concentration of >15 µM significantly reduced fresh weight, dry weight, shoot length, and root length compared to controls, and lower concentrations of selenite were inconsistent. At >15 µM, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Importantly the number of leaves and poorer health grading of plants were only significantly affected at >15 µM compared to controls; lower concentrations were inconsistent.

For sodium selenate (Figs. 5d, 5e, and 5f) at concentrations of >15 µM, there was a significant reduction of fresh weight, dry weight, shoot length, and root length compared to controls and lower concentrations of selenate were inconsistent. At >15 µM, there was also a decrease in water content and increased shoot to root ratio compared to controls, and at times also at lower concentrations of Se. Importantly the number of leaves and poorer health grading of plants were significantly affected at >15 µM compared to controls; lower concentrations were inconsistent.

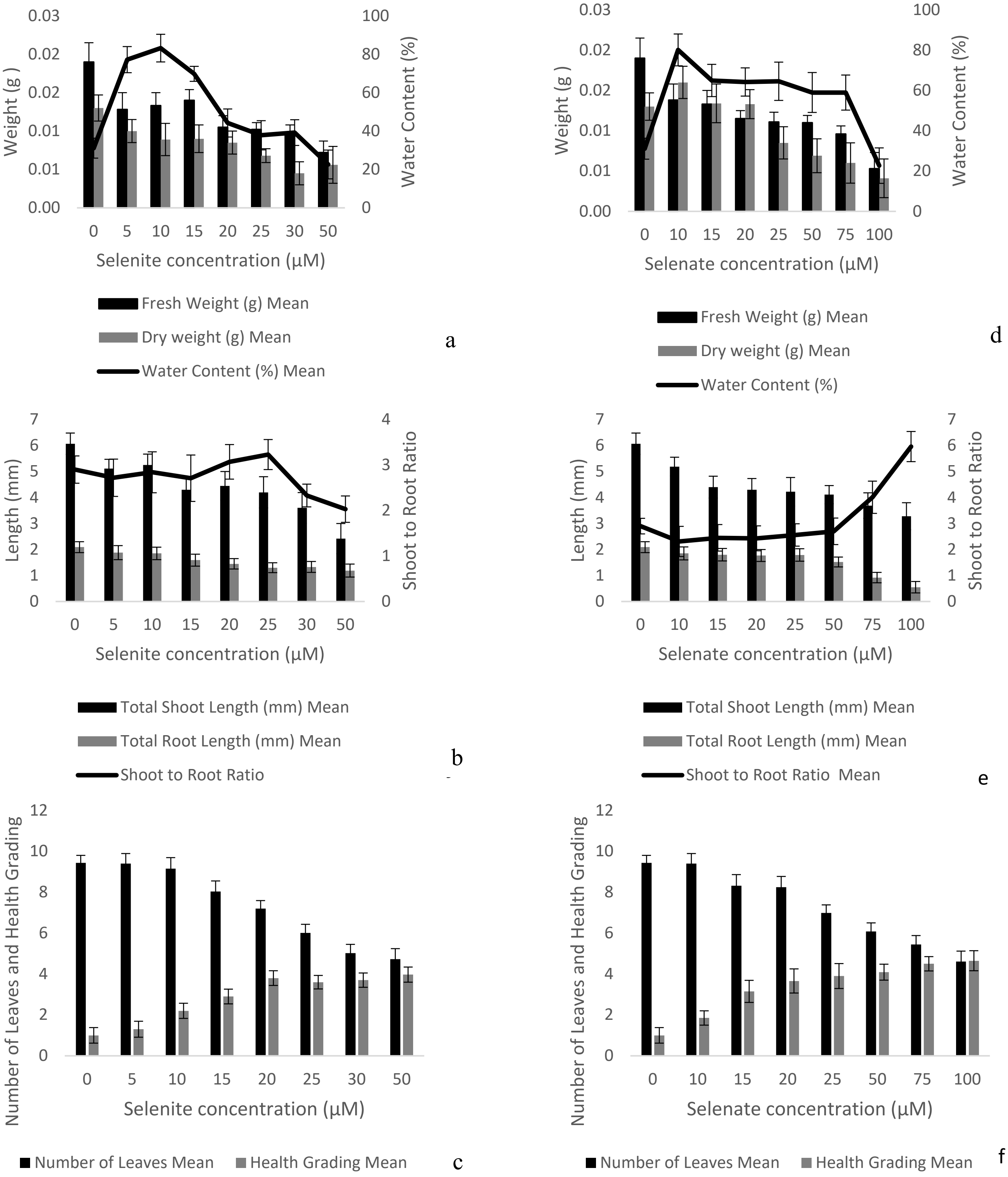

Figure 5. Solanum tuberosum growth variable measurements on exposure to sodium selenite and sodium selenate. a and d) fresh weight, dry weight and water content (%) b and e) total length of shots, roots and shoot to root ratio and (c and f) number of leaves grading where (1-healthy and 5-dead). All values are expressed as means±se, n=6.

The analysis of the data generated through exposing the four plants to increasing concentrations of selenite and selenate salts indicated that *Brassica rapa* was able to tolerate up to 20 µM before there was a notable change in the variables measured. *Helianthus annuus* had a sublethal concentration of 25 µM, *Neptunia amplexicaulis* had a sublethal concentration of 500 µM, and *Solanum tuberosum* had a sublethal concentration of 10 µM for each salt respectively.

2.3 Discussion

The objective of this study was to identify the sublethal concentrations of exposure to sodium selenite and sodium selenate salts to selected edible crop plants. The results and sublethal concentrations deduced were then used to study in greater detail selenium uptake, metabolism, and localization within these plants in the next chapters. Sub-lethal concentrations were used to describe plants where there was no significant mortality induced when compared to the control (Schmidt et al., 1997), but most, if not, all growth variables were adversely affected. The sublethal concentration was defined as "inducing no significant mortality when compared to the untreated control" (Desneux et al., 2007)

2.3.5 *Brassica rapa* sublethal concentrations

Brassica rapa exposed to sodium selenite and sodium selenate at 20 µM concentrations were chosen as being the most appropriate sublethal levels satisfying the criteria above, based on the analyses and plant toxicity variable assessments (Huang et al., 2019). Previous research where *Brassica rapa* varieties have been exposed to Se in either form indicated that they were able to tolerate a range of concentrations of both Se by foliar application, where Se can stimulate growth and enhance tolerance to environmental pressures by inducing oxidative stress, along with offering resistance to pathogens and herbivores: thereby increasing yield and nutritional value (Hawrylak-Nowak et al., Quinn et al., 2007, Pilon-Smits et al., 2009, White and Brown, 2010, El Mehdawi and Pilon-Smits, 2012, Feng et al., 2013).

Studies have also shown that within a narrow range, Se can also become toxic to some plants. White et al. (2004) (White et al., 2004a) and Huang Wu (1991) both described those secondary accumulators as those plants where less than 5-30 mg Se kg dry weight was accumulated, such as in *Brassica juncea* and *Brassica napus* (canola), and species of *Comondra*, *Grayia*, *Gutierrezia*, *Siderenthus,* and *Castilleja*. In a study of 38 genotypic variants of 7 *Brassica oleracea* varieties, it was found that the most effective concentration of selenite and selenate was also 20 µM (Ramos et al., 2011). Forage, crop plants, and some grasses accumulate less than 5 mg Se kg in the biomass and can thus be classified as non-accumulators (Freeman et al., 2006, Baker, 2000, Ernst, 1982). From the current results, the concentration of Se in the biomass cannot be deduced and will be studied quantitatively in chapter 4.

According to Figures 2a and 2b, water content *in Brassica rapa* exposed to selenite showed no significant differences between the control and Se concentrations of 5– 15 µM. At 20 µM, there was a significant difference between not the only control, but also 15 µM, which indicates that at this concentration, *Brassica rapa* is no longer able to process the increasing concentrations of Se, resulting in a decrease in water content which is directly proportional to the decrease in fresh and dry weight in both selenium salt tests. (Prins et al., 2011) demonstrated that in other non-accumulators such as *Brassica juncea*, there were also significant decreases in biomass, pollen germination, seed production, and seed germination. This trend was also evident in the number of leaves produced before the treatment of 20 µM, where the control produced the greatest number of leaves, whilst at the highest tested concentration, *Brassica* produced plants with significantly fewer leaves, with wilting and yellowing evident throughout growth for both forms of Se (Figures 2e and 2f).

2.3.6 *Helianthus annuus* sublethal concentrations

Helianthus annuus exposed to sodium selenite, and sodium selenate at 25 uM concentrations were chosen as being the most appropriate sublethal levels satisfying the criteria above (Huang et al., 2019). *Helianthus annuus* produced a dry weight of 0.07±0.01g with a water content of 87.70% in the control media; however, the first significant difference in dry and fresh weight occurred at the concentration of 25 µM for both selenite and selenate, which indicates that *Helianthus annuus* can tolerate higher concentrations of Se compared to *Solanum tuberosum* and *Brassica rapa*. This finding was supported by previous work conducted by Garousi et al. (2016), where it was found that *Helianthus annuus* on exposure to Se in both forms showed symptoms of toxicity at high doses. However, in contrast to the findings in Figure 3a, *Helianthus annuus* exposed to selenite was more toxic than *Helianthus annuus* exposed to selenate measured by the dry weight of shoots and roots of the plantlets. Nevertheless, the current studies indicated that *Helianthus annuus* had a high Se accumulation capacity. The Se toxicity manifested in poor leaf quality, stem elongation, and root development, as quantitatively shown in Figure 2. This agreed with the studies by (Garousi et al., 2016), where plants supplied with high doses of Se (3mg L^{-}DW of selenate) had black and yellow leaves at higher concentrations (30 mg $\mathrm{L}^{-\circ}$) leaves dried completely. Previous studies indicated that increasing concentrations in growth media could also be used to increase the absorbed absorption of Se in crop plants (Broadley et al., 2006a, Gupta, 1995, Valle et al., 2002).

2.3.7 *Neptunia amplexicaulis* sublethal concentration

Sodium selenite and sodium selenate at 500 µM was chosen as the most appropriate sublethal levels for *Neptunia amplexicaulis,* satisfying the criteria above. *Neptunia amplexicaulis* has been previously studied (Peterson and Butler, 1967, Burnell, 1981) as a hyperaccumulator in natural soil Se concentrations. Still, minimal research has since been done to demonstrate the translocation of Se from the roots to the shoots and its range of toxicity was never studied. This study was conducted in tissue culture and may have resulted in relatively lower Se concentrations than what may be found in contamination sites. The reason for the Se concentration used in the current work was related to the difficulty and health risks of accessing contaminated soils from privately owned power plant properties in the Sydney area.

The first concentration to demonstrate a significant reduction in fresh and dry weight was 500 µM for both selenite and selenate (Figure 4a and 4d). In contrast, *Neptunia amplexicaulis* collected from the natural environment contained up to 10 M of Se. This significant difference between these tolerance levels can be attributed to the short experimental growth period of 21 days in the current work and higher Se concentrations generally found in naturally occurring soil conditions. Shoot and root length also decreased over increasing concentrations of Se (Figure 3b and e). The same trend as *Solanum tuberosum, Brassica rapa,* and *Helianthus annuus* was seen in *Neptunia amplexicaulis* (Figures 4c and 4f), with a decrease in leaf quality grading and the number of leaves produced. After considering the current toxicity assessment, a sublethal concentration of 500 µM appears to be appropriate.

2.3.8 *Solanum tuberosum* sublethal concentration

Compared to *Brassica rapa* and *Helianthus annuus*, the sublethal concentration deduced from the *Solanum tuberosum* assessment was lower, with selenite and selenate concentrations of 15µM identified as sublethal. This finding is in line with the general classification of *Solanum tuberosum* as a non-Se accumulator, with tubers previously measured to contain less than 0.01 µg Se g^{-1} DW (Turakainen, 2007). Other trends observed for *Solanum tuberosum* included the lower amount of biomass produced compared to other plants studied. The control produced a maximum of 0.01 g of biomass compared to six-fold greater production in *Brassica rapa*, seven-fold in comparison *Helianthus annuus* and six-fold in *Neptunia amplexicaulis*. In the current studies, there was no production of tubers within the time frame of 21 days.

Given that dry and fresh weights are the most accepted units for assessing plant health, these were the primary points of reference used to identify a sublethal concentration for the plant varieties studied. The water content (Figures 5a and b) in the *Solanum tuberosum* control was similar to that of plants grown in 50µM concentration. This can be attributed to the growth-stimulating effect of Se, which was also observed in low concentrations; after which it becomes toxic to the plant as seen here. The phenotype of the *Solanum tuberosum* plants (Figures 5c and 5d) demonstrated declines in shoot and root length as the concentration of Se increased. In Figures 5 e and 5f, it was evident that as the concentration of the selenite increases, the number of leaves decreases, and the quality of leaves also diminishes with higher concentrations of Se. After considering fresh and dry weight, the root and shoot length, shoot and root length ratios, and the number of leaves and the grade of leaf material, the sublethal concentration of selenite and selenate for *Solanum tuberosum* was determined to be 15µM.

2.3.9 Comparison to soil Se levels

The test concentrations of Se ranged from 0 in control to concentrations similar to natural and contaminated environments such as those found on coal mining sites (Bond, 1999, Bujdoš et al., 2005, Cutter and Cutter, 1995, Desborough et al., 1999, Khamkhash et al., 2017, Nriagu and Wong, 1983). Previous surveys (Caritat and Cooper, 2011) have found maximum Se concentrations in South Australia and Perth of 4.36 mg/kg, with levels of 0.05 mg/kg occurring widely across Australia. While tissue culture media and soil vary in composition, this comparison is vital to ensure the current tests were relevant to possible applications of the test plants in the phytoremediation of Se contaminated soils in Australia. For ease of comparison, sublethal concentrations were converted to mg/kg. This equates to the sublethal concentrations of selenite for *Brassica rapa, Helianthus annuus*, *Neptunia amplexicaulis,* and *Solanum tuberosum* of 0.96, 1.20, 23.99, and 0.72 mg/kg, respectively. Similarly, the sublethal concentrations of selenate in *Brassica rapa, Helianthus annuus*, *Neptunia amplexicaulis,* and Solanum tuberosum were 1.28, 1.60, 31.99, and 0.96 mg/kg, respectively. The sublethal concentrations of all Se in all these plants fall within the minimum and maximum concentrations detected by previous geochemical soil surveys (Caritat and Cooper, 2011). The limitation of this comparison is that the survey only accounts for the elemental Se and not for ions or compounds of Se, which would therefore occur in lower concentrations than that of elemental Se. As a result, the plants in this study would be suitable for growth in Se concentrations found in soil without considering other elements in the soil composition and climatic conditions. *Neptunia amplexicaulis* can tolerate much higher concentrations of Se, as typically found in contaminated soils such as those surrounding coal mining sites (de Caritat et al., 2011).

Given that the purpose of this study was to analyze the uptake mechanisms and impacts of Se on the selected plants, tissue culture offered a beneficial solution with several advantages. Tissue culture allowed for the rapid expansion of seed material irrespective of seasonal variations, producing disease-free plants in a cost-effective and space-efficient method., thus overcoming the challenges of performing this research in the soil, which has been the accepted means of studying contaminant uptake in plant materials previously (Ponmurugan and Kumar, 2011). Additionally, tissue culture overcame the problems related to the lack of publicly available information regarding Se contamination from coal mining and its associated ash dumping sites for application to Australian Se contaminated sites (Lipski, 2019). It should also be mentioned that these results cannot be directly applied in to field trials due to the mixed composition of contaminated sites, among other factors such as cost; hence it is important to realise the value of these findings lies in the ability to uncover the uptake mechanisms for, and metabolic function of Se in the plant system as a theoretical foundation from which to develop field trials (Doran, 2009).

2.4 Conclusion and Future Direction

Based on impacts upon plant fresh and dry weights, root and shoot lengths, shoot and root length ratios, the number of leaves and the grade of leaf material, the sublethal concentration of each salt of selenite and selenate for *Brassica rapa* was determined to be 20 µM, for *Helianthus annuus* 25µM, for *Neptunia amplexicaulis* 500 µM, and for *Solanum tuberosum* 15 µM. These findings form the basis for the experiments performed in the proceeding chapters. *Solanum tuberosum* was eliminated for further studies as it was challenging to gain sufficient biomass for replication and statistics from this plant.

Other variables could also have been measured, such as chlorophyll content, individual, specific, and total protein quantities, and Se concentration to increase the accuracy of these results; however, the resources for these were not available at the time of experimentation. The novelty of these findings prepares a foundation for the potential use of these plants, pending further research, in phytoremediation.

Chapter 3. Effects of Selenium species on reactive oxygen species enzyme activity

Abstract

Biotic and abiotic pressures induce oxidative stress in plants resulting in fluctuating levels of reactive oxygen species. Selenium is considered essential in animal and human cells but is yet to be found as critical for plant life. However, it has been found to alleviate the detrimental impacts of elevated ROS species in relatively small concentrations, but it can be toxic to plants and animals in high concentrations.

The seeds of *Brassica rapa, Helianthus annuus, Neptunia amplexicaulis* were exposed to sublethal concentrations of selenium salts, sodium selenite, and sodium selenate, which are naturally occurring bioavailable water-soluble forms of selenium, in tissue culture for 21 days to identify the impact of each selenium species on the enzymes responsible for mitigating the effects of an increase in reactive oxygen species, i.e. superoxide dismutase, catalase, lipoxygenase, ascorbic acid peroxidase, glutathione reductase, glutathione, acid, and neutral proteases.

Results indicated significant differences between the selenite and selenate effects on each plant's enzyme activity and in shoots compared to roots. The conclusion was that there was an increased activity of each enzyme in the plants at the pre-determined sublethal concentrations compared to controls except for the proteases. These results confirmed that there are not only physical effects of selenium salts at sublethal concentrations on plants but also physiological impacts.

This increased understanding of the different physiological effects of selenite and selenate exposure through enzyme activity, lay a foundation for further work in genetic modification to enhance the function of enzymes and so to determine if these plants are suitable for remediation purposes. In the context of this thesis, this work also allowed for the substantiation of the protein expression findings.

3.1 Introduction

3.1.1 Antioxidant pathways

Reactive oxygen species (ROS) species are the unsolicited companions of aerobic life when molecular oxygen was introduced to our earth by the O_2 evolving photosynthetic organisms. The O_2 molecule can be a free radical, as it has two unpaired electrons that possess the same spin quantum number, and the result is that O_2 prefers to accept its electrons one at a time leading to the manufacture of ROS that can ultimately damage cells (Halliwell, 2006). ROS are constantly made as by-products of various metabolic pathways and are localized in different cellular compartments. A variety of environmental stresses can also accelerate their production, both biotic and abiotic (Rio et al., 2006). Numerous studies, which will be discussed below, have been performed to demonstrate the effect of various stresses on the reactive oxygen species of plants. Still, there are a limited number of studies that have demonstrated the effects of Se stress on the suite of enzymes seen in this study selected for their role in the enzyme cascade as a stress response.

Reactive oxygen species are formed in various ways in several cellular compartments (Sakac and Sakac, 2000), including mitochondria (Kerksick and Zuhl, 2015), chloroplast (Apel and Hirt, 2004), peroxisomes (Klotz, 2002), endoplasmic reticulum (Moldovan et al., 2000), cytoplasm (Kobayashi et al., 2007), plasma (Kim et al., 2002)membrane and apoplast during normal metabolic processes (Ding et al., 2003), but they can be elevated upon exposure to environmental pressures (Whiteside and Herndon, 2019).

As mentioned earlier, there are few studies to date in which plants have been exposed to sublethal levels of Se. Therefore, existing studies of Se effects on antioxidant enzymes have only tested concentrations of Se at levels where there was thought to be a beneficial effect of Se on the plant in withstanding the respective abiotic stresses discussed in this chapter.

3.1.2 Selenium as a stressor: superoxide dismutase (SOD)

Superoxide dismutase is a metalloenzyme and is the first enzyme in the detoxification process where O_2.- is scavenged by SOD, catalysed by its dismutation, O_2.-is reduced to form H_2O_2, and then other enzymes are used to oxidize H_2O_2 to form O_2.

SODs are classified into three different categories based on their metal cofactors:

1. Fe-SOD (localised in chloroplasts),
2. Mn-SOD (localised in mitochondria and peroxisomes),
3. Cu/Zn-SOD (localised in chloroplasts, peroxisomes, and cytosol)

(Gill and Tuteja, 2010).

Although H_2O_2 is a toxic product of SOD in the cell as it can rapidly damage proteins and is quickly eliminated by ascorbate peroxidase (APX), catalase (CAT), and/or guaiacol peroxidase (GPX) (Eshdat et al., 1997a).

Upon exposure to a range of biotic and/or abiotic environmental stresses, SOD levels are amplified to battle the resulting oxidative stress. Studies performed on salt-stressed Mulberry (Harinsaut et al., 2003), *Cicer arietinum, and Lycopersicon esculentum* (Gapinska et al., 2008), indicated that SOD levels increased significantly. The impact of drought stress on *Glycyrrhiza uralensis* also revealed a significant increase in SOD activity (Pan et al., 2006). Similar results were observed with water-stressed *Trifolium repens L.* (Wang and Li, 2008). Upon exposure to cadmium (Cd) treatment, SOD activity also increased in *Hordeum vulgare* (Guo et al., 2004), *A. thaliana* (Skorzynska-Polit et al., 2003), *Oryza sativa* (Hsu and Kao, 2004), *Triticum aestivum* (Khan et al., 2007) and *Brassica juncea* (Mobin and Khan, 2007). While in most cases a rise in SOD activity has been detected upon exposure to various stresses, including salt, drought, chilling, heat stress, and heavy metal stress, there have been some studies that indicate otherwise, i.e., a decrease in SOD (Ahmad, 2014).

Recently, Se has been found to alleviate oxidative stress caused by water deficit in cucumber roots (Jóźwiak and Politycka, 2019), and Manpreet (2018) demonstrated that on exposure to stress-inducing selenite and selenate in *Triticum aestivum L.* (wheat), there was a significant impact of SOD. Se to date has not been studied at sublethal concentrations to understand the impact on SOD activity in any of the selected plants for this thesis; however, based on previous research related to abiotic stresses cited above, it would be expected that SOD levels across all plants and in all plant, parts would be significantly higher than the controls.

3.1.3 Selenium as a stressor: catalase

Catalase is a tetrameric heme-containing enzyme that catalyzes the decomposition of H_2O_2 to H_2O and O_2, which is primarily formed from the peroxisomes and oxidation involved in beta-oxidation of fatty acids photorespiration and purine catabolism (Duranteau et al., 1998). CAT is an essential part of ROS detoxification during stressed conditions (Gill and Tuteja, 2010). This decomposition process predominantly occurs in the peroxisomes but also the cytosol, mitochondria, and chloroplasts (Elstner, 1982). Like SOD, studies conducted applying various stresses on different plants also showed there was a significant increase in CAT activity.

CAT activity has been observed to fluctuate under metal stress (Gill and Tuteja, 2010b). In *Glycine max* (Balestrasse et al., 2001a), *Phragmites australis* (Iannelli et al., 2002b*), Capsicum annum* (Leon et al., 2002), and *Arabidopsis thaliana* (Cho and Seo, 2005), CAT activity decreased. However, its activity increased in *Oryza sativa* (Hsu and Kao, 2004), Brassica juncea (Mobin and Khan, 2007), *Triticum aestivum* (Khan et al., 2007), *Cicer arietinum* (Hasan et al., 2008), and *Vigna mungo* (Gill et al., 2008). In experiments where rice seedlings were pretreated with H2O2 under non-heat shock conditions, increasing CAT activity, rice seedlings were protected from Cd stress (Hsu and Kao, 2007). Under salt stress conditions, there was an increase in CAT activity in *Cicer arietinum* leaves (Eyidogan and Öz, 2007), and studies by Kukreja et al. (2005) found similar results. A decrease in CAT activity in *Anabaena doliolum* under NaCl and Cu stress (Srivastava et al., 2005) was detected. Under drought conditions, there was an increase in CAT activity in wheat, which was higher in sensitive varieties (Simova-Stoilova et al., 2010b), but in contrast, a study by Sharma and Shanker Dubey (2005) in drought conditions found that CAT levels were lower. Drought stress under high light conditions was also found to produce an increase in CAT activity (Yang et al., 2008), and UV-B stress led to an increase in CAT activity in *Columbia auriculata* seedlings (Agarwal, 2007). Additionally, salt stress combined with drought stress led to a decrease in the CAT activity in *Glycyrrhiza uralensis* seedlings (Pan et al., 2006b).

As stated previously, Se has been previously studied for its stress alleviating effects and not at sublethal concentrations for its effect on CAT in plants. Hasanuzzaman et al. (2011) demonstrated that seedlings treated with selenite improved the tolerance of drought stress in *Brassica napus* by increasing its antioxidative defence ability, evidenced by increased levels of CAT. Additional work by Gupta and Gupta (2016) showed that Se stressed *Brassica juncea* seedlings also had increased levels of CAT enzymes.

3.1.4 Selenium as a stressor: Ascorbic acid peroxidase (APX)

In addition to the production of SOD and CAT as a protection against oxidative stresses, plants will produce ascorbic acid peroxidase as an enzyme specifically responsible for ascorbate oxidation and is another important ROS scavenger. It is thought to also play a critical role in scavenging ROS, and thus protecting cells in higher plants, algae, euglena, and other organisms (Gill and Tuteja, 2010b).

APX enzyme activities are known to be correlated with oxidation tolerance (Batini et al., 1995). When hydrogen peroxide is reduced, APX can maintain the plasticity of the cell wall (Hidalgo et al., 1989). APX also plays a critical role in the ascorbate-glutathione cycle, the main hydrogen peroxide detoxification system in plant chloroplasts (Asada, 1992). Ascorbate is used as a specific electron donor by APX to reduce H2O2 to water. It should also be noted that the role of APX is not restricted to the chloroplasts but also extends to the cytosol, mitochondria, and peroxisomes (Asada, 1992, Asada, 1999, Mittler et al., 2004, Noctor and Foyer, 1998, Shigeoka et al., 2002) Given that APX has a greater affinity for H_2O_2 than CAT and peroxidase (POD) activity, it may have a greater role on ROS mitigation under stress conditions (Gill and Tuteja, 2010b).

Enhanced levels of APX have been detected in plants exposed to various stress conditions. Under Cd stress, *Ceratophyllum demersum* (Arvind and Prasad, 2003), *Brassica juncea* (Mobin and Khan, 2007), *Triticum aestivum* (Khan et al., 2007), and *Vigna mungo* (Singh et al., 2008) demonstrated an increase in APX activity in the leaves. It was reported that there was an increase in APX activity when seedlings of *Oryza sativa* were pre-treated with H2O2 under non-heat shock conditions (Hsu and Kao, 2007). An increase in APX activity was also seen in *Anabaena doliolum* during salt stress (Srivastava et al., 2005). Water stress conditions induced significantly increased levels of APX activity in *Picea asperata* (Yang et al., 2008). During mild drought stress, it was found that plants had higher chloroplastic APX activity than control plants, but the activity decreased under further increased drought stress (Sharma and Shanker Dubey, 2005).

3.1.5 Selenium as a stressor: Guiacaol Peroxidase (GPX)

GPUs are a large family of isozymes that use GSH to reduce H2O2, organic, and lipid hydroperoxides, resulting in the protection of plant cells from oxidative stress (Noctor et al.). In addition to this, GPX decomposes indole-3-acetic acid (IAA) and has a role in the biosynthesis of lignin by consuming H2O2 found in the cytosol, vacuole, cell wall, and extracellular spaces (Gill and Tuteja, 2010a). However, the activity of GPX fluctuates amongst plant species and the stress to which the plant has been exposed. In Picea asperata (Milone et al., 2003), Arabidopsis thaliana (Cho and Seo, 2005), and Ceratophyllum demersum (Arvind and Prasad, 2003), there was an increase in GPX when plants were exposed to increased levels of Cd. Under salinity stress, increased GPX activity has been found in leaves and root tissues in Vigna mungo radiate (Panda, 2001) and Oryza sativa (Yamane et al., 2009).

A study by Balakhnina and Nadezhkina (2017b) found that the effect of Se exposure on GPX activity in wheat included oxidative stress. GPX activity increased on selenite introduction to the soil, but this increase was not observed when selenate was added. However, Balakhnina and Nadezhkina (2017b) concluded that the effect of selenate on growth and overall plant health and, therefore the effect on GPX would vary with the concentration of Se added and the concentration of the stressor. In contrast, studies on selenite and selenate addition to ryegrass showed that the Se concentration in exposed shoots leads to increases in GPX activity (Cartes et al., 2005).

3.1.6 Selenium as a stressor: Lipoxygenase (LOX)

Lipoxygenase (LOX) is a ubiquitous enzyme that catalyzes the hydroperoxidation of unsaturated fatty acids and may also be involved in membrane lipid peroxidation during plant senescence (Feussner and Wastnernack, 2004, Firth et al., 1975, Spiteller, 2003). During the reaction, LOX converts polyunsaturated fatty acids (PUFAS) to form lipid hydroperoxides (LOOHS) in a reaction called lipid peroxidation (LPO). LOX incorporates molecular oxygen into linoleic and linoleic acid-forming lipid peroxides, which decompose to form free oxy-radical compounds such as malondialdehyde and jasmonic acid (Tanou et al., 2009), both of which have been proven to be a contributing factor to plant death. LOX is thought to be a part of a complex and diverse enzyme system, given its existence as several isoforms within a single tissue concerning biochemical characteristics (Gradner, 1991). LOX may serve many functions in the plant developmental processes, such as seed germination and senescence, or during stress-induced responses such as wounding or pathogen attack (Rosahl, 1996).

3.1.7 Selenium as a stressor: Glutathione Reductase (GR)

GR is a flavoprotein, specifically an oxidoreductase, found in prokaryotes as well as eukaryotes, playing an essential role in defence systems by sustaining the reduced status of GSH (glutathione) (Romero-Puertas et al., 2006). While GSH is mainly found in the chloroplasts, trace amounts have also been identified in the mitochondria, cytosol, and endoplasmic reticulum (Edwards et al., 1990, Gill and Tuteja, 2010b, Millar et al., 2003). The primary function of GR is to reduce glutathione disulphide (GSSG) to glutathione (GSH), thereby maintaining cellular redox balance (Hasanuzzaman et al., 2017, Ding et al., 2016).

GR is involved in processes concerning oxidative stress, and GSH plays a critical role in the cell system (Czarnocka and Karpiński, 2018). Together they are vital in determining the tolerance of a plant exposed to various stresses (Jozefczak et al., 2012). The reduction of GSH occurs through the catalysing action of GR, where GSH is the molecule that is heavily involved in the integrated system of metabolic regulation and antioxidative processes in plants (Khan et al., 2015). Glutathione has an important scavenging function, just like those mentioned above, to defend the plant against biotic stresses, specifically by consuming $H_2O_{2,}$ including from the ascorbate- glutathione cycle (Couto et al., 2016). These actions of GSH provide critical duties in cell function such as division, differentiation, death and senescence, enzyme and sulphate transport regulation, xenobiotic detoxification, metabolite conjugation, proteins and nucleotides synthesis, and phytochelatins and underpins the expression of stress-responsive genes (Zeng et al., 2017). In particular, H_2O_2, 1O2, OH•, and O2•- are scavenged by GR through glutathiolation where they are bound with or reduce other biomolecules in the presence of ROS, resulting in the by-product GSSG (Dar et al., 2017). GR in this process catalyzes the NADPH-dependent reaction of the disulphide bonds in oxidized glutathione (CSSG) (K.V.M Rao and Raghavendra, 2006).

Increase in GPX activity in cultivars of *Capsicum annuum* plants (Leon et al., 2002) with decreases in roots. No significant changes in GPX activity were seen in the leaves of Cd-exposed *Pisum sativum* plants (Dixit et al., 2001). Heavy metal stress (Li et al., 2000), oxidative stress (Avsian-Kretchmer et al., 2004), and mechanical stimulation reported an increase in GPX activity (Depège et al., 1998). It is understood broadly that in optimal conditions, there is a balance between GSSH and GSH; however, under stressed conditions, GSSG is stockpiled in higher concentrations (Couto et al., 2016). GSH activity also increases plant tolerance to several stresses such as salinity, drought, heat, low temperatures, and metal toxicity, as found by Hasanuzzaman et al. (2011a), Hasanuzzaman et al. (2012), Luo et al. (2011), Hasanuzzaman and Fujita (2011), Hasanuzzaman and Fujita (2012).

3.1.8 Proteases

Higher plants contain many hundreds of types of proteins, for example, cysteine-, serine-, aspartic-, metallo-, and threonine-proteases, which are involved in the process of photosynthetic energy conversion, growth, and development (Andersson and Barber, 1994). Proteases are a group of enzymes that act to degrade proteins (Van der Hoorn, 2008b). This occurs by the hydrolyzation of the peptide bonds of the proteins, rendering the protein a polypeptide or free amino acid (Alnahdi, 2012). More specifically, the proteinases that act on peptides, sometimes called peptidases, act near the ends of the polypeptide chains (exopeptidases) or within polypeptides (endopeptidases) (Mittler et al., 2004, Palma et al., 2002). Proteases are categorised according to their acid-base behaviour, that is, acid, neutral and alkaline. Acid proteases function optimally at a pH range of 2.0-5.0 and are produced mainly by fungi and plants, neutral proteases perform best at pH 7.0 originating mainly from plants, and those proteases that perform best at a pH above 8.0 are referred to as alkaline proteases (Alnahdi, 2012). The functional flexibility of proteins is provided by changes in the structural states in cells and tissues, and the protein changeover rates are determined by the developmental stage and physiological condition of the plant as well as stress conditions, including oxidative stress imposed on the plant (Uversky and Dunker, 2010).

Protease activity is generally thought to increase with age, exposure to stresses, with the leaves of the plant generally thought to have the highest level of proteolytic enzymes, but the specific proteases that are present remain unclear, which is often justified through enzymatic testing for proteases (Morris et al., 1996). For example, a study by Lusso and Kuc (1995) introduced tobacco mosaic virus (TMV) into *Nicotiana tabacum L.,* which resulted in an increase in phytase in the cytosol and apoplast that induced an immune response to activated in tobacco mosaic virus (TMV)-induced HR in addition to VirD2 cleavage preventing protein transport to the nucleus (Chichkova et al., 2010). In model plant *A. thaliana*, there were increases in proteases where AtMC1 AtMC2 both increased to serve as suppressants of hypersensitive cell death response when infected with avirulent pathogens as such then control runaway cell death (Coll et al., 2010). AtMC9 was also found in elevated levels in *A. thaliana* where it was an effector of PCD activation, xylem cell death, cell content degradation proceeding vacuolar rupture in the nucleus, cytosol, and apoplast (Bollhöner et al., 2013, Tsiatsiani et al., 2013, Watanabe and Lam, 2011, Wrzaczek et al., 2015). Ilyas et al. (2015) and Tian et al. (2007) demonstrated increases in the plasma membrane PIP1 protease, apoplast that had a broad range of extracellular defence against pathogens *C. fulvum*, *P. infestans*, *P. syringae in Solanum lycopersicum* L.

In addition to biotic stresses, protease activity can also be impacted through abiotic stresses such as drought. Some studies have demonstrated that there was a notable increase in intracellular proteases' under drought conditions compared to adequately watered plants consisting largely of cysteine proteases in wheat (Khanna-Chopra et al., 1999, Zagdariska and Wisniewski, 1996). In *Phaseolus vulgaris L.* and *Vigna unguiculata L. Walp,* there was an increase in aspartic proteases (Cruz de Carvalho et al., 2001) and serine-type proteases (Hieng et al., 2004) when placed under drought conditions. Furthermore, experimental data also suggests that drought-sensitive species have a higher protease activity compared to plants that do possess these traits (Hieng et al., 2004, Roy-Macauley et al., 1992, Zagdariska and Wisniewski, 1996). The elevated levels of these proteases highlight them as potential markers of drought resistance (Simova-Stoilova et al., 2010b). In a similar study, copper stressed Phaseolus vulgaris L showed an increase in endoproteases (Karmous et al., 2012). This substantiated previous research by Djebali et al. (2008), who demonstrated an increase in protease activity in the roots and leaves of *Solanum lycopersicum* L., var Ibiza plants on exposure to increased levels of cadmium, particularly cysteine- and metalloendopeptidase activity increased in roots and leaves while serine-endopeptidase activity increased only in leaves. Domash et al. (2008) also demonstrated an increase in protease activity in higher legume and cereal crops where lupin (*Lupinus angustifolius L., cv. Pershatsvet*), pea (*Pisum sativum L., cv. Agat*), and barley (*Hordeum vulgare L., cv. Gonar*) were exposed to increased levels of salinization, heavy metal ions, and phytopathogenic flora. These studies indicated that protease activity increases across a range of plants under stress conditions, often preparing the foundations from which the cascade of enzyme reactions commence the plant stress response, but there is the only study of Se stress on proteases in microorganisms but not in plants (Wu et al., 2016).

The purpose of the experiments in this chapter was to identify trends in the activity of antioxidant enzymes, superoxide dismutase, catalase, lipoxygenase, ascorbic acid peroxidase, glutathione reductase, glutathione, acid, and neutral proteases in *Brassica rapa, Helianthus annuus,* and *Neptunia amplexicaulis*; when exposed to selenite and selenate treatments.

In doing so, they will shed light on the effects of sublethal concentrations of Se in plants and form the foundation of the proteomic studies in chapter 6 of this thesis. More broadly, the understanding of selenite and selenate effects on enzyme activity lays a theoretical understanding of the plants' coping abilities at Se concentrations that are reflective of contaminated power plant soils.

3.2 Method

3.2.1 Sample preparation for enzymes

100g of leaf and root material were collected from pooled samples of each plant type in replicates of 6. The plant material was then made up to 4 mL made up exactly in a final volume graduated cylinder to which K phosphate buffer 50 mM plus 0.5 mM EDTA (pH 7) was added. The removal of phenolics and interfering compounds is required for satisfactory protein extraction from plant tissues. There are two stages at which phenol removal can occur, 1) removing interferences before protein extraction and 2) removal during and after protein extraction. In both stages, the oxidation of phenolic compounds can be prevented with the use of water-soluble PVP or water-insoluble polyvinylpolypyrrolidone (PVPP) (Isaacson et al., 2006, Toth and Pavia, 2001), DTT or 2-ME, sodium ascorbate 22 (Cremer and Van de Walle, 1985), and thiourea 23 (Flengsrud and Kobro, 1989) while performing the tissue extraction. The method selected, utilised 2 -BME which was readily available and stable compared to DTT to perform the same function of eliminating phenols after proteins were directly extracted from the plant tissue in K phosphate buffer 50 mM plus 0.5 mM EDTA, (pH 7).

3.2.2 Total protein

The Bio-Rad protein assay is a colourimetric assay for measuring total protein concentration present in a sample and is based largely on the methods of Bradford dye-binding (Bradford, 1976). It is a widely used and suitable method for determining protein concentrations using the colour change of Coomassie brilliant blue G-250 dye in response to various concentrations of proteins. The dye preferentially binds to basic, aromatic amino acid residues. The Bio-Rad protein assay used 5µL of the ground sample protein solution in 995µL of 1:5 diluted Bio-Rad reagent in clean test tubes (2 mL) and then mixed with a vortex. After 5 min, this solution was measured at 595 nm in glass cuvettes. The diluted due reagent was used as a blank. All cuvettes were rinsed with reagent grade water followed by acetone to ensure the removal of sample and dye residues.

3.2.3 Super oxide dismutase (SOD) (1.15.1.1)

SODs converts superoxide radicals into hydrogen peroxide and molecular oxygen. The oxidation of quercetin at pH 10 is a free radical chain reaction in which superoxide is involved and therefore can be terminated but superoxide dismutase. The extent to which quercetin oxidation is inhibited is a function of SOD concentration based on the methods of Kostyuk and Potapovitch, 1989. 50µL enzyme extract was added to a total reaction volume of 2 mL 30 mM tris-HCL buffer at ph 10, 0.5 mM EDTA, 0.5 mL of 0.8 mM TEMED and 0.4 mL 0.05 mM quercetin. The mixture was then vortexed. The solution was then measured at 406 nm for 20 minutes every 5 minutes after being decanted into a glass cuvette.

3.2.4 Catalase (CAT) (1.11.1.6)

Catalase converts hydrogen peroxide to water and oxygen; therefore, the concentration of catalase can be determined by the decrease in absorbance of hydrogen peroxide at $\lambda = 240$ nM (Li and Schellhorn, 2007, Rørth and Jensen, 1967). 50 µL enzyme extract was added to 2.5 mL K phosphate and EDTA pH 7.0, with 0.05 mL 10 mM H_2O_2, 0.25 mL 10 mM beta-mercaptoethanol (BME) to perform this test and the sample was then measured spectrophotometrically at 240 nm after 1 min every 5 seconds.

3.2.5 Ascorbic Acid Peroxidase (APX) (1.11.1.11)

This assay used ASA as a substrate, both within the cell and in the apoplast (Zheng and Van Huystee, 1992, Mehlhorn et al., 1996), similar to glutathione peroxidases in animals at a pH 7 (Foyer and Halliwell, 1976, Asada, 1992b). This principle is utilised on the assays as described by Nakano and Asada (1981). To perform this assay, 50 µL enzyme extract was added to 2.0 mL K phosphate, EDTA pH 7.0, 0.25 mL 10 mM beta-mercaptoethanol (BME), 0.5 mL 0.5 mM ascorbic acid and 0.05 mL 40mM H_2O_2 and measured at 290 nm after 1 min every 5 seconds.

3.2.6 Guaiacol Peroxidase (GPX) (1.11)

The methods used here were modified from Curtis (1971). GPX uses guaiacol as a hydrogen donor, and the absorbance reflects an increase in tetra-guaiacol at 47-nm, i.e., guaiacol oxidized (Smiri et al., 2013). 50 µL enzyme extract was added to 2 mL K phosphate, EDTA pH 7.0, 0.25 mL 10 mM BME, 0.4 mL 20 mM guaiacol, and 40 mM H_2O_2 and measured at 470 nm after 1 min every 5 seconds.

3.2.7 Lipoxygenase (LOX) (1.13.11.12)

The methods utilized were developed by Fau and Zakut, Sekhar and Reddy, 1982 on the principle that linoleic acid is the most suitable substrate for the assay since LOX uses straight-chain fatty acids, ester alcohols as substrates (Fau and Zakut). 50 µL enzyme extract was added to the 2.5 mL tetraborate buffer pH 10.0- and 0.5-mL linoleic acid. Linoleic acid buffer was made using 100µL of linoleic acid stock, 25 mL of tetraborate buffer, 0.1% tween, which was ultra-sonicated until all components were in solution. The reaction was allowed to take place 5 min, after which the absorbance was read at 550 nm every 30 seconds.

3.2.8 Glutathione Reductase (GR) (1.8.1.7)

The reduction of oxidized glutathione (GSSG) is reduced by glutathione reductase to glutathione (Kaur and Sharma, 2018b). This enzyme is important for the glutathione redox cycle, which maintains a balance of reduced cellular GSH, which serves as an antioxidant to react with free radicals in addition to organic peroxides (Balakhnina and Nadezhkina, 2017). Glutathione reductase activity is determined by the increase in absorption caused by the reduction of dithiobis (2-nitrobenzoic acid) (DTNB) at 412 nm (colorimetric assay). 50µL enzyme extract solution was added to 2.5 mL K phosphate and EDTA at pH 7, 0.25 mL 10 mM beta-mercaptoethanol (BME), 0.2 mL reduced glutathione 1 mM and 0.2 mL DTNB (1 mM in ethanol), and the absorbance was read after 5 min at 412 nm every 30 seconds.

3.2.9 Acid Protease (AP) and Neutral Protease (NP)

500µL enzyme extract solution was added to 0.5 mL 50 mM citric acid buffer at pH 5.4, 0.25 mL 4% haemoglobin solution and 0.25 10 mM BME. The solution was incubated at 40°C for 90 min, after which the reaction was stopped with 50µL 40% TCA and centrifuged for 15 min at 12000g. After centrifugation, 500µL of the clear supernatant was collected, and a ninhydrin reagent was added; this solution was then boiled for 20min, proceeding which, the solution was cooled for 15 min. The same method was applied for the quantifying neutral proteases, with the exception being that 0.5 mL 50 mM K phosphate pH 5.4 buffer was added. At 570 nm, the solution was read for 5 min using a spectrophotometer every 30 seconds (Biochrom Libra S12 UV/Vis). Ninhydrin stock solution was made with 0.0008g stannous chloride, 5 mL citrate buffer at pH 5, 0.2g ninhydrin, and 5 mL ethylene glycol. This solution was made as required daily kept on ice at 4°C for the duration of experiments to ensure stability. Blanks were prepared using TCA with measurements taken before the treatments being read.

3.2.10. Replications and statistics

Enzyme assay experiments were designed to capture enough replicates for statistical analysis. The analysis was conducted on the data using means, standard errors, one way analysis of variance (ANOVA). Duncan's test was applied for analysis proceeding the rejection of the null hypothesis by ANOVA allowing for a comparison of all pairs of means with a significance lever of (P= 0.05). All data is reported as means with standard errors and n = 6.

3.3 Results

Figures 6-17 illustrate the concentrations of enzymes, (a) superoxide dismutase and catalase (b) ascorbic acid peroxidase (APX) and linoleic acid (LOX), (c) guaiacol peroxidase (GPX) and glutathione reductase (GR) (d) acid protease (AP) and neutral protease (NP) involved in reactive oxygen species cycles of *Brassica Vitamin Greens, Helianthus annuus, and Neptunia amplexicualis* shoots and roots on exposure to control, selenite and selenate at sublethal treatments concentrations. Data shown are means ± standard error (n=6).

3.3.1 Selenite and selenate SOD and CAT concentrations in *Brassica rapa*

Shoot and root SOD concentrations were significantly higher in the selenite and selenate treatments compared to the control. Shoot SOD concentration in selenite and selenate was not significantly different compared to SOD concentration in the roots. Shoot and root SOD concentration was not significantly different between selenite and selenate.

Shoot and root CAT concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot CAT concentration was significantly higher in the shoots than in the roots. CAT concentration in shoots was not significantly different compared to CAT concentration in the roots. Shoot and root CAT concentration were not significantly different between selenite and selenate.

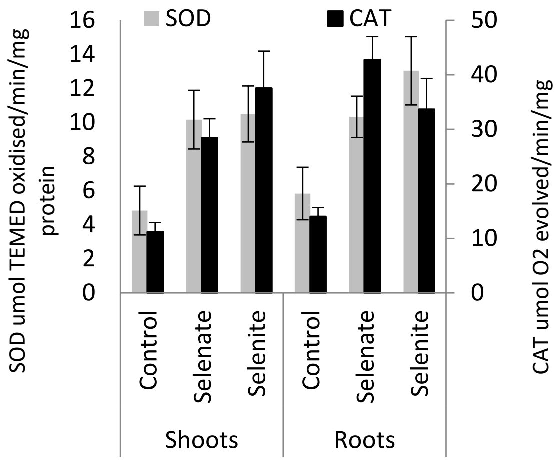

Figure 6. SOD and CAT activity per mg of protein. All data is reported as mean ***±se.*** *n=6*

3.3.2 Selenite and selenate APX and LOX concentrations in *Brassica rapa*

Shoot and root APX concentration was significantly higher in the selenite and selenate treatments compared to the control. Shoot APX concentration in selenite and selenate was not significantly different compared to APX concentration in the roots. Shoot and root APX concentration was not significantly different between selenite and selenate.

Shoot and root LOX concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot LOX concentration was not significantly higher in the shoots than in the roots from selenite treatment. However, LOX concentration in shoots was significantly lower compared to LOX concentration in the roots. Shoot and root LOX concentration with selenite treatment was significantly higher than selenate.

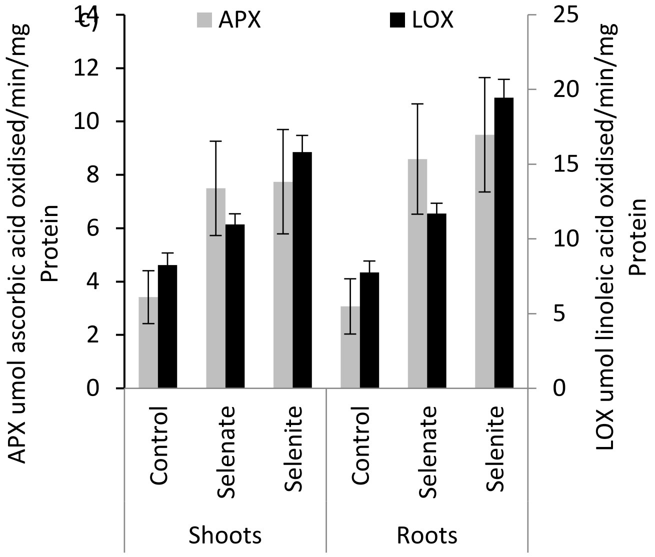

Figure 7. APX and LOX concentrations in Brassica rapa. All data is expressed as mean **±se.** *n=6*

3.3.3 Selenite and selenate GPX and GR concentration in *Brassica rapa*

Shoot and root GR concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot GR concentration in selenite and selenate was not significantly different compared to GR concentration in the roots. Shoot GR concentration was not significantly different from selenite and selenate. Root GR concentration was significantly higher in selenite than selenate.

Shoot and root GPX concentration was significantly higher in the selenite and selenate treatments compared to the control. Shoot GPX concentration in selenite and selenate was not significantly different compared to GPX concentration in the roots. Shoot GPX concentration was not significantly different from selenite and selenate. Root GR concentration was significantly higher in selenite than selenate.

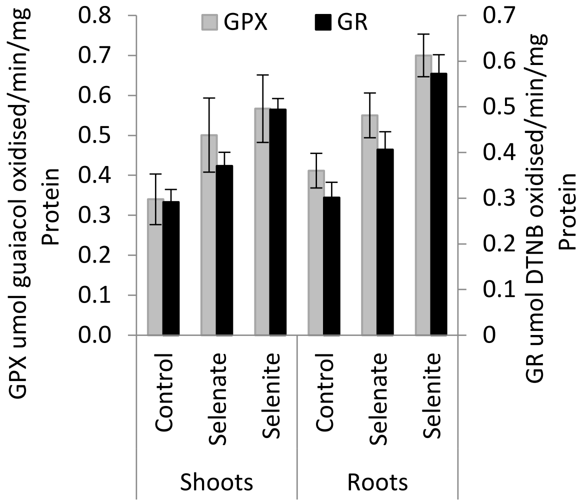

Figure 8. GPX and GR concentrations in Brassica rapa All data is expressed as mean ±***se.*** *n=6*

3.3.4 Selenite and selenate AP and NP concentration in *Brassica rapa*

Shoot and root AP concentration was significantly higher in the selenite and selenate treatments compared to the control. Shoot AP concentration in selenite and selenate was not significantly different compared to AP concentration in the roots. Shoot and root AP concentration was not significantly different between selenite and selenate.

Shoot and root NP concentration was not significantly higher in the selenite and selenate treatments compared to the control. Shoot NP concentration was not significantly different in the shoots than in the roots. NP concentration in shoots was not significantly different compared to NP concentration in the roots. Shoot and root NP concentration was not significantly different between selenite and selenate.

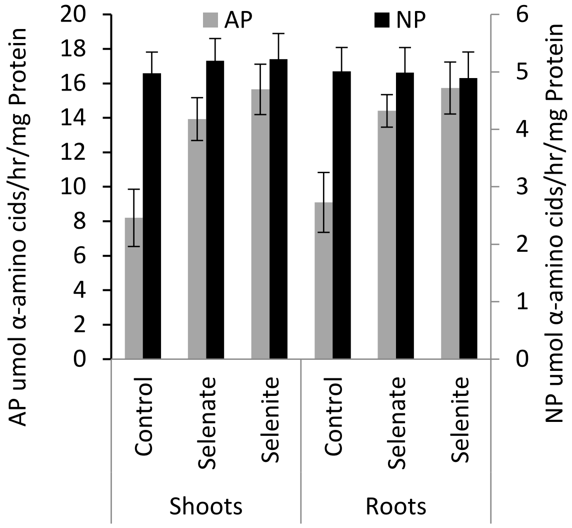

*Figure 9. AP and NP concentrations in Brassica rapa. All data is expressed as mean ±**se.** n=6*

3.3.5 Selenite and selenate SOD and CAT concentration in *Helianthus annuus*

Shoot and root SOD concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot SOD concentration in selenite and selenate was not significantly different compared to SOD concentration in the roots. Shoot and root SOD concentration was not significantly different between selenite and selenate.

Shoot and root CAT concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot CAT concentration in selenite and selenate was not significantly different compared to CAT concentration of selenite and selenate in the roots. Selenite CAT concentration was significantly higher in selenate in shoots, while there were no significant differences between selenite and selenate.

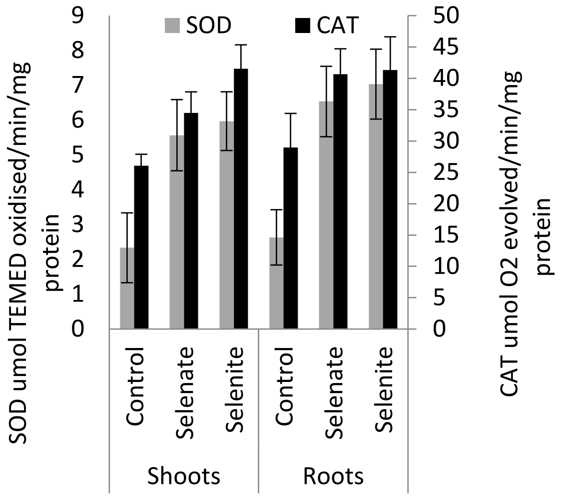

Figure 10. SOD and CAT concentrations in Helianthus annuus. All data is expressed as mean ±***se.*** *n=6*

3.3.6 Selenite and selenate APX and LOX concentration in *Helianthus annuus*

Shoot and root APX concentration was significantly higher in the selenite and selenate treatments compared to the control. Selenite APX concentration was not significantly different between shoots and roots, while selenate was significantly higher in roots than in shoots. There were no significant differences in APX concentration in shoots between selenite; however, the APX levels in roots showed that selenite was significantly higher than selenate.

Shoot and root LOX concentration were significantly higher in the selenite and selenate treatments compared to the control. Selenite LOX concentration was not significantly different between shoots and roots, while selenate was significantly higher in roots than in shoots. Selenite was significantly higher than selenate in shoots and roots.

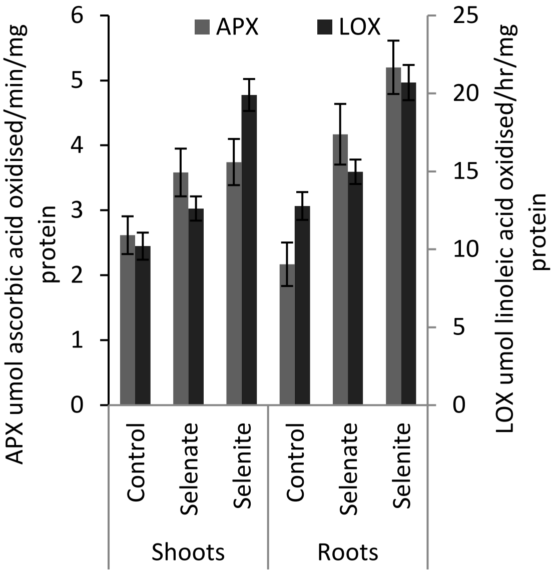

Figure 11. APX and LOX concentrations in Helianthus annuus. All data is expressed as mean ±s. *n=6*

3.3.7 Selenite and selenate GPX and GR concentration in *Helianthus annuus*

Shoot and root GPX concentration was significantly higher in the selenite and selenate treatments compared to the control. Shoot GPX concentration in selenite and selenate was not significantly different compared to GPX concentration of selenite and selenate in the roots. In shoots selenite, GPX concentration was significantly greater than selenate, while there was no significant difference between selenite and selenate.

Shoot and root GR concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot GR concentration in selenite and selenate was not significantly different compared to GR concentration of selenite and selenate in the roots. In shoots, selenite GR concentration was significantly greater than selenate, while there was no significant difference between shoots and roots in selenate. In shoots, selenite was significantly higher than selenate, while there were no significant differences in roots between selenite and selenate.

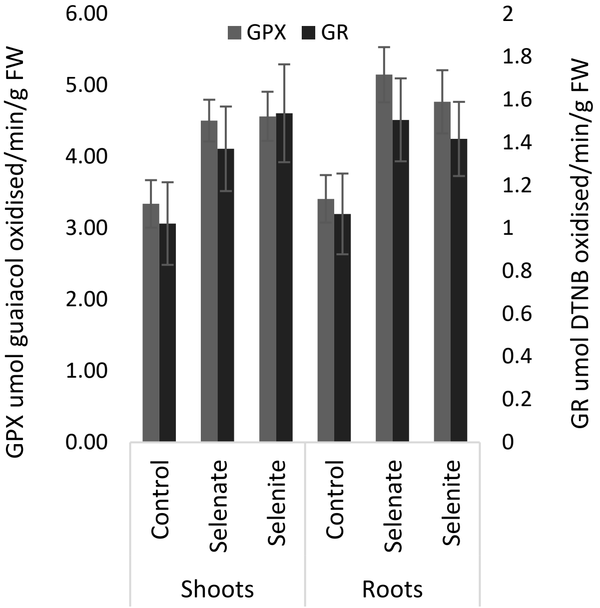

Figure 12. GPX and GR concentrations in Helianthus annuus. All data is expressed as mean ±***se.*** *n=6*

3.3.8 Selenite and selenate AP and NP concentration in *Helianthus annuus*

Shoot and root AP concentration showed no significant differences between selenite and selenate treatments compared to the control. Shoot AP concentration in selenite and selenate was not significantly different compared to AP concentration in the roots. Shoot and root AP concentration was not significantly different between selenite and selenate.

Shoot and root NP concentration was not significantly different in the selenite and selenate treatments compared to the control. Shoot NP concentration was not significantly different in the shoots than in the roots. NP concentration in shoots was not significantly different compared to NP concentration in the roots. Shoot and root NP concentration was not significantly different between selenite and selenate.

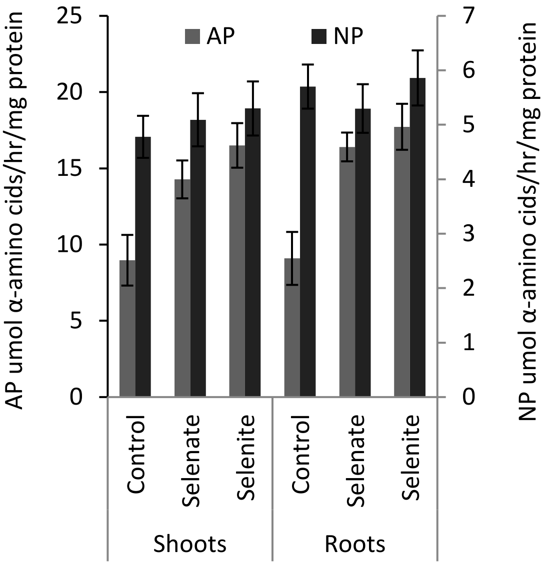

Figure 13. AP and NP concentrations in Helianthus annuus. All data is expressed as mean ***±se*** *n=6.*

3.3.9 Selenite and selenate SOD and CAT concentration in *Neptunia amplexicualis*

Shoot and root SOD concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot SOD concentration in selenite and selenate was not significantly different compared to SOD concentration in the roots. Shoot and root SOD concentration was not significantly different between selenite and selenate.

Shoot and root CAT concentration were significantly higher in the selenite and selenate treatments compared to the control. Shoot CAT concentration was significantly higher in the shoots than in the roots. CAT concentration in shoots was not significantly different compared to CAT concentration in the roots. Shoot CAT concentration was significantly higher in selenite than selenate. Root CAT concentration was not significantly different between selenite and selenate.

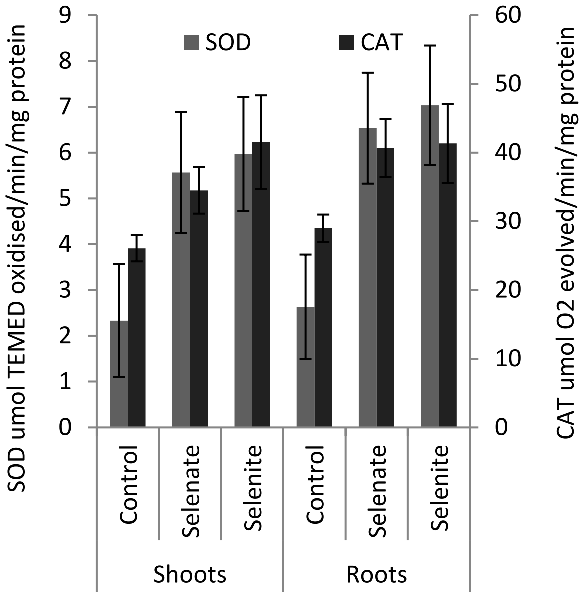

Figure 14. SOD and CAT concentrations in Neptunia amplexicualis. All data is expressed as mean ±se. n=6

3.3.10 Selenite and selenate APX and LOX concentration in *Neptunia amplexicualis*

Shoot and root APX concentration was not significantly different in the selenite and selenate treatments compared to the control. Shoot APX concentration in selenite was not significantly different from the control. However, APX concentration from selenate treatment roots was significantly higher than the control. Shoot and root APX concentration was not significantly different between selenite and selenate.

Shoot and root LOX concentration were significantly higher in the selenite and selenate treatments compared to the control. Selenite shoot LOX concentration was significantly higher in the shoots than the roots; selenate LOX concentration showed no differences between shoots and roots. Selenite in the shoots and was significantly greater in LOX concentration than selenate.

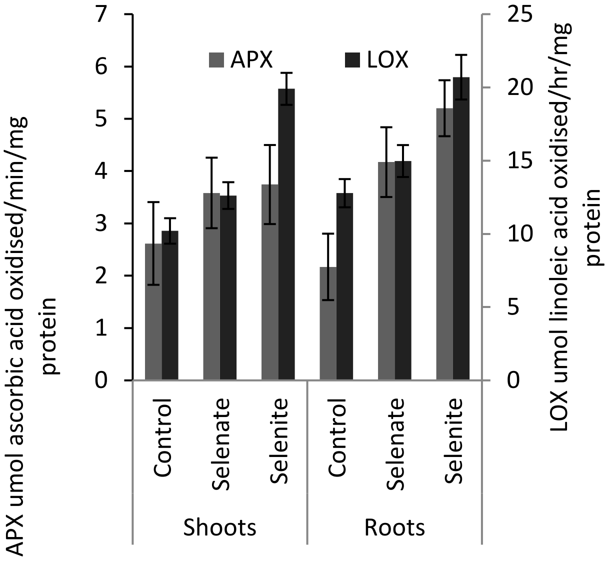

Figure 15. APX and LOX concentrations in Neptunia amplexicualis. All data is expressed as mean ±se. n=6

3.3.11 Selenite and selenate GPX and GR concentration in *Neptunia amplexicualis*

In Figure 16, Shoot and root GPX concentration was significantly higher in the selenite and selenate treatments compared to the control. Selenite and selenate GPX concentration was not significantly different compared between shoots and roots. Shoot GPX concentration was significantly not different in selenite compared to selenate. Root GPX concentration was not significantly different between selenite and selenate.

Shoot and root GPX concentration was significantly higher in the selenite and selenate treatments compared to the control. Selenite shoot GPX concentration in selenite was not greater than selenate, while selenite was significantly higher in shoots compared to GPX concentration in the roots. Shoot and root GPX concentration was not significantly different between selenite and selenate.

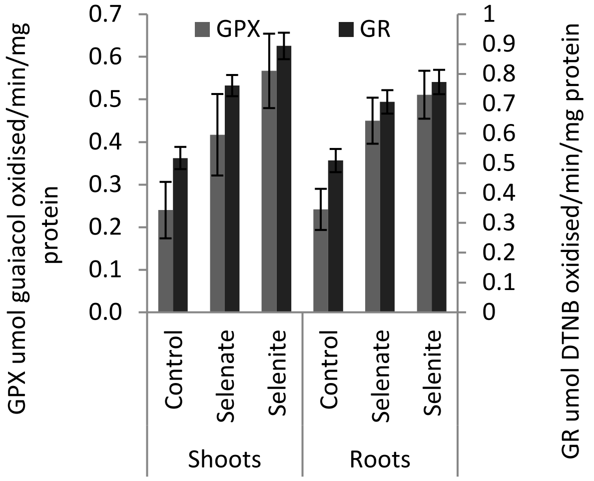

Figure 16.GPX and GR concentrations in Neptunia amplexicualis. All data is expressed as mean ±se. n=6

3.3.12 Selenite and selenate AP and NP concentration in *Neptunia amplexicualis*

Shoot and root AP concentration was not significantly in the selenite compared to the control. Shoot AP concentration was not significantly different from the control; however, AP concentration with selenate treatment in roots was significantly higher compared to the control. Shoot AP concentration in selenite and selenate was not significantly different compared to AP concentration in the roots. Shoot and root AP concentration was not significantly different between selenite and selenate.

Shoot and root NP concentration was not significantly higher in the selenite and selenate treatments compared to the control. Shoot NP concentration was not significantly different in the shoots than in the roots. NP concentration in shoots was not significantly different compared to NP concentration in the roots. Shoot and root NP concentration was not significantly different between selenite and selenate.

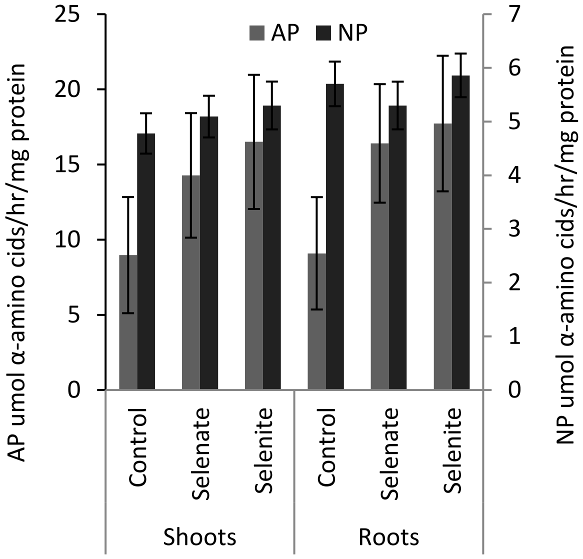

Figure 17. AP and NP concentrations in Neptunia amplexicualis. All data is expressed as mean ±se. n=6

3.4 Discussion

3.4.1 Effect of selenate and selenite on SOD concentration

SOD first demonstrated in maize is a metalloenzyme superoxide dismutase which converts $O_2^{-\bullet}$ to H_2O_2 (Gupta and Gupta, 2017) It is generally upregulated during the increase in oxidative stress caused by abiotic factors, and therefore it has a critical role in the survival of plants (Brown and Shrift, 1982). SOD has been studied in other plants under salt stress in pea, maize, and tea (Ahmad et al., 2010, Tuna et al., 2008, Upadhyaya et al., 2008) but not with Se stress. SOD is the first step triggered by potential oxidative damage and is present in every cell of plants (Brown and Shrift, 1982).

Selenium toxicity can be attributed to two fundamental causes, malformed selenoproteins and the induction of oxidative stress. Se is a pro-oxidant, and as a result, can propagate oxidative stress in plants. Therefore the notable significant increases and decreases in some of the key reactive oxygen species in these experiments are expected based on current literature (Gupta and Gupta, 2017). These findings are similar to those found in this project with selenium salts, where SOD was found to be significantly higher in shoots and roots exposed to selenate and selenite salts compared to the controls ($p<0.05$), and this was seen across all plants selected for this study. Sublethal concentrations are thought to induce an oxidative stress response, during which Se may replace sulphur in protein formation, causing improperly formed protein structure (Brown and Shrift, 1982). This result was also found in studies where Se-induced stress-triggered oxidative stress (Kaur and Sharma, 2018a). Se exposure has been documented to cause an increase in many levels of oxidative species in plants as listed in the studies conducted by the stress of Se (Akbulut and Çakır, 2010, Chen et al., 2008, Gomes-Junior et al., 2007, Schiavon et al., 2012a).

A previous study completed in *Brassica napus* also showed an increase in SOD concentration when increasing doses of arsenic were applied (Liu et al., 2015). In the control species of all plants for there is a balance in the reactive oxygen species; therefore, the removal and the formation of oxygen species are in equilibrium, however, on exposure to sublethal concentrations of 20 µm, 25 µm, and 500 µm of selenite and selenite across *Brassica, Helianthus annuus* and *Neptunia amplexicaulis* varieties the ROS defence system is triggered causing an imbalance. SOD which occurs in the mitochondria, chloroplasts, microsomes, glyoxysomes, peroxisomes, apoplast, and the cytosols, is the first line of defence to restore oxidative stress (Alscher et al., 2002). Plants exposed to selenium as fertilizer after water stress demonstrated an increase in SOD. At low levels of exposure, selenium is thought to have a beneficial impact on plant growth. While both species of selenium salts were higher in treated plants, there was no significant difference between the SOD levels of selenite and selenate treatments. There were no significant differences found between selenite and selenate impact on SOD levels in shoots and roots for all the plants studied. This finding was expected because it is inferred that in alkaline media, selenate uptake is greater than selenite, and the opposite is true in acidic media, and the media prepared was set at pH5.4 (Tian et al.). SOD levels in this study were all elevated given that the plants were all exposed to pre-decided sublethal concentrations in chapter 1.

3.4.2 Effect of selenite and selenate on CAT concentration

A family of heme-containing enzymes contains catalase, an enzyme that is critically important for the dismutation of H_2O_2 into H_2O and O_2, which is important for the metabolic and defence of plants in addition to signalling (Liu et al., 2015). All aerobic eukaryotes contain CAT where it plays a role in the removal of H_2O_2. This is manufactured in peroxisomes by oxidases involved in beta-oxidation, particularly of fatty acids, photorespiration, purine catabolism which takes place during oxidative stress (Sofo et al.) Localized in peroxisomes and mitochondria, CAT is categorized into three main groups; class I localized in photosynthetic tissues, class II catalases associated with vascular tissues and class III catalases are concentrated in seeds and reproductive tissues (Anjum et al.).

Across all plants studied, there were significantly higher levels of catalase in both treatments of selenite and selenite amongst the shoots and the roots. Similar studies completed by Freeman et al. (2010b) demonstrated that proteins, glutathione *S*-transferase, two catalases, a GDP-D-Man pyrophosphorylase, a ferredoxin, a glutathione peroxidase, a glutaredoxin, and an ATP-dependent peroxidase were higher in the selenium hyperaccumulator *S. pinnata* than the secondary accumulator S. *Albescens*; all encoded by antioxidant and redox control genes in the leaves. Amongst the *Brassica*e varieties in other studies exposed to higher concentrations of selenate at 40uM (Ávila et al., 2013, Lyi et al., 2005, Ramos et al., 2011) showed a decrease in CAT concentration which are findings to the contrary found in these studies where all plants showed an increase in CAT concentration upon exposure to the sublethal concentrations; however, this may be attributed to inhibitory effects of Se at higher concentrations (Tian et al., 2017). *Brassica* studies exposed to selenite showed an increase in ROS generation in the roots (Chen et al., 2014, Freeman et al., Mroczek-Zdyrska, and Wojcik). Similarly, ~~to~~ the *Brassica rapa* studied by Chen et al. (2014) demonstrated that selenite caused an increase in ROS in the roots similar to that seen in Figures 1, 2,3, and 4. To date, literature is unclear and inconsistent as to the effect of Se on CAT concentration.

3.4.3 Effect of selenite and selenate on GPX concentration

Figures 1, 2, 3 c, and g demonstrated that *Brassica rapa vitamin greens, Helianthus annuus,* and *Neptunia amplexicaulis* had a significant increase in GPX concentration in treatments with selenite and selenate salts compared to the control. These results are substantiated by similar findings in other varieties of plants where selenium induced an increase in GPX concentration (Balakhnina and Nadezhkina, 2017). This increase in GPX concentration, in combination with other ROS species cascade, help to improve plant resistance against abiotic stresses such as drought, UV, radiation, PCD, and temperature fluctuations; thereby improving plant adaptive capacity (Balakhnina and Borkowska, 2013, Balakhnina et al., 2015, Balakhnina et al., 2005, Hartikainen et al., 2000, Ibrahim, 2014). GPX is thought to be a crucial enzyme triggered by Se in numerous plants that are exposed to stresses (Ali et al., 2015, Lin et al., 2012, Qing et al., 2015). GPX works to convert GSH to CSSG through oxidation, as shown in figure 4. There were significant increases in GPX when exposed specifically to metalloids.

3.4.4 Effect of selenite and selenate on GR concentration

Shoot and root GR concentration were significantly higher in the selenite and selenate treatments compared to the control, as was demonstrated similarly in rapeseed seedlings. (Hasanuzzaman et al., 2011). Shoot GR concentration in selenite and selenate was not significantly different compared to GR concentration of selenite and selenate in the roots. In shoots, selenite GR concentration was significantly greater than selenate, while there was no significant difference between shoots and roots in selenate. These findings were similar to increases in GR in *Triticum Aestivum L* as a result of selenite and selenate exposure (Kaur and Sharma, 2018b).

3.4.5 Effect of selenite and selenate on APX concentration

Plants treated with selenite and selenate both showed and a notable increase in APX compared to the control in shoots and roots. This result at the selected dosages is to be expected since plants are still under stress. APX has the role of detoxifying H_2O_2 in the presence of ascorbate and glutathione (GSH) as part of the ascorbate-glutathione cycle, therefore elevated APX concentration will result in a greater stress response in the plants. Our findings are supported by the studies in *Lemna gibba*, L. minor, and *Spirodela polyrhiza* as described by (Banu Doğanlar, 2013).

3.4.6 Effect of selenite and selenate on protease concentration

Protease concentration plays a critical role in the degradation of proteins, with concentration heavily dependent on a range of factors such as leaf age, localization, and external stresses (van der Hoorn, 2008a) (Simova-Stoilova et al., 2010a). Proteases are best categorized as acidic or neutral protease (Balakrishnan et al., 2011). Under stressed oxidative conditions such as the exposure of sublethal concentrations of selenium, there were no notable increases in AP concentration with selenite and selenate exposure compared to the control in both *Helianthus annuus* and *Brassica*.

It has been shown in Grevillea leaf tissues that high protease concentration has been demonstrated, the result of high oxidative stress (Kennedy and De Filippis, 2004). However, given that all three plant species were exposed to sublethal concentrations of selenium, there were no significant differences in the AP and NP concentration compared to the control except for *Neptunia,* where AP concentration with selenite treatment in roots was significantly higher compared to the control. While no significant difference was calculated, acid protease concentration was still higher in both treatments of selenite and selenate for all three plants. This is attributed to the sublethal concentration exposure to selenium. These elevated levels of stress result in the formation of carbonyl groups which can also be used to measure proteolytic concentration (Reinheckel et al., 1998) under stress conditions but were not measured as a part of this study.

On the other hand, there were no significant differences seen in the concentration of neutral proteases across all three plants. Given that protease consist of a range of protein subclasses, such as acid endopeptidases (Fukayama, Abe & Uchida 2010;) aspartic peptidase (APs) (pepsin-like proteases), and cysteine peptidases (CPs) (papain-like proteases) and/or carboxypeptidases (belong to serine peptidases (SPs) are mostly active at acidic pH (van der Hoorn, 2008a)((Rawlings et al., 2014), the greater detection of protease concentration may be in the acid proteases compared to the relatively neutral proteases.

3.5 Conclusion and future direction

On the comparison of the oxidative stress reaction on the reactive oxygen species across *Helianthus annuus*, *Brassica rapa,* and *Neptunia amplexicaulis,* there were numerous patterns observed and measured that form a foundation for further analysis outside of this experiment.

For all three plants, all reactive oxygen species were elevated compared to the control in line with previous studies that analyzed reactive oxygen species under stress conditions such as chilling, drought stress, metal exposure, temperature, among many others, albeit these elevated levels of enzymatic reactive oxygen species were not always significant.

The inclusion of a greater range of enzymes will enable more enzyme concentration to be accounted for in detail. The limitations of time and resources presented challenges in adding more metabolites and enzymes to this series of experiments. However, the trends observed in the elevated levels of the treatments of selenium to the control demonstrate that at sublethal concentrations, there is an oxidative stress response within each plant that allows it to cope with the added levels of selenium. As mentioned above, ROS are very reactive due to the presence of unpaired valence electrons, and a high concentration of ROS can result in uncontrolled oxidation in cells, during which cellular components, including (Mittler, 2002) DNA, protein, membrane lipids, may become damaged (Cassells and Curry, 2001). Evidence suggests that the functional roles of these defence ROS responses include the protection of the photosynthetic mechanisms, the preservation of membrane integrity, and the protection of the DNA and proteins (Alscher et al., 1997, Gill and Tuteja, 2010a, Kovalchuk, 2010, Apel and Hirt, 2003, Gill and Tuteja, 2010b).

The information gathered from these experiments confirms a stress response at sublethal concentrations supported by previous studies discussed above. To determine the proteins being impacted by the uptake of Se at sublethal concentrations in Chapter 4, seed storage proteins will be studied.

Chapter 4. Selenium distribution in plants

Abstract

Selenium has emerged as a contaminant in terrestrial and aquatic environments in many regions around the world as a result of mining. Extensive research has concluded that selenium is an essential micronutrient to humans, animals, and some microorganisms, but to date, limited research has been conducted to determine the essentiality of selenium to higher plants concerning selenium translocation and accumulation on a molecular level in Australia.

The current work thus investigated: 1) patterns of translocation of radiolabelled selenite and selenate by whole plant autoradiography, 2) the quantity of the 75Se accumulated in DNA, RNA, lipids, carbohydrates, and various classes of proteins. The plants, *Brassica rapa* 'Vitamin Greens', *Helianthus annuus* 'Dwarf *Helianthus annuus*' and *Neptunia amplexicaulis* were grown in tissue culture growth media and harvested after 21 days of exposure to a range of increasing concentrations of selenite and selenate salts of 5-50µM and 10-100µM, respectively, after which sublethal exposure concentrations were selected.

The sublethal concentrations were 20µM of each selenium salt for *Brassica rapa* and 25µM of each selenium salt for *Helianthus annuus*. Autoradiography displayed Se75 translocation of selenite in relatively greater proportions in the leaves than roots and selenate in the roots than leaves. These patterns of translocation coupled with further fractionation studies in chapter 5 will assist in the selenoprotein studies at a later stage to determine if selenium is incorporated into proteins or protein-bound in these plants. The patterns of selenium translocation, its accumulation, and possible phytovolatilization in plants have implications on their suitability as tools for phytoremediation and biofortification.

4.1 Introduction

4.1.1 Selenium species

Se is a naturally occurring element that is essential for optimal health and maintenance of human and animal tissue function; it is yet to be classified as biologically essential for plants. As described in chapter 1, selenium is analogous to sulphur. It forms a part for the chalcogens group with the most common valence states being, -2, 0, +2, +4, +6 and occurs as Se^{2-} (selenite), Se^0 (elemental selenium), $Se_2O_3^{2-}$ (thioselenate), SeO_3^{2-} (selenite), and SeO_4^{2-} (selenate) respectively. The bioavailability, uptake, distribution, metabolism, and volatilisation within living organisms are influenced by the chemical state of Se, the type of vegetation, the chemical form of Se in soil, pH of the soil, moisture content, the concentration of Se, and the composition of the soil. The two most bioavailable forms of Se are selenite and selenate, but some plants can also absorb selenomethionine from the decay of selenium contained in decaying plant material. Most often, selenium is found in its elemental form associated with shale, sulphide ores, and coal mining; on exposure to air is converted to selenite and selenate, making it bioavailable to animals and plants, which within a narrow, low range, can be detrimental to ecosystem health.

As described in chapter 1, the plants selected for this study were *Brassica vitamin green*, *Helianthus annuus,* and *Neptunia amplexicaulis*. The sublethal concentrations that the plants were subjected are the same as those in chapter 1.

4.1.2 Selenium distribution

These experiments were performed on a whole plant level to provide a qualitative and quantitative analysis, visually the uptake of selenium in three plants known for varying levels of selenium absorption, the patterns of uptake in the leaves, stems, and roots. Additionally, an understanding of the location of Se accumulation has the potential to include the plant used in this study for phytoremediation. Toxic contaminants can be absorbed by the roots and transported to the upper organs of the plants, which can be later harvested or volatilized into a less toxic form (Newman and Reynolds, 2004). It is considered cheaper and has a less likely hood to disrupt the fertility of the soil (Robinson et al., 2000). *Stanleya pinnata* and *Astragalus bisulcatus* have an abundance of research to indicate their strong ability to accumulate Se;

however, the slow rate of growth and low biomass production limit the phytoremediation potential (Germ et al., 2007a), presenting a need to consider alternative plants for potential use in phytoremediation.

To date, there are almost no existing studies of Se in higher plants using autoradiographic methods, and as mentioned previously, the few existing studies study Se at concentrations that are considered to benefit plant growth (Mounicou et al., 2006) but not at the sublethal concentrations studied in this thesis. Given that some species can cope well with the increased concentration of Se, other species manifest signs of stunted growth, leading to early or eventual death (Chéry et al., 2002). There is a need to gain an understanding of Se function in individual plant species. Furthermore, the identification and understanding of the ability of plants to accumulate Se also allow the opportunity to use the plants for biofortification as different plants have a varying ability to tolerate and accumulate Se. It is important to focus on plants based on their ability to accumulate Se in their edible parts (Lynch, 2007).

4.1.2 Selenium distribution methods

To date, numerous analytical and instrumental methods such as microwave digestion and fluorescence detection have been utilized to find selenium in samples from the environment(Nahir and Sheffield, 2002, Pyrzyńska, 2002). Many challenges in the detection of Se have been identified, and in recent years some have been overcome stemming from the narrow range between essential Se and the toxic concentration, including developments in detection sensor material, electrode surface modifiers, chelators, along with improved detection limit, range, reproducibility, stability, selectivity, and sensitivity (Devi et al., 2017). Yet, there are still many complexities presented by the samples matrix and the end goal of Se quantification and/speciation (Paikaray, 2016).

Broadly, selenium detection methods can be applied through destroying samples such as inductively coupled plasma mass spectroscopy (ICP-MS), a hydride generation atomic fluorescence spectrometer (HG-AFS), or the hydride generation atomic absorption spectroscopy (HGAAS) or Instrumental Neutron Activation Analysis (INAA) which is gaining popularity, not considered destructive to the sample but an only be used on-air samples currently (Ballihaut et al., 2007). ICP -MS is most suitable when Se detection in samples is routine and multi-elemental, while INNA is best used when Se is expected to be found at minute concentrations (10^{-8}–10^{-9} g) (Uden, 2002). Comparatively, HGAAS can be utilised when lower detection limits are thought to be present (0.1 µg) (Buchberger, 2001, Mazej et al., 2006, Vassileva et al., 2001).

More conventional methods of Se detection are gas chromatography (GC), high-performance liquid chromatography (HPLC), ICP-MS, neutron activation analysis (NAA), atomic fluorescence spectrophotometry (AFS), AAS, electrothermal AAS, and graphite furnace AAS (GF-AAS), which have been used on plant samples to detect Se concentrations widely. For improved speciation and detection, hyphenated separation methods such as Ion chromatography (IC) and capillary electrophoresis (CE) have been applied with atomic spectrophotometry detection systems such as Electrothermal AAS and plasmic optical emission spectroscopy (POES) (Bailey, 2017).

Increasingly when attempting to detect trace analysis of toxic elements, it is vital to find the total content of the element at lower concentrations while also determining the binding state. Determination of the binding state will influence its toxicity as seen with selenite and selenate, which are both more bioavailable through their water solubility compared to elemental Se, metabolism in environmental and biological systems (Fairweather-Tait et al., 2010)

Some methods depend on sample interaction and a source of X-ray excitation, which include energy-dispersive X-ray spectroscopy (EDXRS), Fourier-transform infrared spectroscopy (FT-IR), powered X-ray diffraction (PXRD), extended X-Ray absorption fine structure (EXAFS) and X-ray absorption spectroscopy (XAS) to determine mechanisms of selenium sorption, (Zhang, 2007). To date, X-ray Absorption Spectroscopy has been used on *A. bisulcatus* plants initially grown for three weeks in selenate (Pickering et al., 2000), but there are no distribution studies that reflect visually or quantitatively selenium distribution within the plants in this study. Additionally, in the existing studies, despite the improvements to methodology, the presence and absence of previously identified selenium species remain inconsistent, highlighting the need for selenium distribution studies using an alternative technique such as radiotracers as used in Chapters 4 and 5 in this thesis.

ICP- MS has been previously used to determine Se concentrations in *Brassica juncea* species (Sharma and Sohn, 2009, Szpunar, 2005) along with size exclusion chromatography studies (Mounicou et al., 2006), all yielding varied results in determining the distribution of Se within the plants, therefore, using autoradiography as a technique for determining the distribution of Se in plants was worth exploration.

Implementing the use of short half-life radioisotopes from the whole plant to genetic, molecular, organismal, and ecosystem studies can be used to determine the process that binds the enzyme-based biochemical reactions to the physiological responses of plants to environmental stimuli and stresses (Osmond, 1989). Positron emission tomography (PET) has been widely used in medical diagnostic imaging (Antoch et al., 2003, Phelps, 2000, Schaller, 2004) with an increasing number of studies using this method on plants coupled with other imaging methods, for instance, photoswitch imaging detector was utilised for both positron- and γ-ray-emitting nuclide imaging (Wu and Tai, 2011). Where live imaging systems were needed PlanTIS and PETIS were used to determine the movement of radionuclides in tomato (Suwa et al., 2008), broad bean (Matsuhashi et al., 2006), beet and bulbs (Jahnke et al., 2009), soybean (Kawachi et al., 2011) barley (Tsukamoto et al. 2006), wheat (Matsuhashi et al., 2006) and rice (Ishikawa et al., 2011). ^{11}C (Minchin and Thorpe, 2003), ^{13}N (Ohtake et al., 2001), ^{15}O (Kiyomiya et al., 2001), ^{18}F (Nakanishi et al., 2001), ^{52}Fe (Tsukamoto et al., 2009), ^{52}Mn (Tsukamoto et al., 2006) and ^{107}Cd (Fujimaki et al., 2010) were used as the positron emitters for these imaging methods.

Where live tracing of a radiotracer isn't needed, radiotracers can also be used on a whole plant level to a genetic level, but to date, at the time of writing, there are no studies of selenium at sublethal concentrations. Additionally, even with improvement to detection of minute concentrations of Se, there are varying conclusions on the existence of major Se-containing compound in the leaves is Se-methyl selenocysteine, with smaller amounts of selenocystathione and γ-glutamyl-Se-methyl selenocysteine in *Astragalus bisculcatus* (Neuhierl et al., 1999, Nigam and McConnell, 1969). Even more robust studies using micro X-ray Fluorescence Microscopy (μXRF) and scanning electron microscopy (SEM-EDS), as well as on chemical forms of Se in various tissues using liquid chromatography-mass spectrometry (LC-MS) and synchrotron X-ray absorption spectroscopy (XAS) has only shown methyl-selenocysteine and seleno-methionine in the foliar tissues but not Se-methyl selenocysteine (Harvey et al., 2020).

4.1.3 Aims

These experiments aimed to determine Se concentrations in plants using radiotracers. This study investigated the uptake, accumulation, and distribution of selenium species in *Brassica rapa.cv vitamin green*, *Helianthus annuus cv. Helianthus annuus* and *Neptunia amplexicaulis,* through the entire plant and plant organs, upon a 21-day exposure to selenite and selenate salts and autoradiography followed by a detailed analysis of plant protein fractions in chapter 5.

4.2 Methods

4.2.1 Culture Preparation.

1L of 1/4 strength media solution was prepared with 1.0825g MS salts, 7.5g sucrose and stirred over a hot plate to ensure that all components were dissolved to set media for tissue culture. 600 mL was then placed into a 1L stock bottle with 0.8g of agar to set media for tissue culture. All bottles of tissue culture were placed in an autoclave on an agar setting for 30 min at 121°C, 104kPa, and left to set at room temperature to cool. Culture media was used the same day as preparation.

4.2.2 Seed preparation.

Seeds of the *Brassica rapa* cv. Vitamin greens and *Helianthus annuus* were sourced from Rangeview seeds, Derby, Tasmania. *Neptunia amplexicaulis* seeds were sourced from QLD by Dr Ashwa Nanjappa Central Queensland University, Australia. Hard seed shells were removed by hand without damaging the seed inside. Once the seed shell was removed, all seeds were surface sterilised with 70 % (v/v) ethanol for 3 min, followed by five autoclaved MQ water rinses. Seeds were then washed in 2% sodium hypochlorite solution for 5 min, after which they were rinsed five times autoclaved MQ water again. All seeds were used immediately after sterilisation. *Neptunia amplexicaulis* seeds were nicked with a nail clipper to break open the seed coating to ensure penetration of nutrients and initiate the first stage of the triphasic germination begin, i.e. rapid initial uptake (Bewley, 1997, Manz et al., 2005, Schopfer and Plachy, 1984). This was not necessary for the softer seed coatings. Plants were grown for 21 days at a temperature of 24°C, relative humidity of 85-95 %, and a day/night cycle of 12 hours/12 hours. A data logger was used to ensuring that humidity and temperature were consistent throughout the experiment.

4.2.3 Radiotracer stock preparation.

0.1g sodium selenite Na_2SeO_3 and 0.10023g sodium selenate Na_2SeO_4 (BioReagent $\geq$ 98%, Sigma-Aldrich, Germany) were measured on a weighing scale added to a titanium capsule. After neutron activation to produce radioisotopes ^{75}SeIV and ^{75}SeVI at the OPAL reactor, ANSTO, Sydney, Australia. Activated salts were dissolved in Milli-Q water to produce stock solutions for both Se species. Selenium speciation of each stock solution was confirmed using ion chromatography. Inductively coupled plasma mass spectrometry (ICP) was used to validate the concentration of Se in each stock solution using internal standards. Radiotracers were then further diluted with stable sodium selenite and selenate stock solutions to form required sublethal concentrations determined in chapter 1, i.e. *Brassica rapa*, 20µM for both selenite and selenate salts, *Helianthus annuus* 25µM for both selenite and selenate and 500µM for both selenium salts for *Neptunia amplexicaulis*. The selenium stocks were diluted into 300 mL autoclaved agar and distributed into (n=6) 200 mL tissue culture jars. Six seeds of each plant variety were embedded into the surface of the agar and sealed with a lid for fractionation studies. The same procedure was followed for whole plant autoradiography except one plant variety per treatment of selenite, and selenate was placed in tissue jars (n=3).

4.2.4 Autoradiography

After the completion of 21 days, all three plants of each variety were removed from the tissue culture for autoradiography. The roots of all plants were individually cleaned on paper towels and patted to remove agar media. The roots were then rinsed with MQ water on the paper towel and dried. This process was repeated four times for each plant. Each plant was then spread onto an A4 sheet with double-sided tape and covered with a plastic sleeve to prevent direct contact with the phosphor plate. Each plant was then exposed to a blank phosphor plate for 66.5 hours for optimal signal; 3 different periods were used to determine the optimal sample exposure time to the phosphor plate.

Each plant was removed from the three plants from each plant variety, extracted from the tissue culture, and placed on double-sided tape on an A4 paper for exposure to a phosphor plate for 66.5 hours with standards and calibrated on Image J.

4.3 Results

The results presented below in Figures 18,19, and 20 show the patterns of Se uptake in the three plants, *Brassica rapa*, *Helianthus annuus,* and *Neptunia amplexicaulis.*

Figure 18 indicates that qualitatively selenium uptake in leaves on the *Brassica rapa* exposed to selenite is less than that of the *Brassica rapa* that was exposed to selenate by two times whereas based on the images alone, the Se content in the roots in the *Brassica rapa* looks similar (this will be discussed in greater detail in chapter 5).

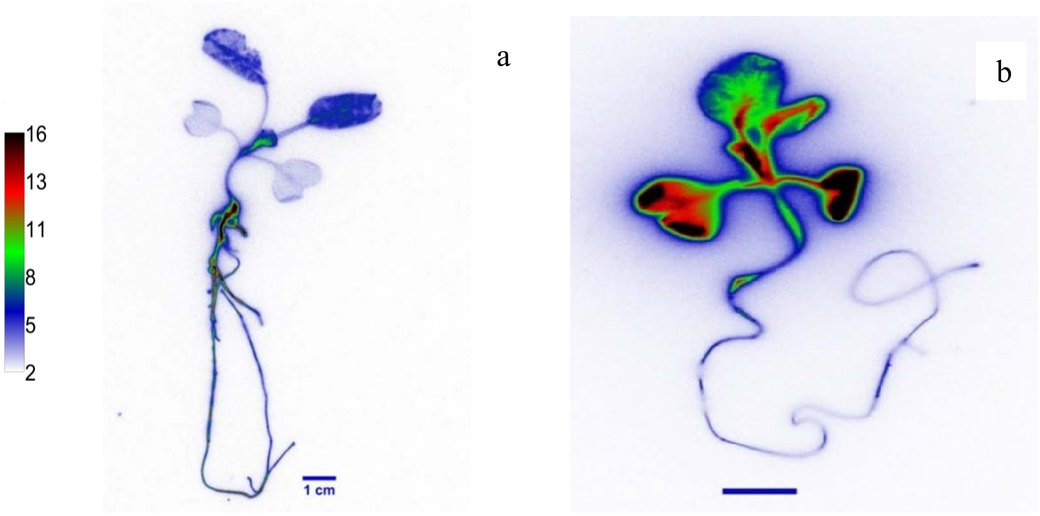

Figure 18. Selenium uptake as selenite (a) Se pg/pixel and selenate (b) Se pg/pixel in Brassica rapa.

Figure 19 indicates that qualitatively selenium uptake in leaves on the *Helianthus annuus* exposed to selenite is less than that of the *Helianthus annuus* that was exposed to selenate by two times, whereas based on the images alone, the Se content in the roots in the *Helianthus annuus* exposed to selenite is greater than the selenium uptake in the plants exposed to selenate.

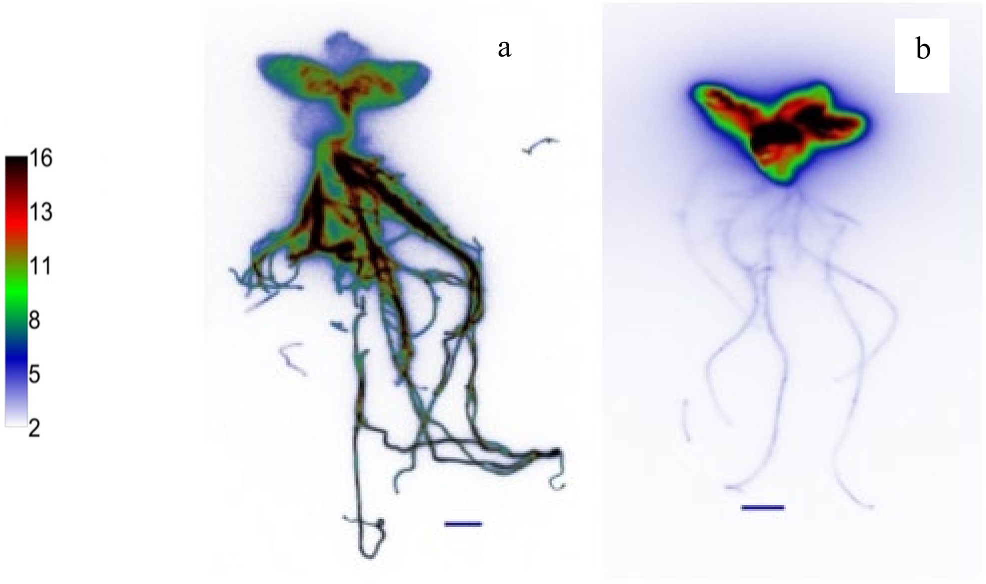

Figure 19. Selenium uptake as selenite (a) Se pg/pixel and selenate (b) Se pg/pixel in Helianthus annuus.

Figure 20 indicates that qualitatively selenium uptake in leaves on the *Neptunia amplexicaulis* exposed to selenite is less than that of the *Neptunia amplexicaulis* that was exposed to selenate by two times,whereas based on the images alone, the Se content in the roots in the *Neptunia amplexicaulis* looks similar (this will be discussed in greater detail in chapter 5).

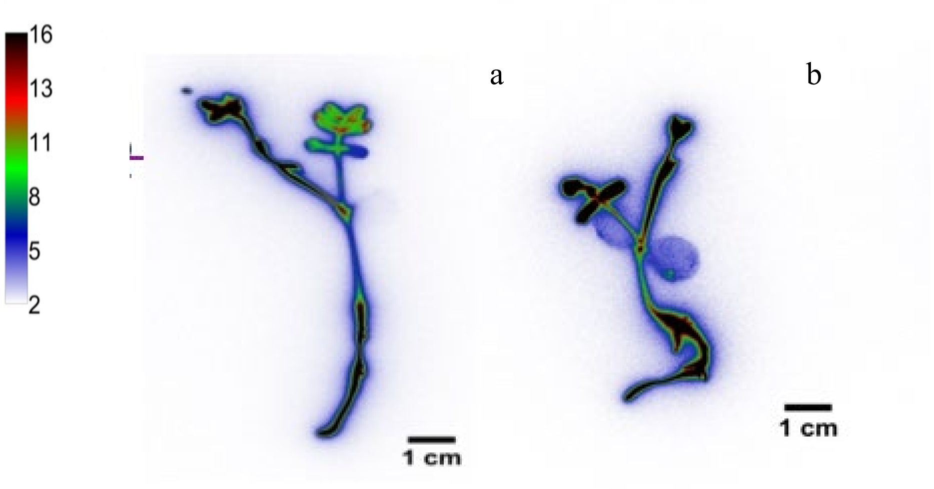

Figure 20. Selenium uptake as selenite (a) Se pg/pixel and selenate (b) Se pg/pixel in Neptunia amplexicaulis

4.4 Discussion

In previous chapters, Se was mentioned as having a beneficial impact on plants through the stimulation of growth, increasing tolerance to abiotic stresses, thereby increasing pathogen resistance (Feng et al., 2013, Pilon-Smits et al., 2009a, Quinn et al., 2007a). On the other hand, Se has been observed in high concentrations to be toxic to plant growth (Fu et al., 2011, Longchamp et al., 2015, Mao et al., 2011, Ximénez-Embún et al., 2004).

4.4.1 Effect of selenite and selenate exposure on Se uptake in *Brassica rapa*

*Brassica*ceae species have been long considered useful for their Se accumulation trait. However, this can vary significantly between species from non-accumulators to Se hyperaccumulator species, and therefore it is necessary to trial the Se uptake capability of each variety.

Based on the autoradiographic images (figure 18b compared to 18a), uptake in *Brassica rapa* exposed to selenate was two times greater with 16ug/pixel in the leaves than when exposed the *Brassica rapa* was exposed to selenite salts in the leaves, which contained only eight ug/pixel. In comparison, Se uptake was greater in the roots of *Brassica rapa* exposed to selenite salts than the selenate exposed *Brassica rapa*. Numerous studies have indicated that at low concentrations, Se has a growth promotion effect and at high concentrations an inhibitory effect on plants as seen in radish, Chinese cabbage, rapeseed, Indian mustard, and spinach which was demonstrated in chapter 2. In this series of experiments, the selenium uptake in *Brassica rapa* is in agreeance with findings from other studies where selenium uptake from selenate exposure was greater than selenite in the leaves(Longchamp et al., 2015).

Brassica rapa selenate roots contain the highest concentration of inorganic selenium compared to the leaves from exposure to selenite, while the leaves of *Brassica rapa* grown in selenate contain the greatest concentrations of inorganic selenium. A study using selenite and selenate uptake using hydride generation-atomic fluorescence spectrometry (HG-AFS) turnip leaves treated by Se (VI) accumulated significantly more Se compared turnip treated with by Se (IV) under the same concentrations (Xiong et al., 2015) which are in agreeance with the findings in this study as seen in figure 5a. The increased accumulation of selenium from the exposure of selenate compared to selenite is driven by the relative mobility of selenate in media compared to selenite. As a result, it is thought that selenate facilitates faster selenium uptake while selenite is a source of long term selenium (Broadley et al., 2006b). In particular, it was found that whole-plant maize accumulated greater concentrations of selenite than selenate, which was reflected in roots and grains while stems and leaves Se concentration from plants exposed with selenate were notably higher than plants conditioned with selenite (Longchamp et al., 2015). The results also confirm, similar to other *Brassica* varieties, that selenium uptake is dependent on the bioavailable supply of selenite and selenate in the growth medium as seen in pakchoi (Fu et al., 2011) and Ulva australis (Schiavon et al., 2016). It should be noted that while the findings of this experiment were consistent with the research conducted by Ximénez-Embún et al., 2004, research has also demonstrated that rice, wheat soybean, and maize can also accumulate Se in equal concentrations regardless of selenite or selenate exposure (Li et al., 2008, Zayed et al., 1998) and some experiments Se was accumulated in greater concentrations from selenite exposure than selenate. In particular, it was found that whole-plant maize accumulated greater concentrations of selenite than selenate, which was reflected in roots and grains while stems and leaves Se concentration from plants exposed with selenate were notably higher than plants conditioned with selenite (Longchamp et al., 2015). The results also confirm, similar to other *Brassica* varieties, that selenium uptake is dependent on the bioavailable supply of selenite and selenate in the growth medium as seen in pakchoi (Fu et al., 2011) and Ulva australis (Schiavon et al., 2016).

The findings from this experiment, however, suggest that the supply of selenium in its selenate form is translocated in greater concentrations than when selenium is supplied in its selenite form. The results from exposing *Brassica rapa* to Se through selenite and selenate exposure demonstrate its ability to accumulate Se, and given that *Brassica rapa* is an edible crop, there is primary potential for this to be biofortified with Se. Further research is required to determine the ability of *Brassica rapa* to absorb other metals and metalloids, which will assist in determining its suitability for the phytoremediation of soil.

4.4.2 Effect of selenite and selenate exposure on Se uptake in *Helianthus annuus*

Selenium uptake in *Helianthus annuus* exposed to selenate was two times greater than the *Helianthus annuus* when exposed to selenite salts in the leaves based on the autoradiographic images in figure 19a compared to 19b. In comparison, Se uptake is greater in the roots of the

Helianthus annuus exposed to selenate salts than the selenite exposed *Helianthus annuus.* These findings were consistent with studies where selenium salts, when exposed to Indian mustard and white lupin (Ximénez-Embún et al., 2004) and selenate uptake was greater than selenite. This pattern of uptake and translocation varies according to the selenium form supplementing the plants, the plant species and varieties, phase of plant growth, and Se exposure concentration (Gupta and Gupta, 2017). The higher uptake of Se from selenate exposure is recognized as being the result of easy transfer from the roots to the shoots than selenite. The observation of selenate, although not quantitative, agrees with the findings where selenate uptake occurs through sulphate transporters, whereas selenite was found to be translocated by the phosphate transport mechanism (Terry et al., 2000).

The relatively higher Se uptake based on the autoradiography results and existing literature suggests that selenium addition in the form of selenate allows for the faster translocation of Se, and therefore it is worth investigating further the potential of *Helianthus annuus* for Se enriched *Helianthus annuus* for supplementation of the human diet and phytoremediation.

4.4.3 Effect of selenite and selenate exposure on Se uptake in *Neptunia amplexicaulis*

Selenium uptake in the leaves of *Neptunia amplexicaulis* appeared to be equal regardless of which Se species the plants were subjected to. Given how tightly packed the new young leaves are compared to the older leaves, clear patterns of Se accumulation were not visible. Older lamina leaves displayed lower Se accumulation compared to the relatively younger leaves indicating the possibility of Se cycling into younger leaves.

Similar to other species of Se hyperaccumulators, such as *A.biscultas* and *S.pinnata*, *Neptunia amplexicualis* accumulated Se in its youngest tissues, implying a possibility that the plant used Se as a herbivory defence mechanism. Recent research suggests that Se in the young leaves is organic Se and may be translocated in organic form from the roots where the assimilation of Se is thought to take place (Yu et al., 2019b).

4.4.4 Autoradiography technique used for Se detection in plants

Autoradiography was the selected method of detecting a Se distribution method because of its high sensitivity, which is especially useful where trace elements may occur in minute concentrations and the availability of Se^{75} with a 119-day half-life. The next step should have been to perform ICP-MS on each plant, i.e., leaves, stem, and roots, to substantiate the qualitative observations of Se concentrations in all three plants species. However, ICP-MS for ultra-trace element detection of radioactive Se^{75} from an agar matrix was not available. There was also a need to undertake gamma counts of Se^{75} on a gamma counter to measure ad determine Se concentration in each plant section, so while the autoradiography contributes to previously minimal knowledge of Se distribution within the *Brassica rapa*, *Helianthus annuus,* and *Neptunia amplexicualis,* the concentration of Se within each plant section could not be determined. If this series of experiments was to be repeated, each plant section should be either weighed individually, and ICP-MS used to confirm the concentration of Se absorbed by each plant.

4.5 Conclusion and Future Direction

Whilst Se concentration could not be determined in this experiment using the autoradiography technique for quantification; this study does demonstrate the relative distribution and uptake patterns of Se at the sublethal concentrations that were previously determined comparing secondary Se accumulators compared to a confirmed hyperaccumulator. The significance of the uptake pattern in *Brassica rapa* and *Helianthus annuus,* and *Neptunia amplexicualis* may assist in developing these species as phytoremediation plants and potentially for use as cattle fodder where soils are deficient in Se.

Chapter 5 Effect of selenite and selenate exposure on seed storage protein selenium uptake

Abstract

Seed storage proteins are the basis of fuel for the germination of seeds into seedlings. During this process, they provide a source of nitrogen, among other starting nutrients. Eventually, these proteins are metabolised and revived in a new form to serve another purpose within the plant.

Selenium has been identified to affect protein formation in plants through malformed proteins when sulphur is replaced, and therefore it is possible to observe selenium in secondary and hyperaccumulators where plants are exposed to selenium at sublethal concentrations determination in chapter 2.

The objective of this experimental work was to determine to identify the relationships between selenium and seed storage proteins since no work has been done to date on the selected plants, *Helianthus annuus, Brassica rapa,* and *Neptunia amplexicaulis.* The results of this chapter were then used to inform the experimental design in chapter 6. The seed storage proteins of focus were focused on globulins, albumins, glutelins, and prolamins along with residue lipids as part of the defatting process and the crude starch which remained after each plant was processed.

This series of experiments determined that where the plants were exposed to Se75 radiotracer, there was an association of selenium to plant seed storage proteins; however, contrary to the findings seen in chapter 4, while selenium was detected in distributions, there is no binding to any plant protein at this stage. Prolamins and albumins contained the greatest concentration of selenium regardless of the form of selenium the plants were exposed to, but all plants also contained selenium in an inorganic form.

Since very little is known about seed storage proteins in plants that are not cereal crops, it was difficult to draw a conclusion linking seed storage proteins and their relationship with selenium once absorbed and distributed within the plants.

These results indicate that selenium is distributed in association with protein concentration, but its metabolism is still unclear.

5 Introduction

5.5.1 Seed storage proteins (SSPs)

Selenium has been widely recognized as vital to humans and animals (Brown and Arthur, 2001). Selenium has been identified as selenocysteine and selenomethionine in humans and animals, best described as a compound where Se replaces S in the important amino acid cysteine (Sors et al., 2005). Early in the chapter, the role of selenocysteine was described in the human body; however, to recap, cysteine is incorporated into selenoenzymes and eventually selenoproteins through co-translational processes guided by a UGA codon (Kryukov et al., 2003, Osaka et al., 2003). If selenoproteins are misincorporated or translated incorrectly, it can lead to defective protein function in humans and animals. In plants, cysteine is vital as a metabolic precursor of important biomolecules, vitamins, cofactor, antioxidants, and many other defence-related compounds (Domínguez-Solís et al., 2004, Wirtz and Droux, 2005). Since the role of Se in plants is unknown, by identifying if Se is incorporated into proteins, then it may be possible to take advantage of the mechanism for phytoremediation through genetic engineering (Altenbach et al., 1992). There are millions of protein sequences in plants, and therefore any attempt to observe potential Se in seed storage proteins serves as a starting point to more in-depth protein studies discussed in greater detail below.

Seed storage proteins are present in seeds as the source of sulfur and nitrogen for developing seedlings after germination (Youle and Huang, 1978) and have been proven to be a vital nutrition source for humans and animals (Young and Pellett, 1994). It is accepted that one type of seed storage protein is prevalent in seeds (Galland et al., 2014, Rödin and Rask, 1990), and in the early stages of germination of several dicotyledonous species, these proteins belong to the 11–12 S globulins protein class (Shutov et al., 2003), an understanding of which is more important to inform the proteomics studies carried out in chapter 6. More broadly the fate and redevelopment of seed storage proteins may be useful to determine the end use of the plant for livestock feed nutrient supplementation. As a result of the studies conducted in this thesis, the results presented at the very least provide the theoretical foundation for genetically engineering the plants for phytoremediation of Se and addition to livestock feed which was the historical purpose of identifying SSPs (Shewry et al., 1995).

Below is an overview of the broad classes of the seed storage proteins (SSP), some of which may play an important role in defence and metabolism or solely function as a source of nutrients post-germination. For example, in wheat, the seed storage proteins are in the form of the gluten fraction, which it attributes to the bread-making qualities of bread (Biesiekierski, 2017); however in legumes have been found to contain low levels of amino acids such as lysine, threonine, and methionine and therefore livestock supplementation for livestock feed with the certain legume seeds may not be appropriate (Galili and Amir, 2013).

Research to date has shown a distinct difference in the structures, but all seed storage proteins share common properties 1) they are developed in high concentration in certain plant tissues and at specific stages of development, 2) most contain sulphur and methionine, and 3) synthesis is dependent on the availability of nitrogen (Shewry et al., 1995). However, some seeds contain naturally occurring higher concentrations of sulphur amino acids allowing the plants that contain these amino acids to maintain higher levels of SSPs; it is thought that tuber varieties may develop the seed storage proteins in the vegetative tissues rather than the tuber (Shewry et al., 1995). Additionally, it is also thought that SSPs are present in mature seeds in smaller protein bodies, but the origin and transformation of the SSPs are not well understood. Finally, and perhaps most importantly, all SSPs are subject to polymorphism due to proteolytic processing and glycolisation, which means they can be precursors to other functional proteins in plant development which vary significantly between and within species (Mylne et al., 2014).

Although the first SSP were identified in wheat as a glutelin isolate and then globulins from Brazil nuts (Shewry et al., 1995), Osborne (1924a) was the first to classify SSPs, this was based on their extraction and solubility, which has remained the most widely accepted practice of isolating and SSPs to this day. The classes of SSPs are the following: albumins (water-soluble), globulins (diluted saline water-soluble), a prolamin (alcohol and water mixture soluble), and glutelin (dilute acid or alkali) (Osborne, 1924b, Shewry et al., 1995, Shewry and Pandya, 1999, Shewry and Tatham, 1990).

Albumins are widely present in Cruciferae, particularly oilseed rape and in Arabidopsis by Shewry et al. (1995) but more recently. Moreno and Clemente (2008) studied the PTMs of albumins and their translocation, the importance of which will be described in greater detail in Chapter 6 when discussing findings from FASTA sequences and protein matches. As germination occurs, the albumins undergo post-translational modification and transport for deposition in dedicated vacuoles (Mylne et al., 2014). In plants, albumin is vital for maintaining appropriate osmotic pressure but is also broken down and processed for other functions within plants, which can vary widely within a single species. In *Helianthus annuus*, the albumin genes exist in 6 known forms HaG5 (Allen et al., 1987), HaB1B2 (GenBank AJ275962), pHAO (Thoyts et al., 1996), SFA8 (Kortt et al., 1991), PawS1, and PawS2 (Mylne et al., 2011) which all go on to post-translational modifications (PTMs) to encode various albumins that play a role in signalling, gene expression, stability of plant proteins and enzyme kinetics (Friso and van Wijk, 2015). To the time of this thesis being written, studies that focused on the albumin in plants identified leucine, legumin, phaseolin, ricin,

Globulins are major seed storage proteins consisting of two major classes, vicilins and legumins, which are involved in the dehydration and hydration process in both fungi and plant cells (Kriz and Schwartz, 1986). This class of proteins can be dissolved in a buffered aqueous salt solution (Müntz, 1998). As mentioned in an earlier chapter, the effect of Se on plants has only been studied when selenite and selenate concentrations were considered beneficial but not at sublethal concentrations. Taking into consideration previous studies where Se was studied for its beneficial effects, it is thought that globulin and prolamin contain higher concentrations of S than the other SSP fractions and therefore more likely to accumulate Se from S metabolism (Chanput et al., 2009), but this observation is highly controversial to date with many studies providing various conclusions(Aureli et al., 2012, Chanput et al., 2009, Fang et al., 2010a, Tao, et al., 2008) from experiments which will be discussed in at the end of this section to take into consideration the known effects of Se on all SSPs.

Prolamins were initially distinguished from other seeds storage proteins soluble in 70% ethanol and sometimes referred to as reserve proteins, poor essential amino acids (Larkins et al., 2017) but contain large concentrations of proline and glutamine. The prolamins vary between 0 000 to almost 100 000 in their molecular mass. Given the range of size variation of prolamin, they possess a range of proteomic structures, so far identified in Triticeae (wheat, barley, rye) and the Panicoideae (maize, sorghum, millets) (Tao et al., 2008). Additionally, they possess two similar structural features of their structure. The first feature is the distinction of regions and domains which have undergone various changes and come from different origins (Shewry and Halford, 2002). The second feature is the amino acid sequence that contains at least one or more short peptide motifs or is enriched in specific amino acid residues (Anderson et al., 1989, Finch-Savage and Leubner-Metzger, 2006). These features are important as they are foundational for developing the high proportions of glutamine, proline, and other amino acids such as histidine, glycine, methionine, phenylalanine) essential for plant function (Bartels et al., 1986).

The glutelins are larger and much more complicated than prolamins (Shewry and Pandya, 1999), known as the largest polymers in natural gluten in wheat (Wrigley, 1996). To date, most glutelins have been studied for optimization of gluten for food processing but not for the effect of Se; therefore, the results of Se effects on glutelin in the selected plants will be the first (Johansson et al., 2013, Kuktaite et al., 2011)

In summary, SSPs are vital for the role they play in early germination, and the PTMs that take place after their use may be per cursors to other well-identified proteins, but there has been limited information to determine the toxic effects of Se as selenite and selenate on the selected plants. The reason for the additional step of using seed storage proteins is that there is a range of complexities in identifying the proteins associated with plants as outlined by ranging from structural formations and the range of PTMs that can take place during plant development (Rasheed et al., 2020).

Starch is an insoluble carbohydrate synthesis by plants and algae as a means of energy storage in an osmotically inert form. It is critical for consumption in the human diet. Starch is divided into main categories based on their location of formation, i.e. photosynthetic organs and non – photosynthesis organs, as transitory starch and storage starch (Pal et al., 2013). Transitory starch is made in plant leaves resulting from Photosynthates formed during the day but is degraded during the night to assist in processes of metabolism, energy production and biosynthesis when photosynthesis processes are not occurring. Starch formed from non-photosynthetic tissues such as seeds, stems, roots, and tubers are generally stored for longer periods and termed as storage starch. The storage starch is transported during germination, regrowth, and sprouting, especially when photosynthesis is unable to meet the demand for energy (Pfister and Zeeman, 2016). *Helianthus annuus* proteins have been found to contain mostly globulin and albumins (Raymond et al., 1991). The main use of *Helianthus annuus* proteins being for animal feed (González-Pérez, 2015).

Outside of the selenium that may be bound/incorporated into the seed storage proteins, selenium may also remain inorganic within plants depending on the geochemistry of surrounding soil (Broadley et al., 2006a, Combs, 2001, Frankenberger and Engberg, 1998, Gupta and Gupta, 2002, Lyons et al., 2003). While selenium has been studied for its plant benefits and not at sublethal concentrations, it is still controversial as to its fate within the plant. Some studies indicate that where Se is not bound to other minerals, as occurs when selenium is applied as foliar fertilizer, it may be accumulated as inorganic Se, which is absorbed but not integrated into the plant in an organic form (Premarathna et al., 2012). The metabolism of selenium for this study, if incorporated into protein as organic selenium, offers the additional opportunity for selenium to be used for biofortification of livestock feed (Broadley et al., 2006b).

The few studies that have studied selenium in seed storage proteins have been measured purely in seeds instead of seedlings. For example, studies in *Lecythidaceae* varieties focussed on *B. excelsa* (Jayasinghe and Caruso, 2011) contain 50 % of proteins that are predominately rich in sulfur-containing amino acids (Zuo and Sun, 1996) two main types of seed storage proteins albumin and globulin (Zuo and Sun, 1996). Rice has been studied to some extent for selenium accumulation(Antunes and Markakis, 1977). However, to date these studies have not been studied in any of the plants studied in this research. In the studies of Lecythis minor seeds, 11S globulin,2S albumin , 2S sulfur-rich seed storage proteins and 11S globulin-like protein were identified using shotgun proteomics(SM SUN et al., 1987). In one other study of rice, selenomethionine (SeMet) and selenocysteine (SeCys) residue were identified in 19 kDa globulin, granule-bound starch synthase, and the family of glutelin-type seed storage protein (Fang et al., 2010). It should be noted however as mentioned these studies have all been studied in seeds and grains but not in seedlings therefore this research was a pilot study of selenium accumulation and speciation of selenium in the selected plant seedlings. An understanding of the ability of the accumulation of selenium in the seedlings will determine if there is a chance of observing selenoproteins or selenium containing proteins in chapter 6.

Aims

Given there is no research studying the effects of Se uptake on seed storage proteins in any plants other than rice, this chapter aimed to quantify uptake selenium uptake patterns in

Brassica rapa, Helianthus annuus, and *Neptunia amplexicualis* leaves, stems and roots at the seedlings stage of growth at 21 days. The descriptive conclusions drawn in this chapter were then used to inform the selection of plants for the final chapter of this thesis on proteomics.

5.6 Method

5.6.1 Culture Preparation.

1L of 1/4/ strength media solution was prepared with 1.08 g MS salts, 7.5g sucrose, and stirred over a hot plate to ensure that all components were dissolved to make the tissue culture media. 600 mL was then placed into a 1L stock bottle with 0.8g of agar to set media for tissue culture. All bottles of tissue culture were placed in an autoclave on an agar setting for 30 min at 121°C, 104kPa, and left to set at room temperature to cool. Culture media was used the same day as preparation.

5.6.2 Seed preparation.

Seeds of the *Brassica rapa* cv. Vitamin greens and *Helianthus annuus* were sourced from Rangeview seeds, Derby, Tasmania. *Neptunia amplexicaulis* seeds were sourced from QLD by Dr Ashwa Nanjappa Central Queensland University, Australia. Hard seed shells were removed by hand without damaging the seed inside. Once the seed shell was removed, all seeds were surface sterilised with 70 % (v/v) ethanol for 3 min, followed by five autoclaved MQ water rinses. Seeds were then washed in 2% sodium hypochlorite solution for 5 min, after which they were rinsed five times with autoclaved MQ water again. All seeds were used immediately after sterilisation. *Neptunia amplexicaulis* seeds were nicked with a nail clipper to break open the seed coating to ensure penetration of nutrients and initiate the first stage of the triphasic germination begin, i.e. rapid initial uptake (Bewley, 1997, Manz et al., 2005, Schopfer and Plachy, 1984). This was not necessary for the softer seed coatings. Plants were grown for 21 days at a temperature of 24°C, relative humidity of 85-95 %, and a day/night cycle of 12 hours/12 hours. A data logger was used to ensuring that humidity and temperature were consistent throughout the experiment.

5.2.3 Radiotracer stock preparation.

0.1 g Sodium selenite Na_2SeO_3 and 0.10023g sodium selenate Na2SeO4 (BioReagent ≥ 98%, Sigma-Aldrich, Germany) were measured out on a weighing scale added to a titanium capsule. After neutron activation to produce radioisotopes 75SeIV and 75SeVI at the OPAL reactor, ANSTO, Sydney, Australia. Activated salts were dissolved in Milli-Q water to produce stock solutions for both Se species. Selenium speciation of each stock solution was confirmed using ion chromatography. Inductively coupled plasma mass spectrometry (ICP) was used to validate the concentration of Se in each stock solution using internal standards. Radiotracers were then further diluted with stable sodium selenite and selenate stock solutions to form required sublethal concentrations determined in chapter 1, i.e. *Brassica rapa*, 20 µM for both selenite and selenate salts, *Helianthus annuus* 25µM for both selenite and selenate and 500 µM for both selenium salts for *Neptunia amplexicaulis*. The selenium stocks were diluted into 300 mL autoclaved agar and distributed into (n=6) 200 mL tissue culture jars. Six seeds of each plant variety were embedded into the surface of the agar and sealed with a lid for fractionation studies.

5.2.4 Seed storage protein fractionation.

The fractionation methods followed procedures by (Fang et al., 2010b) with some modifications to include the use of the radiotracer. After 21 days in tissue culture exposed to either selenite and selenate, all plants were removed from the culture, and the roots were cleaned with MQ water to ensure removal of agar and patted dry with paper towels.

All plants were then weighed on an analytical balance. The leaves, stems and roots were separated, and 100mg of each plant part was weighed from each sample. Samples were then ground with a mortar and pestle with LN2, which is considered the most suitable and most efficient means of crude protein extraction (Walker, 1996).

After grinding each sample, the mortar and pestle were both cleaned and checked with a hand-held gamma counter to prevent cross-contamination of samples. In between individual sample preparation, the mortar and pestle were wiped twice with 80% v/v ethanol to prevent cross-contamination between samples and transferred to a 3 mL borosilicate vial (kimbel tube).

Each extraction step was performed twice with 1.5 mL of solvent added twice to form a total of 3 mL for radio analyses of each protein fraction.

Lipid (Fraction A) extraction was performed by suspending the plant pellet in hexane and allowing this to soak for 4 hours on a shaking tray., the supernatant was then removed and placed in a 6 mL kimbel tube. The samples were placed in a fume hood to air dry for 24 hours to ensure that all hexane had evaporated from the sample for defatting.

1.5 mL of MQ water was then added to the sample with shaking twice for 3 hours each time followed by 10 mins of centrifugation at 3500g to separate water-soluble inorganic Se Fraction B and albumin Fraction C.

Albumin (Fraction C) was determined by subtracting the inorganic count from the total Se fraction after Inorganic selenium (Fraction B) was passed through a 0.45µM Whatman filter. The sample pellet was then mixed with 1.5 mL 5% NaCl solution to extract salt soluble proteins two times for 3 hours, followed by centrifugation for 10 min at 3500g, after which the supernatant was then collected into the eppendorf tube to form Fraction D, globulin. The pellet was then resuspended in 1.5 mL 0.02M NaOH for 1 hour twice; after each soak time, the sample was centrifuged at 3500g for 10 min to form the alkali extract. Fraction E, glutelin. The pellet was then subjected to the 70% v/v ethanol on a shaking tray 3 hours 2 times; the supernatant was then extracted to form the Fraction F, prolamin and the remaining pellet consisted of proteins that were not dissolved in the above solutions that are bound to crude starch and the residual plant cells to form Fraction G.

All 3 mL samples were then placed in a 6 mL borosilicate glass vial for the detection of Se^{75} using an automated gamma counter (Wizard^2, Perkin-Elmer, Australia) on a pre-set program with a 5 min counting time; in-house standards were prepared that were geometrically identical to sample geometry.

5.3 Results

The results below illustrate the uptake of selenium into four protein fractions in addition to crude starch and associated inorganic selenium on exposure to selenite and selenate radiotracer as $Se^{75.}$ Standards were not used for these experiments; therefore, all values can only be described as trends rather than as absolute concentrations. L= leaves, S= stems and R=roots.

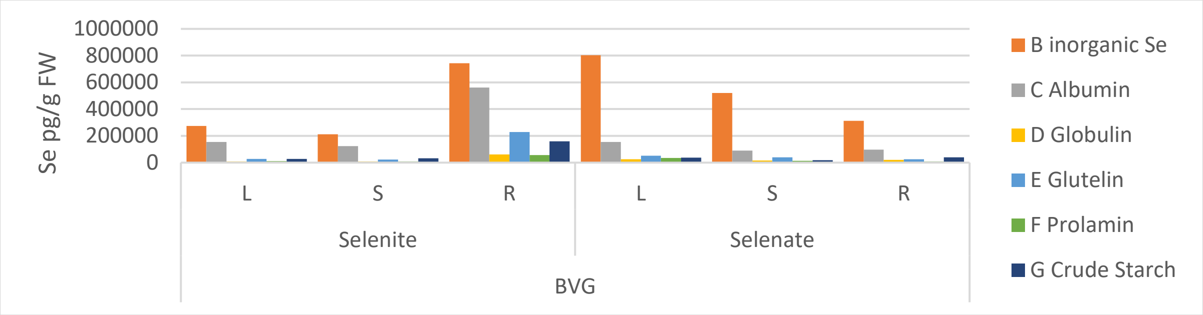

Figure 19 Selenium concentration in leaves, stems and roots in Brassica rapa on exposure to selenite and selenate n=6.

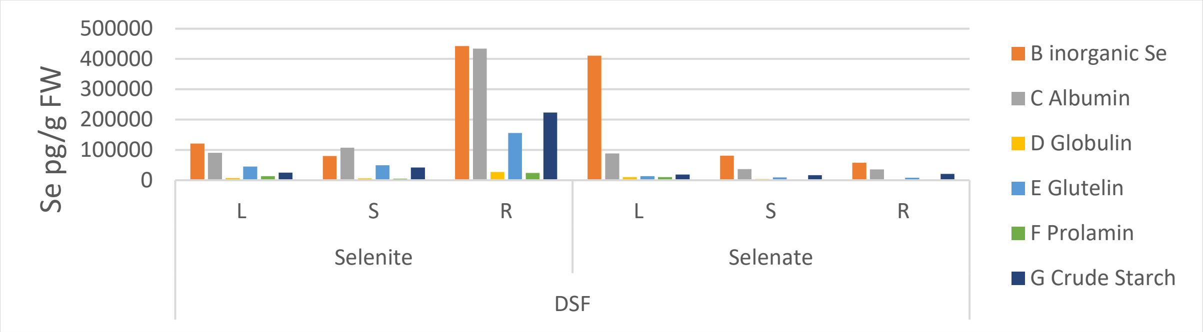

Figure 20 Selenium concentration in leaves, stems and roots in Helianthus annuus on exposure to selenite and selenate. n=6

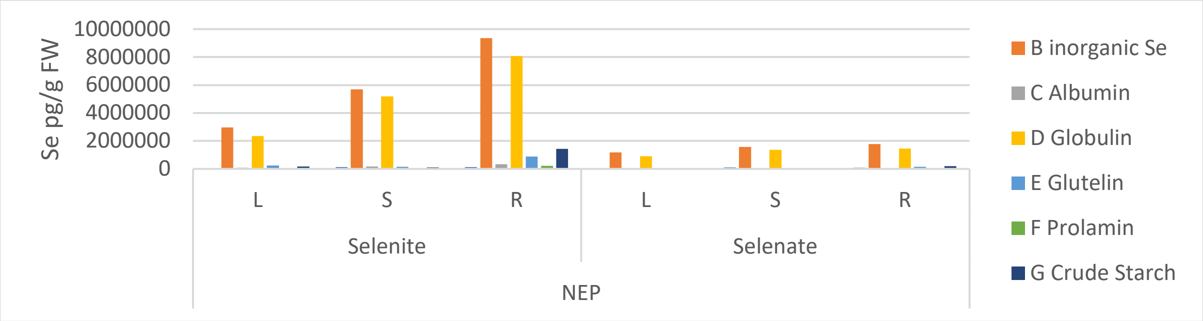

Figure 21 Selenium concentration in leaves, stems, and roots in Neptunia amplexicaulis on exposure to selenite and selenate.n=6

5.4 Discussion

The current study attempted to quantify the selenium content in *Brassica rapa, Helianthus annuus,* and *Neptunia amplexicualis* leaves, stems and roots at the seedling stage of growth at 21 days, since research so far has only identified the majority of genes responsible for SSPs synthesis and accumulation in seeds of Arabidopsis thaliana, model species that share high collinearity with *B. napus* (Borisjuk et al., 2013b) but not in *Brassica rapa, Helianthus annuus or Neptunia amplexicaulis*. Therefore, the results produced in this chapter are only preliminary results to inform the next chapter. Most commonly SSPs have been studied for their mobilization in vetch seed (Schlereth et al., 2000), but in more recent years, the post-germination process (Eastmond and Graham, 2001, Pritchard et al., 2002)has been studied a little more to understand the process of SSP's breakdown and mobilization including starch degradation (Galland et al., 2017), protein (Rosental et al., 2014, Yang et al., 2007) and lipids (Sreenivasulu et al., 2008) including their fate after seedling establishment (Bourgne et al., 2000, Gallardo et al., 2001, Job et al., 1997). The process between seed storage protein formation, germination, and post-germination has been focussed on economically critical species including, *Oryza sativa* (Sun et al., 2015), *Glycine max* (Bellaloui et al., 2017) , *Sorghum bicolour, Linum usitatissimum,* and model species *Medicago truncatula* (Vandecasteele et al., 2011) and *Arabidopsis thaliana* (Shrestha et al., 2016) and only in recent years grasses (Ataíde et al., 2013, Goyoaga et al., 2011, Kim et al., 2009, Lopes et al., 2013, Shaik et al., 2014, Zhao et al., 2018) but there are no studies that identify the effect of sublethal concentrations on SSPs break down and post-germination mobilization.

5.4.1 Selenite and selenate distribution in *Brassica rapa*

As described in chapter 4, selenium uptake in *Brassica rapa* exposed to selenate was two times greater with 16ug/pixel than the *Brassica rapa* exposed to selenite salts in the leaves based on the autoradiographic images in figure 4b compared to 4a. In comparison, Se uptake was greater in the roots of *Brassica rapa* exposed to selenite salts than the selenate exposed *Brassica rapa*. These qualitative results are substantiated indirectly with the attempted quantification of Se in plant parts leaves, stems, and roots.

When selenium concentrations were studied at a proteomic level when exposed to selenite and selenate, the results do not change from chapter 4. SSPs combined with starch result in seed storage sources that collect in the matured embryo of *B. napus* seeds. These act as a source of energy for growth, including seed germination, in addition to providing energy for humans and animals (Borisjuk et al., 2013a).

Research so far has only identified the majority of genes responsible for SSPs synthesis and accumulation in seeds of Arabidopsis thaliana, model species that share high collinearity with

B. napus (Borisjuk et al., 2013b) but not in *Brassica rapa;* therefore, the results produced in this chapter are only preliminary results for *Brassica rapa* with albumin napin genes only being identified extremely recently.

While the results indicate that there is selenium association in the organic form with the SSP's with albumin being the highest in the leaves, stems, and roots in *Brassica rapa* exposed to selenite compared to selenate, there is little evidence to conclude that selenium had been incorporated into these proteins with this research alone as no further protein analysis was available on the SSPs. However, it appears that *Brassica rapa* can store selenium in an inorganic state in the roots, which are in agreeance with research on other *Brassica* varieties to date (Liu et al., 2017, Liu et al., 2016, Schiavon et al., 2012b, Yu et al., 2019a).

5.4.2 Selenite and selenate distribution in *Helianthus annuus*

The quantification of selenium in *Helianthus annuus* the uptake in the form of selenite was less than the total uptake of on exposure to selenate, which was in agreeance with selenate uptake patterns in *Brassica rapa* in a hydroponic solution(Lyons et al., 2009) and through the foliar application (Germ et al., 2007b). The results obtained here were also similarly reflected in experiments by Singh et al. (1980), which demonstrated relatively similar concentrations of Se in Indian mustard (*Brassica juncea L.)* and lettuce (*Lactuca Sativa L.*) (Hartikainen et al., 1997) and soybean*, Glycine max L.* (Djanaguiraman et al., 2005) at beneficial Se concentrations. On the contrary to the beneficial concentrations on Se at sublethal concentrations showed inhibitory effects on tomato, lettuce, and radish (Cruz de Carvalho et al., 2001), which confirmed that Se is linked to germination and therefore, aside from investigating SSP selenium links for informing proteomic studies, an understanding of SSPs and the effect of Se on them is important to plant development studies. However, the mechanisms of the Se and its association to SSPs in germination is not clearly understood, particularly shown in studies by (Mirza et al., 2010), where Se showed improvements to germination rates and antioxidant concentration and selenite improved bitter gourd growth *(Momordica charantia L.).*

Given that studies to date on SSPs have been focused on cereal crops, there is a shortage of comparable studies to substantiate the findings of selenium association with the SSPs found in this study, and even in on the study at the time of this thesis being written only observes the protein in *Helianthus annuus* cultivars on a whole SSPs level rather than individual classes of proteins. Regarding the findings in the results, although *Helianthus annuus* accumulates lower concentrations of Se than *Brassica*, there is also a lower concentration of individual proteins, which agrees with the findings that due to *Helianthus annuus* containing a greater protein content generally, the protein is also more likely to lysed by proteinases faster into amino acids faster than *Brassica* (Erbaş et al., 2016).

5.4.3 Selenite and selenate distribution in *Neptunia annuus*

Interestingly, *Neptunia amplexicaulis* absorbed more selenium in the form of selenite than selenate, which does align with existing research of other hyperaccumulators such as *Stannleya pinata* (El Mehdawi et al., 2015, Harvey et al., 2020, Schiavon et al., 2016, Schiavon et al., 2012b)

Method optimization

Selenite and selenate concentrations in seed storage proteins could not be determined using this method, 1) the original methodology assumes that there is no loss of selenium during the first protein extraction step; 2) when leaves, stems, and roots were mixed individually using radiotracer, the final powdered weight should be the true weight throughout all experiments to account for biomass losses in the processing of the tissue.

It should also be noted that the protein content of each seed storage protein fraction needed to be measured which would have allowed the results to be displayed with the concentration of the selenite or selenate per milligram of protein at the very least. ICP- MS is unable to detect selenium in plant tissue without highly specialised equipment which was not available at a nuclear research facility. If this experiment was to be repeated, it could be done with ICP-MS on the plant materials without the Se75 radioisotope exposure which would have resulted in the clear quantification of not only the Se species, selenite, and selenate in plant organs, but also within the seed storage proteins. Then a new experiment could have been completed to validate the IMS findings for a qualitative identification on the translocation of Se within thaterials.

5.5 Conclusion and Future direction

5.5.1 Significance of seed storage proteins for Se accumulation

The seed storage proteins that were studied were albumins, globulins, glutelins, prolamins, and crude starch after lipids were removed from the plant materials. The concentration of each seed storage protein class can vary significantly between species of plants. The analysis of seed storage proteins is important in exploring the prospect of supplementing the human diet with selenium as an essential micronutrient for humans and animals. Similar studies conducted to analyze plants for seed storage proteins have been completed *Helianthus annuus, Brassica rapa,* and to a lesser extent, *Neptunia amplexicaulis*.

Seed storage proteins are critical in plant development and a source of human nutrition. They accumulate in high concentration seedlings to assist in germination and remain a significant source of protein for human and animal consumption. Developing an understanding of the potential binding of Se to these proteins can translate into relevant information regarding protein longevity content, stress. Given that storage proteins undergo post-translational modifications understanding Se binding would show ere Se could transfer into other proteins through the five types of post-translational modifications, phosphorylation, glycosylation, lipid modification, ubiquitination, and redox-related modifications (Bond et al., 2011).

Chapter 6- Effects of selenium on protein regulation in plants

Abstract

Selenoproteins to date have been well researched and their role well understood in the human body and animals, and this level of detail is yet to be developed in plants. There is physiological evidence that these selenoproteins have been seen in viruses, bacteria, archaea, and mammals but not in plants or yeasts.

Genes encoding GPX-like homologues from *Chlamydomonas* and some higher plants have been isolated in the past few years; however, they all contain cysteine residues in their catalytic site, unlike the animal selenoproteins, which contain selenocysteine.

This chapter aimed to determine if selenite or selenate could be incorporated into plant proteins. If not, what effect do selenite and selenate have on protein regulation compared to the control based on the previously established sublethal concentrations? Suppose plants could incorporate selenium into the plant proteins. In that case, the plants may be used for phytoremediation of old contaminated mine site soils and as a secondary application, these plants could be used to biofortify livestock feed to compensate for selenium deficiency.

Plant proteins concentrations were established using gels, followed by shotgun protein sequencing and MS analysis. These methods were used to compare protein regulation on exposure to selenite or selenate compared to the control, if selenoproteins could not be detected.

The results indicated no selenoproteins were detected in this experiment; however, the results did identify some species of the reactive oxygen enzymes as seen in chapter 3.

A recommendation for future research on this topic would be to further optimize plant protein extraction protocols for high fibre plants and follow up shotgun analysis with protein markers to identify specific proteins in a targeted manner to capture and hone in to capture low abundance high coverage proteins. Due to funding and time limitations, this could not be achieved for this series of experiments.

Overall, while selenoproteins were not identified in this experiment, the results indicate a significant upregulation of plant enzymes identified between the selenite and selenate exposed plants compared to the control in all three plants. This indicates and confirms that there is enhanced defence mechanisms for the plant. While other proteins were downregulated, the

sublethal concentrations determined in previous chapters result in physiological limitations for the plants. The mechanisms of this function could not be determined in this experiment but present an opportunity for further work in this area.

6.1 Introduction

6.1.1 Selenoproteins

To date, it remains unconfirmed if selenium is incorporated into plant materials, with selenoproteins such as GPX garnering minimal attention over the years (Bartling et al., 1993). Given that some plants can accumulate Se, plants that can incorporate Se into their proteins offer possibilities for the use of plants for phytoremediation and biofortification of livestock feed.

Exposure of various plants to a range of Se concentrations has demonstrated that Se at optimal concentration can, in fact, have an enhancing effect on plant growth and defence against abiotic stresses where Se acts as a pro-oxidant (Hugouvieux et al., 2009).

Sublethal concentrations of Se, as discussed in Chapter 1-3, show that there is an increase in antioxidant concentration in *Helianthus annuus, Brassica rapa* and *Neptunia amplexicaulis*. To date, there is no research to suggest how Se is incorporated into these plant varieties and the influence this has on essential protein regulation. Some reports have shown that some plant extracts have shown GPX like concentration, but this can also be attributed to the abundance of glutathione s-transferases (Eshdat et al., 1997b). Several researchers have also identified genes encoding GPX like homologues from higher plants and *Chlamydomonas* over the course of the last few years; however, these encode Cys over Sec in their catalytic site (Leisinger et al., 1999, Li et al., 2000, Miyasaka et al., 2000) which impacts the formation of the protein structure and therefore its function. Studies also indicate that GPX may, in fact, contain selenocysteine in plants as purified proteins showing selenium-dependent classical GPX concentration have been observed in *Chlamydomonas reinhardtii (Shigeoka et al., 1991, Yokota et al., 1988)* in germinating barley (Huang et al., 1994)**,** and in *Aloe vera* (Sabeh et al., 1993). In all of these species, an inorganic selenium atom is linked to proteins that catalyse peroxidase concentration, to which selenium is associated in various chemical forms (Behne and Kyriakopoulos, 2001). This inference suggested that while Se remains disputed as essential, it may be present in a range of photosynthetic organisms.

Selenoproteins in mammals, virus's bacteria, archaea, and mammals have been identified to possess the rare amino acid selenocysteine in their primary structure, but this has not been found widely in plants and yeasts. The most investigated selenoproteins of enzymes are the group of GPXs. In particular, *Chlamydomonas* and higher plants have been shown to possess genes encoding GPX-like homologues from which cysteine is present in their catalytic site but not as selenocysteine. Selenocysteine is encoded by a UGA opal codon that is most commonly a stop codon in all organisms that possess selenocysteine. It was reported in *Chlamydomonas reinhardtii that the* cDNA-cloned sequence of a GPX homologue also possesses a TGA codon in the frame to the ATG internally. Critical functions such as the expression of mRNA expression, protein synthesis, and enzymatic concentration are selenium-dependent. Proteolytic digestion performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on the peptides synthesised by proteolytic digestion substantiated the presence of selenocysteine residue at the predicted site, which also suggested that this takes place in the mitochondria (Behne and Kyriakopoulos, 2001). This research, for the first time, provided proof that the UGA opal codon is decoded in the plant kingdom to incorporate selenocysteine (Fu et al., 2002).

6.1.2 Aims

The aim of this investigation was to find out which proteins, if any, in *Helianthus annuus*, *Brassica rapa* and *Neptunia amplexicaulis* may be associated with Se incorporation, the impact of the sublethal concentration on protein regulation in these plants and the significance of the proteins impacted using data gathered from the preceding chapter.

6.2 Methods

6.2.1 Sample preparation.

Seeds of *Helianthus annuus*, *Neptunia amplexicaulis* and *Brassica rapa* were sterilized as seen in chapter 1 and extracted from agar media after 21 days. Roots were cleaned of agar with distilled water and patted dry with paper towels.

6.2.2 Protein extraction.

According to the methods described by Nasim et al. 2019, the leaves of *Neptunia amplexicaulis* were weighed and placed in a cryomill capsule. Liquid nitrogen (LN2) was poured over the capsule and plant material until all LN2 had evaporated. The capsule was then placed on the cryomill for 2 minutes to form a powder; 100 mg of leaves and roots were collected for each sample i.e stems, leaves and roots. The powdered samples were stored at -80°C in 15 mL falcon tubes overnight for the next step. 100 mg FW of plant material, 25 mL of extraction buffer was prepared with the to the following components: 100 mM Tris 100 mM EDTA, 100 mM EDTA, 50 mM Borax, 50 mM ascorbic acid, 30% sucrose, 0.5% SDS, 1.5% Triton X-100 v/v, protease inhibitor (2 tablets), 1% PVPP and 2% B-mercaptoethanol, protease inhibitor tablets, polyvinylpolypyrrolidone (PVPP) were added only on the day of use. Extraction buffer was prepared fresh for each experiment

After the addition of all components, the extraction buffer placed into a sonicator bath for 10 min to ensure that all components were fully solubilized. 2 mL of extraction buffer was added to each powdered sample with two volumes of tris saturated phenol (ph7.8). The solution was then vortexed for 5 min. After vortexing, the sample was centrifuged for 15 min at 15000 rcf at 4°C. The supernatant was collected, avoiding the interface, and the remaining material was disposed. Borax/PVPP/Phe (BPP) extraction buffer was added in equal volumes to the supernatant. Samples were again vortexed for 5 min followed by centrifugation at 15000 rcf for 15 min at 4°C. The supernatant was collected again and placed in a 50 mL falcon tube. 50 mL of 0.1M ammonium acetate in methanol was prepared and four volumes was addedto the supernatant. Samples were then vortexed for 5 min again and left overnight in a -20 freezer to allow the protein to precipitate. After 12 hours, the frozen samples were defrosted in the fridge and centrifuged again for 5 min at 15000 rcf at 4 °C after the overnight incubation. The supernatant was discarded, leaving behind a protein pellet. The protein pellet was resuspended and washed with 20 mL of 0.1M ammonium acetate, vortexed for 5 min, and centrifuged for 5 min at 15000 rcf at 4°C to wash any debris, this step was repeated 3 times. After the final ammonium acetate wash, protein pellets were resuspended in an 80% acetone solution. Resuspension in acetone was followed by centrifugation at 15000 rcf at four °C for 5 min. After the last acetone wash, samples were transferred to 1.5 mL tubes (for easier resuspension using a pipette) and left for acetone evaporation in the fume hood for 20 min. Samples were left in the freezer at -80°C overnight

6.2.3 Protein Pellet Rehydration and Solubilisation.

Protein samples were rehydrated and solubilised in a solution of 100µL of 100 mM ammonium bicarbonate (AMBIC) and 8M urea. All samples were placed in a sonicator bath to ensure that protein pellets were resuspended.

6.2.4 Alkylation and reduction reaction.

2.5µL 5 mM tris (2-carboxyethyl)phosphine (TCEP) was added to the sample, followed by 2.5µL 20 mM AMBIC. All samples were then incubated at room temperature (RT) for 90 min. To stop the reaction, all samples were quenched with 2 µL of 20 mM dithiothreitol (DTT).

6.2.5 **Protein assay gel preparation.**

5 mL of separating gel was prepared with 1.25 mL 1.5M Tris hydrochloric acid (HCL) ph 8.8, 1.5 mL of 30% acrylamide solution, 2.1 mL of DH_20, 25µL 20% sodium dodecyl sulfate (SDS) solution was blended gently to prevent suds from forming and distorting protein quantification. Just before the addition of the separating gel solution was added to the gel plates, 5µL tetramethylethylenediamine (TEMED) and 25µL of 20% w/v APS were added to the separating gel solution. Biorad glass 0.75 mM glass panels were assembled for running the gels. The gel solution was added to the brim of assembled panels. Standards were prepared in ammonium bicarbonate (AMBIC) with 8M to replicate the protein assay conditions. 10µg/µL BSW was prepared in H_2O to make eight standards. All standards were prepared in AMBIC with 8M urea as the buffer. 10ug/ul BSW was prepared with, 0.0052g per520µL H_2O. Standards were prepared excluding the stock solution. Standards were used to prepare serial dilutions. Serial dilutions were prepared by adding 5µL ammonium bicarbonate (AMBIC) with 8M urea to the Bradford assay (BSA).

6.2.5 Samples preparation for the protein assay

To ensure proteins concentrations could be captured within the standard curve, samples were prepared undiluted and as 1 in 5 dilutions. All loading dye was diluted to 1x dye diluted from 4x lammeli loading dye buffer. Neat protein samples were prepared by adding a 5µL sample to a 0.5 mL centrifuge tube, with 5µL loading dye . 1-in 5-dilution samples were prepared by adding 2µL sample to 0.5 mL centrifuge tube containing 8µL AMBIC and 8M urea. 5µL of the total 1 in 5 dilution sample was transferred to new 0.5 mL tubes, after which 5µL loading dye was added. All samples were then boiled for 5 min at 95°C in a heating block then removed to cool at room RT. Gel containers were filled with a running buffer which was diluted 10x to 1x. Samples were loaded into each well.. Samples were then secured with clamps . Electrophoresis was started at 200mV for 5 min and was run until the dye was halfway down the gel.

6.2.6 Staining technique

Gels were removed from plates and were soaked in a fixing solution for 30 min at RT. After this, the gel was rinsed twice with mqH_2O. Gels were then left overnight to soak in a solution of 1-part methanol:4 parts coomassie stain on a shaking stand. After overnight staining, the coomassie stain was drained away. All residue was rinsed with H_2O, then 100 mL of water was added to gels and left to soak for 1 hour to allow all residual stains to be removed. The gels was processed on image J to determine protein concentrations based on the standards.

6.2.7 Trypsin digestion

Trypsin digestion was performed using the protein concentration from the assays. To calculate the volume of sample required for the trypsin digest, 50µg of protein was divided by the protein concentration. Once the volume of sample required for 50µg protein was calculated, AMBIC (no urea) was calculated to dilute the samples from AMBIC with eight mM urea to 1M urea. This reduction in urea concentration was required to prevent interferences with mass spectrometry (MS) and then vortexed for 20 seconds at 15000 relative centrifugal force (rcf) at 37°C.

6.2.8 Modified 6-minute Trypsin Digest

Following trypsin digestion above for protein quantification, 2 µg protein was required for MS loading. This was calculated by adding the calculated volume of 200 mM AMBIC and 1µL trypsin. The samples were placed into a foam tube holder in a beaker filled with water, placed into the microwave at power 20, for 6 minutes.. Samples were then loaded with the corresponding MS loading buffer volume into sample vials. .The modified trypsin digestion was used to decrease the time needed for digestion for MS run optimisation.

6.2.9 Loading for MS preparation

A total of 5µL of each sample was needed for the MS with 1µg of protein in each sample. The trypsin digested sample was loaded into the MS based on the calculated volume (µL) required for 1µg of protein. This was completed for all samples before running samples in the MS sample vials.

6.2.10 Protein analysis

An autosampler was used linked to a nanoLC system (Tempo Eksigent, USA), where 10 µl of the sample was loaded at 20 µL/min with MS loading solvent (2% Acetonitrile + 0.2% Trifluoroacetic Acid) onto a C8 trap column (CapTrap, Michrom Biosciences, USA). After cleaning the trap for three minutes, the peptides were washed off the trap at 300 ml/min onto a PicoFrit column (75 µm × 100 mM) tightly packed with Magic C18AQ resin (Michrom Biosciences, USA). Peptides were eluted from the column and into the source of a QSTAR Elite hybrid quadrupole-time-of-flight mass spectrometer (Applied Biosystems/MDS Sciex) using the following program: 5–50% MS solvent B (98% Acetonitrile + 0.2% formic acid) over 8 min, 50–80%MS buffer B over 5min, 80% MS buffer B for 2min, 80–5% for 3min. MS solvent A consisted of 2% acetonitrile + 0.2% formic acid. The eluting peptides were ionised with a 75 µm ID emitter tip that was connected to 15 µm (New Objective) at 2300V. An Intelligent Data Acquisition (IDA) experiment was undertaken, with a mass range of 375–1500 Da continuously scanned for peptides of charge state 2+, 5+ with an intensity of more than 30 counts/s. Selected peptides were fragmented, and the production fragment masses were measured over a mass range of 100–1500 Da. The mass of the precursor peptide was then excluded for 15 s.

6.2.11 Protein identification

Peptides fragments were identified, and protein identity was inferred using both MS and PEAKS Studio software (Peak Studio 7.5, Bioinformatics Solution Inc., Waterloo, ON, Canada). The MS and MS/MS data produced by the QSTAR were searched with a taxonomic restriction of *Viridiplantae* (green plants), legumes, soy, and arabidopsis using the software Mascot Daemon (version 2.4) and searched against the MSPnr100 databases (comprised of all known reference protein sequences including NCBI, RefSeq, UniProt, EuPathDB and Ensembl). The variables and settings used were the following Fixed Post-translational modifications Deamidation (NQ); Oxidation (M); Propionamide: Variable Modifications: carbamidomethyl, propionamide, oxidized methionine; Enzyme: semi-trypsin; Number of Allowed Missed Cleavages: 3; Peptide Mass Tolerance: 100 ppm; MS/MS Mass Tolerance: 0.2 Da; Charge State: 2+and 3+. All protein scores were normalized to the control.

6.2.12 Bioinformatic analysis

Identified proteins with a 100% match to the database and excluding $p > 0.05$ were assigned to Gene Ontology (GO) using Blast2GO software (https://www.blast2go.com/), coupled with the UniProt GO annotation program to determine the biological role, cellular and molecular function. The GO database, BLAST annotations, and information reported in the literature were used to analyze and classify the identified proteins based on their cellular localization, molecular functions, and biological processes and alignment to the scope of this research. Based on the searches, specific up-regulated and down-regulated proteins were identified.

6.3 Results

The following results sections illustrate the functional role of the proteins identified with 100% matches with GO annotations. While there is overlap with the proteins identified, all proteins have been identified as having a role in molecular and cellular capacities when searched against the *Arabidopsis thaliana* database.

Figure 22. GO annotations of all proteins that were identified by GO functions.

Table 3. Helianthus annuus comparison of control and selenite treatment with 100% matches

Putative calreticulin 3	Up-regulated	Roots
Putative berberine/berberine-like FAD-binding type 2	Up-regulated	Roots
Serine hydroxymethyltransferase	Up-regulated	Roots

Helianthus annuus control and selenate

Table 4. Helianthus annuus comparison of control and selenate treatment with 100% matches

Putative calreticulin 3	Downregulated	Roots
Putative berberine/berberine-like FAD-binding type 2	Downregulated	Roots
Serine hydroxymethyltransferase	Downregulated	Roots

Table 5. Helianthus annuus comparison of selenite and selenate treatment with 100% matches

Serine hydroxymethyltransferase 4	Up regulated	Leaves
Calmodulin	Down regulated	Roots
Calmodulin-2	Down regulated	Roots

Table 6. Neptunia amplexicaulis comparison of control and selenite treatment with 100% matches

Heat shock protein 90-5 chloroplast	Up-regulated	Leaves

Heat shock protein 90-1	Up-regulated	Leaves
Serine--glyoxylate aminotransferase	Up-regulated	Leaves
Oxygen-evolving enhancer protein 1-1 chloroplastic	Up-regulated	Leaves
Small ubiquitin-related modifier 2	Up-regulated	Leaves
Chaperonin 60 subunit alpha 1 chloroplastic	Up regulated	Leaves
Catalase	Up regulated	Leaves
Catalase-2	Up regulated	Leaves
Oxygen-evolving enhancer protein 1-1 chloroplastic	Up regulated	Roots
Catalase	Downregulated	Roots
Catalase-2	Downregulated	Roots
Small ubiquitin-related modifier	Downregulated	Roots
Small ubiquitin-related modifier	Downregulated	leaves

Table 7. Neptunia amplexicaulis comparison of control and selenate treatment with 100% matches

Protein	Regulation in treatment	Plant part
Sucrose synthase 1	Up regulated	leaves
Heat shock protein 90-1	Up regulated	leaves
Chaperone protein htpG family protein	Up regulated	leaves
ATP sulfurylase 1 chloroplastic	Up regulated	leaves
L-ascorbate peroxidase 3	Up regulated	leaves
Heat shock protein 60-2	Up regulated	leaves
Chaperonin CPN60-like 1 mitochondrial	Downregulated	leaves
Sucrose synthase 4	Up regulated	leaves
Small ubiquitin-related modifier 2	Downregulated	leaves
Catalase	Down regulated	Roots
Catalase-2	Down regulated	Roots
Oxygen-evolving enhancer protein	Up-regulated	Roots
Sucrose synthase	Downregulated	Roots
Sucrose synthase	Downregulated	Roots
Putative heat shock protein Hsp90 family	Downregulated	Roots

Table 8. Neptunia amplexicaulis comparison of selenite and selenate treatment with 100% matches

Chaperonin CPN60 mitochondrial	Up regulated	Roots
Small ubiquitin-related modifier 2	Up regulated	Roots
Putative chaperonin Cpn60/TCP-1 family	Downregulated	Roots

6.4 Discussion

Literature to date suggests that selenoproteins have been found in other hyperaccumulators, but the results obtained in this research did not detect any selenoproteins. This could be explained by the need for further method development to consider low abundance and low coverage proteins. Instead, the most significant proteins identified with 100% matches have been discussed about their existence (Appendix 1 contains all other proteins identified with 30% match to existing proteins or are not within the scope of the objective).

Putative calreticulin 3* was up-regulated in the roots of *Helianthus annuus* treated exposed to selenite and selenate compared to the control as seen in (Table 3 & 4)

Putative calreticulin 3 is a protein that is responsible for molecular level calcium-binding and chaperone promoting folding in the calreticulin/calnexin cycle. Its role within this cycle is vital in the mono glycosylated glycoproteins, which are synthesized in the ER (Lopes et al., 2013). The downregulation of this protein affected by selenite suggests that the proteomic effect of selenium uptake at sublethal concentrations leads to a decrease in calcium ion binding (Kim et al., 2009), carbohydrate-binding, and protein binding (Li et al., 2009). On a whole plant level, the effects of these molecular binding mechanisms being impacted suggest that the defence response to a bacterium (Gaudet et al., 2011) leading to hypersensitivity (Saijo et al., 2009) in plants. The impact of these processes being disrupted, although it cannot be determined at this stage of growth to the degree of damage to *Helianthus annuus*, current research suggests that a disruption of putative calreticulin 3 may lead to a decrease in seedling growth inhibition response to the pathogen-associated molecular pattern (PAMP) and increase vulnerability to pythogenetic bacteria (Li et al., 2009).

Berberine/berberine-like FAD-binding type[1] 2 enzyme was up-regulated in the roots of *Helianthus annuus* exposed to selenite and selenate treatments compared to the control (Table 3 & 4)

Berberine/berberine-like FAD-binding type 2 is a protein that is largely unexplored but known to consist of 27 classes of FAD-binding proteins that catalyzes the formation of the berberine bridge by oxidation of the N-methyl group of (S)-reticuline (Winkler et al., 2006). While the effects of selenium uptake are unknown at this point, stress studies on *Helianthus annuus* and *Lactuca sativa* have shown that BBE-like enzymes (Custers et al., 2004)are thought to oxidize cellulose-derived fragments as well as cellobiose and glucose. In the model species *Arabidopsis (Winkler et al., 2006),* one of the functions of this protein built by At2g34790/BBE15, which also codes monolignol oxidase, is manipulated as a defence marker (Andrade et al., 2014), the result of which is the production of conversion of indole-3-acetaldoxime (IAOx) to indole-3-carbonyl nitrile (ICN) (Daniel et al., 2015) which then acts as a metabolite in inducing pathogen defence indicating that BBE plays a role in plant immunity(Rajniak et al., 2015). These findings in current research do not align with the findings in this research since BBE has been downregulated, which may indicate that selenium uptake regardless of its form affects plant susceptibility to pathogens; however, without the availability of more literature about the function of the BBE family, this understanding cannot be drawn conclusively.

Serine hydroxymethyltransferase was up-regulated in the roots of *Helianthus annuus* exposed selenite and selenate treatments compared to the control (Table 3 & 4).

Serine hydroxymethyltransferase was up-regulated in the roots of *Helianthus annuus* exposed selenite and selenate treatments compared to the control (Table 3 & 4). During photorespiration, serine--glyoxylate aminotransferase is responsible for converting glyoxylate and serine into glycine and hydroxypyruvate in the presence of asparagine and other substrates. Studies utilising barley and tobacco indicate that Asn aminotransferase is linked to a photorespiratory enzyme, i.e. serine glyoxylate aminotransferase coded by AGT1. The increased expression of Serine--glyoxylate aminotransferase in the treatment of selenite suggests that there is a benefit of selenite treatment in *Helianthus annuus* with its ability to produce increased levels of Asn. This has been recorded as a major storage form of organic nitrogen that is relocated through plant sink tissue (Lea et al., 2007). Studies that eliminated Asn still grow normally even without the major nitrogen storage ability (Zhang et al., 2013). Young leaves, particularly such as those observed in the tissue culture of *Helianthus annuus* contain Asn while there are greater concentrations of Asn aminotransferase in the leaves mature (Ireland and Joy, 1981). These studies also indicated that Asn aminotransferase, when characterised, was structurally similar to Ser: glyoxylate aminotransferase about substrate preference and subcellular localization

(Ireland and Joy, 1983a, Ireland and Joy, 1983b). In varieties of tobacco and barley lacking Ser:glyoxylate aminotransferase, there was also an absence of Asn aminotransferase, suggesting that there may be a greater ability for the plant to have extended nitrogen storing abilities. (Havir and McHale, 1988, Murray et al., 1987) Similarly, the selenite stress allowed for greater expression of the Ser: glyoxylate aminotransferase suggesting that the nitrogen storage ability in *Helianthus annuus* as a result of sublethal concentration exposure is increased compared to the control.

Putative calreticulin 3 was up-regulated in the roots of *Helianthus annuus* treated exposed to selenite and selenate compared to the control as seen in (Table 3 & 4).

Putative calreticulin 3 was up-regulated in the roots of Helianthus annuus treated exposed to selenite and selenate compared to the control. This is another commonly conserved and abundant multifunctional protein is Calreticulin (CRT) which is encoded by a small gene family, most often associated with abiotic and/biotic stress responses in plants. (Xiang et al., 2015). It has been well documented as a Ca^{2+}-binding molecular chaperone that is responsible for the folding of newly synthesized glycoproteins and the regulation of calcium homeostasis in the ER lumen (Qiu et al., 2012). There are three main domains of the CRT protein as follows: N-terminal region (N-domain), the central proline-rich domain (P-domain), and the carboxyl-terminal region (C-domain) and an ER retention signal H/KDEL (Mesaeli et al., 1999). Two distinct forms of CRT in higher plants are also present AtCRT1 and AtCRT2, which assist in regulating plant defence against pathogens. The results suggest that there is the upregulation in the selenate in the roots that activates a defence mechanism for the roots at the sublethal concentration of 25µM compared to the control. Putative berberine/berberine-like FAD-binding type 2 was downregulated in the control compared to the selenate *Helianthus annuus* roots-. Vanillyl alcohol-oxidase fold is tethered by Berberine bridge enzyme-like (BBE-like) to the FAD cofactor bound to cysteine and histidine residue (Daniel et al., 2016). The activity of these enzymes in the model plant, *Arabidopsis thaliana, indicates that they function as* monolignol oxidoreductases suggesting a link to the fluctuations in the extracellular monolignol pool. This can affect cell wall metabolism and lignin formation. There is little data to date to demonstrate the direct function of BBE like enzymes; however, recently, it has gained attention for its increased regulation in the presence of pathogenic plant defence response (Attila et al., 2008, Coram et al., 2010, González-Candelas et al., 2010) and during salt stress (Daniel et al., 2015, Zhang et al., 2014). Additionally, as demonstrated above, this family of proteins is upregulated in the selenate-stressed *Helianthus annuus* plant roots compared to the control substantiating the limited previous findings. Serine hydroxymethyltransferase (SHMT) was down-regulated in the control selenate-treated roots compared to the selenate *Helianthus annuus* root. This protein is a pyridoxal 5′-phosphate (PLP)-the dependent enzyme that is responsible for the catalysis of the reversible serine glycine conversion in tetrahydrofolate dependent or independent methods (Bouché et al., 2005). Additionally, this protein also cleaves β-hydroxy amino acids that are tetrahydrofolate-independent and is critical in the main source of activated one-carbon units suggesting its role in cell proliferation (Girgis et al., 1997, Townsend et al., 2004, Wu et al., 2017). The role of SHMT in plants is more challenging than those present in bacterial or mammalian cells because there is a greater variety of SMHT encodes that are aimed at different subcellular compartments such as the cytosol, mitochondria, plastids, and nucleus (Ranty et al., 2006). The elevated levels of this protein in the *Helianthus annuus* roots suggest that the plant is more vulnerable to Se stress.

Lipoxygenase was downregulated in the leaves of *Helianthus annuus* exposed to selenite compared to the control.

Lipoxygenase is upregulated in the control compared to the selenite treated *Helianthus annuus* leaves. These correspond with elevated levels of lipoxygenase Chapter 2. Lipoxygenase oxidizes fatty acids as part of the lipoxygenase family (LOXs; EC 1.13.11.12), including non-genomic iron-compatible dioxygenases. These are responsible for the catalysis of regio- and stereo deoxygenation of polyunsaturated fatty acid (PUFA) with a range of cis, cis-1,4-pentadiene fragments as well as the production of fatty acid hydroperoxides (Andreou and Feussner, 2009, Feussner and Wasternack, 2002, Porta and Rocha-Sosa, 2002). Because of LOX activity, a conjugated cis-trans complex is formed. Linoleic acid oxidation is the first step in the enzyme cascade producing biologically active compounds called oxylipins. These are active in the production of plant organism responses to abiotic and biotic stresses effects such as regulation of ageing and apoptosis (Göbel et al., 2001, Maccarrone et al., 2001, Mosblech et al., 2009, Santino et al., 2013, Savchenko et al., 2014, Schwarz et al., 2001, Taki et al., 2005, Vellosillo et al., 2007, Wasternack and Hause, 2013). LOX activity differs according to various stressors; for example, in studies by Lakin (2004), LOX expression increased as a result of low-temperature stress encouraging membrane phospholipids degradation and release of the LOX substrate, PUFA [106]. On the other hand, the functional activity of LOX in corn decreased in the control but returned to a stable level when salt stress was reduced (Zhao et al., 2010). Cold stress also indicated a decreased level of 9-LOX, but there was an increase in 13-LOX, indicating the wider interactions of two different sites of the lipoxygenase cascade during the production of biologically active products resulting from abiotic stresses (M et al., 2015). This variation in the pattern of LOX expression is also visible in the comparison of the control of *Helianthus annuus* leaves to the selenite treated leaves where there was an upregulation in the control, with a similar pattern not observed in the treatment. Seedlings of stressor-sensitive plant *Festuca pratensis Huds*. Contained the low rates of lipoxygenase activity in the control but contained substantially lower concentrations in the treatments with higher and lower temperatures. Our results suggest that the selenite treated leaves indicated decrease LOX activity in agreeance with other stressors on model plants. Carbonic anhydrase is downregulated in the control compared to the selenite treated *Helianthus annuus* leaves. Deemed a critical enzyme involved photosynthesis for its ability to convert CO_2 to HCO_3^- reversibly (Bhat et al., 2017). Carbonic anhydrase is a zinc-containing metalloenzyme. It plays a role in carboxylation and stomatal closure in plant leaves (Neish). It is the second most abundant enzyme in the chloroplast and is one the most efficiently reacting enzymes in plants (Badger, 2003). Carbonic anhydrase plays an important role as an efficient zinc metalloenzyme, with many studies concentrating on the role of Carbonic anhydrase in inorganic carbon fixation, respiration, and carbon dioxide transport, and electrolyte secretion. It was also found that this enzyme assists in defending the stroma against enzymes (Ludwig). Studies in *Arabidopsis* plants are also present in Arabidopsis leaves that show an upregulation in carbonic anhydrase (Kawamura and Uemura). The upregulation in the selenite treated *Helianthus annuus* leaves suggests that selenite at the sublethal concentration provides an increase in oxidative stress protection based on the role of carbonic anhydrase as a critical enzyme in photosynthesis.

Similar to the control and selenate comparison, serine hydroxymethyltransferase 4 was also identified in the comparison of selenite and selenate treated plants, where it upregulated in the selenite leaves of *Helianthus annuus* compared to the selenate leaves.

Calmodulin (CaM) downregulated in the selenite compared to the selenate treated *Helianthus annuus* roots*

Calmodulin (CaM) proteins are a major group of calcium sensors, and these play a critical role in cellular signalling cascades (Poli et al., 2018). Calmodulin was downregulated in the selenite compared to the selenate treated *Helianthus annuus* roots. Calmodulin-2 is downregulated in the selenite compared to the selenate *Helianthus annuus* treated roots. These function through the regulation of numerous target proteins. While CaM proteins are conserved throughout eukaryotes some characteristics are unique to plants (Hill and Hemmingsen, 2001). It is a growing group of plant proteins that is thought to also regulate cell death as part of the defence signalling pathways (Yamamoto, 2016). Toxic concentrations of metalloids can adversely affect protein structure and functionality through the sulfhydryl group's disruption, thereby destroying the integrity of the plasma membrane. This interference with photosynthesis and respiration decreases the efficiency of metabolic processes (Emamverdian et al., 2015, Ovečka and Takáč, 2014). For example, studies by Sunkar et al. (2000) demonstrated that the removal of CaM and cyclic nucleotide-binding regions abolished Pb^{2+}-hypersensitivity in transgenic plants. This finding indicates that the CaM binding property of the channel proteins is a potentially viable method for phytoremediation of soils with the ability to minimize sensitivity in crop plants to toxic metalloids with further research. The elevated levels of CaM and CaM-2 proteins in the selenate treated *Helianthus annuus* roots compared to selenite suggest that *Helianthus annuus* transgenic varieties with further research may be used to remediate contaminated soils.

These results indicate that there is a need for further research into each of these plants to further identify protein regulation within the plants. While there was upregulation of critical protein that indicated enhanced defence mechanisms for the plant there were also proteins that crippled at these sublethal concentrations resulting in inhibitory effects on plant materials.

Heat shock protein 90-5 chloroplast was up-regulated in the leaves of *Neptunia amplexicaulis* exposed to selenite compared to the control.

Heat shock protein 90-1 was up-regulated in the leaves of *Neptunia amplexicaulis* exposed to selenite compared to the control (Table 6)

The activation of heat shock proteins can be induced because of the number of stresses not limited by heat, such as but not limited to sub-optimal temperature, osmotic, salinity, oxidative, drought, high-intensity irradiations, wounding, and heavy metals stresses (Swindell et al., 2007). These are referred to as "heat shock proteins (HsP)," stress proteins," or "stress-induced proteins" (Gupta et al., 2010, Lindquist and Craig, 1988, Morimoto et al., 1994). There is a range of heat shock proteins characterized by the carboxylic terminal called the heat-shock domain (Helm et al., 1993). Chloroplastic heat shock protein 90-5 can function in protein signalling and trafficking where it regulates glucocorticoid receptor activity (Pratt et al., 2004). The upregulation of this protein in control instead of the treatment suggests that *Neptunia amplexicaulis* is better adapted to the stress of selenite exposure than the control, and the absence of Se in the control *Neptunia* induces the activation of the stress proteins. In particular, HsP 90 can bind to Hsp70 in various chaperone complexes to provide resistance against pathogens. It has been reported in thaliana, tobacco varieties, and rice that the HsP 90 was essential for innate immune response and pathogenic resistance substantiating experimental findings (Hubert et al., 2003, Liu et al., 2004, Schulze-Lefert, 2004, Thao et al., 2007, Yamada et al., 2007). Chloroplastic heat shock protein 90-5 was upregulated in control and the selenite treated *Neptunia amplexicaulis*. This protein performs both biological and molecular functions, with roles in ATP binding, protein homodimerization activity, and unfolded protein binding. Its biological functions include desolation, protein folding, and import into the chloroplast stroma. It also reacts to chlorate, heat, salt, and drought stress (Cao et al., 2003, Feng et al., 2014, Inoue et al., 2013, Oh et al., 2014). Heat shock protein 90-1 was also upregulated in the control and selenite exposed *Neptunia amplexcaulis* leaves, and this is responsible for the chaperone-mediated protein folding and the defence response to the bacterium.

ATP sulfurylase one chloroplastic- upregulated in leaves of *Neptunia amplexicualis* selenate treatment compared to the control*

ATP sulfurylase 1 ATPS (ATP: sulfate adenylyltransferase) is the first enzyme involved in the sulfate assimilation pathway, and till recently, its role in fungi and bacteria was widely available, it is only in more recent years that its role in phototrophic organisms (especially of algae) is gaining more attention. In particular, sulfate reduction (adenosine 5′-phosphosulfate reductase, APR) and cysteine synthesis (cysteine synthase or O-acetylserine (thiol)lyase, OAS-TL) processes are not clearly understood concerning the role of enzymes. The upregulation of this ATP sulfurylase 1 in the leave of *Neptunia amplexicaulis* treated with the selenate indicates is interesting because there is sulfate assimilation taking place, which aligns with existing research however, given that selenoproteins were detected, it may mean that *Neptunia* amplexicualis has evolved as a means of storing the selenium in an organic form.

Sucrose synthase 1 downregulated in selenate compared to the control*

Sucrose synthase 1 is responsible for sucrose cleaving to provide UDP- glucose and fructose for use in numerous metabolic pathways. It is upregulated in control compared to selenate *Neptunia amplexicaulis*. It forms part of the GT-4 glycosyltransferase subfamily and so far studied extensively in Arabidopsis thaliana, where it is regulated differently depending on the form of stress imposed, i.e. temperature, drought, and oxygen deficiency (Déjardin et al., 1999). The lower levels of protein regulation in the treatment indicate that the stress is affecting the ability of the plant to cope with the sublethal concentrations. These results also suggest that there may be a decreased level of starch production substantiated by studies in potatoes (Zrenner et al., 1995) and carrots (Tang and Sturm, 1999). Sucrose synthase 4 was also up-regulated in control compared to the selenate-treated leaves. This upregulation in the leaves of the control compared to the selenate treated leaves was also observed under stress conditions in model plants where an increased expression of SUS demonstrated an increase in plant biomass and growth rate, taller plants, and early fruit induction and tobacco (Coleman et al., 2006, Xu and Joshi, 2010).

Heat shock protein 90 downregulated in selenate exposed plants compared to the control* *Neptunia amplexicaulis* leaves

Heat shock protein 90 was also upregulated in the control compared to the selenate treated *Neptunia amplexicaulis* leaves. While there are many classes of heat shock protein, the type of heat shock protein identified determines the ability of the plant to handle stress. (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60, and (5) small heat-shock proteins (sHsps) (Al-Whaibi, 2011), according to the molecular weight are induced in most living organisms to cope with heat stress. Additionally, the genetic variations in the morphogenetic pathway are buffered by Hsp90 (Kadota and Shirasu, 2012).

Chaperone protein htpG family protein is downregulated in the control leaves of *Neptunia amplexicaulis**

Chaperone protein htpG family protein is upregulated in the control leaves compared to the selenate *Neptunia amplexicaulis* treated leaves. This is responsible for the regulation of meristem size and organization (Klein et al.). An increase in this protein in the control suggests that there is an impairment of the meristem elongation in the selenate-treated plant leaves.

Heat shock protein 60 down-regulated in the control compared to the selenate treated leaves *

Heat shock protein 60 was also down-regulated in the control compared to the selenate-treated leaves. This protein is found in the mitochondria and is responsible for protein folding and the prevention of accumulation of aggregation of denatured proteins. It is critical in assisting plastid proteins, including Rubisco (Wang et al., 2004). This protein acts by binding with other proteins after they are transcripted and before folding to prevent protein clusters from forming (Parsell and Lindquist, 1993). The detection of this protein upregulation in the treatment suggests that there is greater Rubisco activity in the treatment compared to the control.

Mitochondrial chaperonin CPN60-like 1 down-regulated in the control compared to the selenate treated leaves*

Mitochondrial chaperonin CPN60-like 1 downregulated in the control compared to the selenate treated leaves. This protein is responsible for mitochondrial protein imports and macromolecular structuring. It is also thought to play critical roles in the prevention of misfolding while promoting the refolding and the methodical assembly of polypeptides that are produced under stress conditions (Carrie et al., 2015, Heazlewood et al., 2004). In *Neptunia* leaves exposed to selenate showed greater up-regulation of this protein, suggesting an adaptive capability for this sublethal concentration of selenium exposure. Small ubiquitin-related modifier 2 is down-regulated in the control compared to the selenate treated leaves. SUMO modifiers are similar to ubiquitin in their structure and mechanism. Catalase down-regulated in the control compared to the selenate treated roots. This is compliant with previous studies where it was demonstrated an increase in CAT proteins in high yield mutants from lower yield mutants of rice varieties (Poli et al.) Catalase-2 down-regulated in the control compared to the selenate treated roots. Catalase down-regulated in the control compared to the selenate treated roots. These results are substantiated by widely documented data that indicates an increase in catalase expression when exposed to a/biotic stresses and by the research in the chapters with the literature review and enzymes analysis as seen in chapters 1 and 2, respectively. Oxygen-evolving enhancer protein was upregulated in the control compared to the selenate treated *Neptunia* roots. To date, limited data has been produced to indicate the reasons for fluctuating levels of OEEC; however, its increase is also attributed to increased stress levels, particularly heat stress in plants (Yamamoto, 2016). It also stabilizes the manganese cluster, which serves as the main site of water splitting (Murakami et al., 2002, Yi et al., 2005). Sucrose synthase was down-regulated in the control compared to the selenate-treated leaves. Putative heat shock protein Hsp90 family down-regulated in the control compared to the selenate treated leaves.

6.5 Conclusion and Future Direction

These results indicate that there is a need for further research into each of these plants to further identify protein regulation within the plants. While there was upregulation of critical protein that indicated enhanced defence mechanisms for the plant, there were also proteins that crippled at these sublethal concentrations resulting in inhibitory effects on plant materials. It should be noted, however that this proteomics should be further optimized with proteomics markers to identify specific proteins in a targeted manner to capture and hone in to capture low abundance high coverage proteins, but due to funding and time limitations, this could not be achieved for this series of experiments.

Chapter 7 General discussion, the significance of findings and conclusions

The detection of effective Se accumulator species is an area with vast benefits to animal and human health. Given that very little research to date has addressed the physiological impacts of selenite and selenate on the selected plants, there is an opportunity through this research to contribute to addressing the gaps in knowledge on reactive oxygen species concentration patterns under Se stress, to attempt autoradiographic imaging for Se quantification, and perform comparative proteomics analysis between Se non-accumulator species and accumulator species. By doing so, the value of using plants as a means of Se phytoremediation was assessed, along with developing a greater understanding of Se metabolism mechanisms.

The current research aimed to address these gaps in knowledge by:

1. Identifying sublethal concentrations of selenite and selenate exposure in Se accumulator and non-accumulator plant
2. Identifying patterns of ROS related enzyme concentration in a Se accumulator and non-accumulator plant
3. Quantifying Se in Se accumulator and non-accumulator plants through creating an autoradiographic image
4. Identifying relationships between seed storage proteins and selenium uptake as a precursor to proteomic studies
5. Identifying up and downregulating proteins on exposure to sublethal concentration of Se

The overall objective of this study was to use the theoretical knowledge gained to inform plant selection for use for field trials on Se contaminated soils.

Chapter 2 experiments aimed to determine the sublethal concentrations of sodium selenite and sodium selenate in *Solanum tuberosum*, *Helianthus annuus*, *Brassica rapa. var* vitamin greens and *Neptunia amplexicualis* by measuring a range of growth variables on exposure to increasing concentrations of sodium selenite and sodium selenate in tissue culture. The findings of this chapter were then used as the basis for further investigations into the antioxidation cycle enzymes and metabolites, uptake, quantification of Se within the plants, and trends in the upregulation and downregulation of proteins.

Based on considerations and analyses, and variables acceptable for plant toxicity determination variables (Huang et al., 2019), i.e. fresh weight and dry weight, the root and shoot length, shoot and root length ratios, and the number of leaves and the grade of leaf material, the sublethal concentration of each salt of selenite and selenate for *Brassica rapa* was 20µM, for *Helianthus annuus* 25µM, for *Neptunia amplexicaulis* 500µM, and *Solanum tuberosum* 15µM. At a critical point of the research and experimental investigations, *Solanum tuberosum* was eliminated for further studies as the plant was challenging to gain sufficient biomass for replication and statistics. These findings formed the basis for the experiments performed in the preceding chapters. To enhance the accuracy of these results, other variables could also have been measured, such as chlorophyll content, individual, specific, and total protein quantities; and Se concentration should also have been measured. The novelty of these findings prepares a foundation for the potential use of these plants, pending further research, in phytoremediation.

The purpose of the experiments in chapter three was to identify trends in the concentration of antioxidant enzymes, superoxide dismutase, catalase, lipoxygenase, ascorbic acid peroxidase, glutathione reductase, glutathione, acid, and neutral proteases in *Brassica rapa, Helianthus annuus,* and *Neptunia amplexicaulis*; when exposed to selenite and selenate treatments. In doing so, they shed light on the role of Se when exposed to plants at sublethal concentrations for the foundation to proteomic studies in chapter 6 of this thesis. All findings aligned with existing data where plants exposed to stress factors both abiotic and biotic resulted in oxidative stress in the plants, and as expected, enzymes in the reactive oxygen species cascade were elicited. More broadly, the understanding of selenite and selenate effects on enzyme concentration lays the theoretical understanding of the plants coping ability if plants are used at and in concentrations that are reflective of contaminated power plant soils.

These experiments in chapter 4 aimed to determine Se concentrations in plants using radiotracers. This study investigated the uptake, accumulation, and distribution of selenium species in *Brassica rapa.cv vitamin green*, *Helianthus annuus cv. Helianthus annuus* and *Neptunia amplexicaulis,* through the entire plant, plant sections, and protein fractions upon a 21-day exposure to selenite and selenate salts and autoradiography. To date, numerous analytical and instrumental methods such as microwave digestion and fluorescence detection have been utilized to find selenium in samples from the environment (Nahir and Sheffield, 2002, Pyrzyńska, 2002). Many challenges in the detection of Se have been identified, and in recent years some have been overcome stemming from the narrow range between essential Se and the toxic concentration, including developments in detection sensor material, electrode surface modifiers, chelators, along with improved detection limit, range, reproducibility, stability, selectivity, and sensitivity (Devi et al., 2017). Yet, there are still many complexities presented by the samples matrix and the end goal of Se quantification and/speciation (Paikaray, 2016).

Broadly, selenium detection methods can be applied through destroying samples such as inductively coupled plasma mass spectroscopy (ICP-MS), a hydride generation atomic fluorescence spectrometer (HG-AFS), or the hydride generation atomic absorption spectroscopy (HGAAS) or Instrumental Neutron Activation Analysis (INAA) which is gaining popularity, not considered destructive to the sample but an only be used on-air samples currently (Ballihaut et al., 2007). ICP -MS is most suitable when Se detection in samples is routine and multi-elemental, while INNA is best used when Se is expected to be found at minute concentrations (10^{-8}–10^{-9} g) (Uden, 2002). Comparatively, HGAAS can be utilized when lower detection limits are thought to be present (0.1 µg) (Buchberger, 2001, Mazej et al., 2006, Vassileva et al., 2001).

More conventional methods of Se detection are gas chromatography (GC), high-performance liquid chromatography (HPLC), ICP-MS, neutron activation analysis (NAA), atomic fluorescence spectrophotometry (AFS), AAS, electrothermal AAS, and graphite furnace AAS (GF-AAS), which have been used on plant samples to detect Se concentrations widely. For improved speciation and detection, hyphenated separation methods such as Ion chromatography (IC) and capillary electrophoresis (CE) have been applied with atomic spectrophotometry detection systems such as Electrothermal AAS and plasma optical emission spectroscopy (POES) (Bailey, 2017).

Increasingly when attempting to detect trace analysis of toxic elements, it is vital to find the total content of the element at lower concentrations while also determining the binding state. Determination of the binding state will influence its toxicity as seen with selenite and selenate, which are both more bioavailable through their water solubility compared to elemental Se, metabolism in environmental and biological systems (Fairweather-Tait et al., 2010)

To determine mechanisms of selenium sorption, some methods depend on sample interaction and a source of X-ray excitation, which include energy-dispersive X-ray spectroscopy (EDXRS), Fourier-transform infrared spectroscopy (FT-IR), powered X-ray diffraction (PXRD), extended X-Ray absorption fine structure (EXAFS) and X-ray absorption spectroscopy (XAS) (Zhang, 2007).

To date, X-ray Absorption Spectroscopy has been used on *A. bisulcatus* plants initially grown for 3 weeks in selenate (Pickering et al., 2000), but there are no distribution studies that reflect visually or quantitatively selenium distribution within the plants in this study. Additionally, in the existing studies, despite the improvements to methodology, the presence and absence of previously identified selenium species remain inconsistent, highlighting the need for selenium distribution studies using an alternative technique such as radiotracers as used in Chapters 4 and 5 in this thesis.

ICP- MS has been previously used to determine Se concentrations in *Brassica juncea* species (Sharma and Sohn, 2009, Szpunar, 2005) along with size exclusion chromatography studies (Mounicou et al., 2006), all yielding varied results in determining the distribution of Se within the plants, therefore, using autoradiography as a technique for determining the distribution of Se in plants was worth exploration.

Implementing the use of short half-life radioisotopes from the whole plant to genetic, molecular, organismal, and ecosystem studies can be used to determine the process that binds the enzyme-based biochemical reactions to the physiological responses of plants to environmental stimuli and stresses (Osmond, 1989). Positron emission tomography (PET) has been widely used in medical diagnostic imaging (Antoch et al., 2003, Phelps, 2000, Schaller, 2004) with an increasing number of studies using this method on plants coupled with other imaging methods, for instance, photoswitch imaging detector was utilized for both positron- and γ-ray-emitting nuclide imaging (Wu and Tai, 2011). Where live imaging systems were needed PlanTIS and PETIS were used to determine the movement of radionuclides in tomato (Suwa et al., 2008), broad bean (Matsuhashi et al., 2006), beet and bulbs (Jahnke et al., 2009), soybean (Kawachi et al., 2011) barley (Tsukamoto et al. 2006), wheat (Matsuhashi et al., 2006) and rice (Ishikawa et al., 2011). ^{11}C (Minchin and Thorpe, 2003), ^{13}N (Ohtake et al., 2001), ^{15}O (Kiyomiya et al., 2001), ^{18}F (Nakanishi et al., 2001), ^{52}Fe (Tsukamoto et al., 2009), ^{52}Mn (Tsukamoto et al., 2006) and ^{107}Cd (Fujimaki et al., 2010) were used as the positron emitters for these imaging methods.

Where live tracing of a radiotracer isn't needed, radiotracers can also be used on a whole plant level to a genetic level, but to date, at the time of writing, there are no studies of selenium at sublethal concentrations. Additionally, even with improvement to detection of minute concentrations of Se, there are varying conclusions on the existence of major Se-containing compound in the leaves is Se-methyl selenocysteine, with smaller amounts of selenocysteine and γ-glutamyl-Se-methyl selenocysteine in *Astragalus bisculcatus* (Neuhierl et al., 1999, Nigam and McConnell, 1969). Even more robust studies using micro X-ray Fluorescence Microscopy (µXRF) and scanning electron microscopy (SEM-EDS), as well as on chemical forms of Se in various tissues using liquid chromatography-mass spectrometry (LC-MS) and synchrotron X-ray absorption spectroscopy (XAS) has only shown methyl-selenocysteine and seleno-methionine in the foliar tissues but not Se-methyl selenocysteine (Harvey et al., 2020).

Autoradiography was the selected method of producing a Se distribution method because of its high sensitivity and the wide range of detection, especially where trace elements may occur in minute concentrations and the availability of Se^{75} with a 119-day half-life. To substantiate the qualitative observations of Se concentrations in all three plant species, the next step should be to perform ICP-MS on each plant, i.e. leaves, stems, and roots however, ICP-MS for ultra-trace element detection of radioactive Se^{75} from an agar matrix was not available. There was also a need to undertake gamma counts of Se^{75} on a gamma counter to measure and determine Se concentration in each plant section, so while the autoradiography contributes to previously minimal knowledge of Se distribution within the *Brassica rapa*, *Helianthus annuus,* and *Neptunia amplexicualis,* the concentration of Se within each plant section could not be determined. If this series of experiments was to be repeated, each plant section should be either weighed individually, and ICP-MS used to confirm the concentration of Se absorbed by each plant.

Given there is no research studying the effects of Se uptake on seed storage proteins in any plants other than rice, this chapter aimed to quantify uptake selenium uptake patterns in *Brassica rapa* and *Helianthus annuus,* and *Neptunia amplexicualis*. The descriptive conclusions drawn in this chapter were then used to inform the selection of plants for the final chapter of this thesis on proteomics. The seed storage proteins that were studied were albumins, globulins, glutelins, prolamins, and crude starch after lipids were removed from the plant materials. The concentration of each seed storage protein class can vary significantly between species of plants. The analysis of seed storage proteins is important in exploring the prospect of supplementing the human diet with selenium as an essential micronutrient for humans and animals. Similar studies conducted to analyse plants for seed storage proteins have been completed by *Brassica rapa* and *Helianthus annuus* and, to a lesser extent, *Neptunia amplexicaulis*.

Seed storage proteins are critical in plant development and a source of human nutrition. They accumulate in high concentration seedlings to assist in germination and remain a significant source of protein for human and animal consumption. Developing an understanding of the potential binding of Se to these proteins can translate into relevant information regarding protein longevity content, stress. Given that storage proteins undergo post-translational modifications understanding Se binding would show ere Se could transfer into other proteins through the five types of post-translational modifications, phosphorylation, glycosylation, lipid modification, ubiquitination, and redox-related modifications (Bond et al., 2011).

This investigation aimed to find out which proteins, if any, in *Helianthus annuus*, *Brassica rapa* and *Neptunia amplexicaulis* may be associated with Se incorporation, the impact of the sublethal concentration on protein regulation, and the significance of the proteins impacted using data gathered from the preceding chapter. Selenoproteins in mammals, virus bacteria, archaea, and have been identified to possess the rare amino acid selenocysteine in their primary structure, but this has not been found in plants and yeasts. The most co mMonly investigated selenoproteins of enzymes are the group of GPXs. In particular, *Chlamydomonas* and higher plants have been shown to possess genes encoding GPX-like homologs from which cysteine is present in their catalytic site but not as selenocysteine. Selenocysteine is encoded by a UGA opal codon that is most commonly a stop codon in all organisms that possess selenocysteine. It was reported in *Chlamydomonas reinhardtii that* the cDNA-cloned sequence of a GPX homologue also possesses a TGA codon in the frame to the ATG internally. Critical functions such as the expression of mRNA expression, protein synthesis, and enzymatic concentration are selenium dependent. Proteolytic digestion performed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) on the peptides synthesized by proteolytic digestion substantiated the presence of selenocysteine residue at the predicted site, which also suggested that this takes place in the mitochondria. For the first time, this research provided proof that the UGA opal codon is decoded in the plant kingdom to incorporate selenocysteine (Fu et al., 2002).

Even though these experiments did not detect selenoproteins Putative calreticulin 3, putative berberine/berberine-like FAD-binding type 2, Serine hydroxymethyltransferase, Serine hydroxymethyltransferase 4, Calmodulin, Calmodulin-2, Heat shock protein 90-5 chloroplast Heat shock protein 90-1, Serine--glyoxylate aminotransferase, Oxygen-evolving enhancer protein 1-1 chloroplastic, Small ubiquitin-related modifier 2, Chaperonin 60 subunit alpha 1 chloroplastic and catalase, which all to varying degrees, whether up or down-regulated, affects the germination and development of each plant. These results indicate a need for further research into each of these plants to identify protein regulation within the plants further. While there was upregulation of critical protein that indicated enhanced defence mechanisms for the plant, there were also proteins that crippled at this sublethal concentration resulting in inhibitory effects on plant materials.

Limitations and challenges

This research undoubtedly offered new and novel insight into the patterns of oxidative stress when secondary and primary accumulators are germinated at sublethal concentrations. While specific selenoproteins could not be identified or detected in this method, it is worth noting that this may be due to several reasons outside of method optimization which has been discussed in the previous chapters. These reasons include but are not limited to using an increased starting biomass, insufficient plant proteomic data compared to animal and human proteins, and the natural difficulty of selenium metabolization. These are widely acknowledged in existing protocols whereby even more robust methods such as synchrotron usage has failed to detect certain selenoproteins (Corzo Remigio et al., 2020) in plants known to accumulate selenium compared to previous research, which substantiates the conclusion of this thesis which is that selenium metabolism remains unclear along with its effect on individual proteins (Peterson and

Butler, 1967). In broader terms, however, *Helianthus annuus* and *Neptunia amplexicaulis* both are still feasible to be trialled in field studies for selenium.

Given that the purpose of this study was to analyze the uptake mechanisms and impacts of Se on the selected plants, tissue culture offered a beneficial solution with several advantages. Tissue culture allowed for the rapid expansion of seed material irrespective of seasonal variations, producing disease-free plants in a cost-effective and space-efficient method., thus overcoming the challenges of performing this research in the soil, which has been the accepted means of studying contaminant uptake in plant materials previously (Ponmurugan and Kumar, 2011). Additionally, tissue culture overcame the problems related to the lack of publicly available information regarding Se contamination from coal mining and its associated ash dumping sites for application to Australian Se contaminated sites (Lipski, 2019). It should also be mentioned that these results cannot be directly applied in to field trials due to the mixed composition of contaminated sites, among other factors such as cost; hence it is important to realise the value of these findings lies in the ability to uncover the uptake mechanisms for, and metabolic function of Se in the plant system as a theoretical foundation from which to develop field trials (Doran, 2009).

Autoradiography was the selected method of detecting a Se distribution method because of its high sensitivity, which is especially useful where trace elements may occur in minute concentrations and the availability of Se^{75} with a 119-day half-life. The next step should have been to perform ICP-MS on each plant, i.e., leaves, stem, and roots, to substantiate the qualitative observations of Se concentrations in all three plants species. However, ICP-MS for ultra-trace element detection of radioactive Se^{75} from an agar matrix was not available. There was also a need to undertake gamma counts of Se^{75} on a gamma counter to measure ad determine Se concentration in each plant section, so while the autoradiography contributes to previously minimal knowledge of Se distribution within the *Brassica rapa*, *Helianthus annuus,* and *Neptunia amplexicualis,* the concentration of Se within each plant section could not be determined. If this series of experiments was to be repeated, each plant section should be either weighed individually, and ICP-MS used to confirm the concentration of Se absorbed by each plant.

Selenite and selenate concentrations in seed storage proteins could not be determined using this method, 1) the original methodology assumes that there is no loss of selenium during the first protein extraction step; 2) when leaves, stems, and roots were mixed individually using radiotracer, the final powdered weight should be the true weight throughout all experiments to account for biomass losses in the processing of the tissue.

It should also be noted that the protein content of each seed storage protein fraction needed to be measured which would have allowed the results to be displayed with the concentration of the selenite or selenate per milligram of protein at the very least. ICP- MS is unable to detect selenium in plant tissue without highly specialised equipment which was not available at a nuclear research facility. If this experiment was to be repeated, it could be done with ICP-MS on the plant materials without the Se75 radioisotope exposure which would have resulted in the clear quantification of not only the Se species, selenite, and selenate in plant organs, but also within the seed storage proteins. Then a new experiment could have been completed to validate the ICP-MS findings for a qualitative identification on the translocation of Se within the plant materials.

Research significance and contribution to field

The results of this research demonstrate the potential use of *Neptunia amplexicaulis* and *Helianthus annuus* phytoremediation of selenium. The results of the experiments discussed above show that these plants can absorb selenium from the medium to the roots of the plant and transport them further to the aerial organs of the plants. Research to date illustrates that from the leaves, the selenium can be removed through harvesting, or volatilised into the atmosphere in less toxic forms as dimethyl selenide (DMSe) and dimethyl diselenide (DMDSe) which are estimated to 600 times less toxic than elemental selenium (Newman and Reynolds, 2004, Dumont et al., 2006). This method of soil remediation is cost effective without altering the fertility of the soil compared to physical methods of soil remediation (Robinson et al., 2000; Pilon-Smits and Freeman, 2006).

The relatively high tolerance of selenium in *Neptunia amplexicaulis* to selenium places this plant as a potentially greater option for phytoremediation compared to). *Stanleya pinnata* and *Astragalus bisulcatus* are well-known Se-accumulators. *Stanleya pinnata and Astragalus bisulcatus*, although well researched for their selenium uptake properties, are slow growing and low in biomass production. Furthermore, given that the initial shotgun proteomics method did not identify any misformed selenoproteins in *Neptunia amplexicaulis*, it may be possible that *Neptunia amplexicaulis* can volatilise selenium however this was out of scope for this research.

Similarly, *Helianthus annuus,* also demonstrated significant uptake of selenium although at a lower concentration compared to selenium and selenoproteins were not identified. While this plant may also be used for phytoremediation, it may also be used for biofortification which was the secondary objective of this research. While phytoremediation is an efficient method of means of restoring soil health, one of the challenges is the disposal of the contaminated plant waste. If left undisposed then the plants can be toxic to humans and animals. Selenium biofortification involves using the selenium enriched plants for addition to agricultural soil through which food plants can be enriched with selenium, leading to an increase in dietary intake of selenium. Additionally, these findings have highlighted an opportunity to genetic engineering and manipulation of agronomic practices. This practice is safe and addresses the selenium as a nutrient deficiency through the consumption of the edible portions of the plant therefore *Helianthus annuus* may be a suitable plant for biofortification purposes (Nestel et al., 2006; Mayer et al., 2008; Zhao and McGrath, 2009). Until now such studies have only been seen in tomatoes, some brassica varieties and certain rice varieties to address selenium nutrient deficiency.

The *Neptunia amplexicaulis* also presents an opportunity for the creation of a transgenic plant variety that would increase the tolerance of Se concentration, uptake and transportation to the shoots, acccumuation in the shoot tissues and increase in selenium volatilisation (Morgan et al., 2005; Lynch, 2007).

Chapter 8 Conclusion and Future directions

The current research aimed to address these gaps in knowledge by identifying sublethal concentrations of selenite and selenate exposure in Se accumulator and non-accumulator plant, identifying patterns of ROS related enzyme concentration in a Se accumulator and non-accumulator plant, quantifying Se in Se accumulator and non-accumulator plants through creating an autoradiographic image, identifying relationships between seed storage proteins and selenium uptake as a precursor to proteomic studies and identifying up and downregulating proteins on exposure to sublethal concentration of Se.

While not all objectives were met with positive findings, each objective presented opportunities for further optimization, efficiency, and depth that can be pursued post this research to reveal to a greater extent the insights of Se metabolism in plants, effects of Se metabolism on plant physiology and potential for phytoremediation.

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Appendix 1. Protein densitometry gels for *Neptunia amplexicualis* shoots and roots and *Helianthus annuus.*

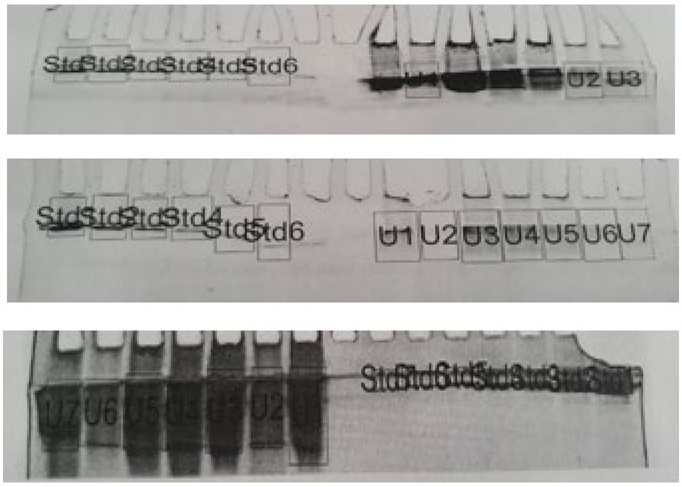
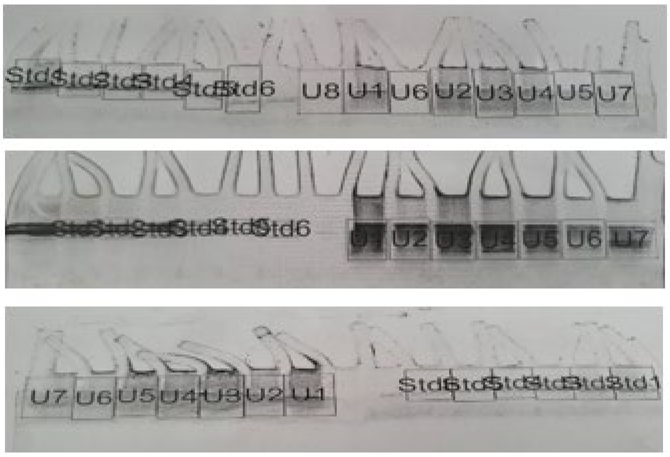


*Figure SEQ Figure * ARABIC 23. 1-D gel of Neptunia amplexicaulis for protein concentration*



Appendix 2	Protein mg/g FW		
Protein concentrations	*Brassica rapa*	*Helianthus annuus*	*Neptunia amplexicaulis*
contr shoot	5.8	5.1	0.6
senate shoot	3.4	3.6	0.4
senite shoot	3.9	4.1	0.5
contr root	5.1	4.0	0.5
senate root	3.8	3.1	0.4
senite root	3.4	3.0	0.5

Appendix 3

Appendix one contains all proteins searched from *Helianthus annuus* and *Neptunia amplexicaulis* samples

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
F4IVZ7|F4IVZ7_ARATH	Rubisco activase OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P25857|G3PB_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPB chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPB PE=1 SV=2
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9SIF2|HS905_ARATH	Heat shock protein 90-5 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=HSP90-5 PE=1 SV=1 (ur c senite nept leav)
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P27323|HS901_ARATH	Heat shock protein 90-1 OS=Arabidopsis thaliana OX=3702 GN=HSP90-1 PE=1 SV=3 (ur c senite nept leav)
sp|Q9SI75|EFGC_ARATH	Elongation factor G chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPEFG PE=1 SV=1
sp|Q43127|GLNA2_ARATH	Glutamine synthetase chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLN2 PE=1 SV=1
A0A1P8ANC0|A0A1P8ANC0_ARATH	Hydroxypyruvate reductase OS=Arabidopsis thaliana OX=3702 GN=HPR PE=3 SV=1

sp|Q9C9W5|HPR1_ARATH	Glycerate dehydrogenase HPR peroxisomal OS=Arabidopsis thaliana OX=3702 GN=HPR PE=1 SV=1
sp|Q9SA52|CP41B_ARATH	Chloroplast stem-loop binding protein of 41 kDa b chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CSP41B PE=1 SV=1
A0A1P8ATL2|A0A1P8ATL2_ARATH	Chloroplast RNA binding protein OS=Arabidopsis thaliana OX=3702 GN=CRB PE=4 SV=1
sp|Q93VR3|GME_ARATH	GDP-mannose 3 5-epimerase OS=Arabidopsis thaliana OX=3702 GN=At5g28840 PE=1 SV=1
sp|Q56YA5|SGAT_ARATH	Serine--glyoxylate aminotransferase OS=Arabidopsis thaliana OX=3702 GN=AGT1 PE=1 SV=2 (ur c senite nept leav)
sp|P34788|RS18_ARATH	40S ribosomal protein S18 OS=Arabidopsis thaliana OX=3702 GN=RPS18A PE=1 SV=1
sp|Q93W22|RL103_ARATH	60S ribosomal protein L10-3 OS=Arabidopsis thaliana OX=3702 GN=RPL10C PE=2 SV=1
sp|Q9LZH9|RL7A2_ARATH	60S ribosomal protein L7a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL7AB PE=2 SV=1
sp|P56771|CYF_ARATH	Cytochrome f OS=Arabidopsis thaliana OX=3702 GN=petA PE=3 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2
sp|P42794|RL112_ARATH	60S ribosomal protein L11-2 OS=Arabidopsis thaliana OX=3702 GN=RPL11B PE=2 SV=2
sp|P42795|RL111_ARATH	60S ribosomal protein L11-1 OS=Arabidopsis thaliana OX=3702 GN=RPL11A PE=2 SV=2
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur c senite nept leav)
sp|Q9FLP6|SUMO2_ARATH	Small ubiquitin-related modifier 2 OS=Arabidopsis thaliana OX=3702 GN=SUMO2 PE=1 SV=1 (dr c senite nept leav)
sp|P21238|CPNA1_ARATH	Chaperonin 60 subunit alpha 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPN60A1 PE=1 SV=2 (ur c senite nept sun)
sp|O65396|GCST_ARATH	Aminomethyltransferase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GDCST PE=2 SV=1
A0A2H1ZEA9|A0A2H1ZEA9_ARATH	Aminomethyltransferase OS=Arabidopsis thaliana OX=3702 GN=At1g11860 PE=3 SV=1

sp|Q8W593|LGUC_ARATH	Probable lactoylglutathione lyase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g67280 PE=1 SV=1
sp|Q07473|CB4A_ARATH	Chlorophyll a-b binding protein CP29.1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=LHCB4.1 PE=1 SV=1
A0A1P8B564|A0A1P8B564_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur c senite nept leav)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (ur c senite nept leav)
F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur c senite nept leav)
sp|P41916|RAN1_ARATH	GTP-binding nuclear protein Ran-1 OS=Arabidopsis thaliana OX=3702 GN=RAN1 PE=1 SV=1
sp|P41917|RAN2_ARATH	GTP-binding nuclear protein Ran-2 OS=Arabidopsis thaliana OX=3702 GN=RAN2 PE=1 SV=3
sp|Q8H156|RAN3_ARATH	GTP-binding nuclear protein Ran-3 OS=Arabidopsis thaliana OX=3702 GN=RAN3 PE=1 SV=2
Q2V3Q0|Q2V3Q0_ARATH	Proteasome subunit alpha type OS=Arabidopsis thaliana OX=3702 GN=PAD1 PE=3 SV=1
sp|P30186|PSA7A_ARATH	Proteasome subunit alpha type-7-A OS=Arabidopsis thaliana OX=3702 GN=PAD1 PE=1 SV=1
sp|P62090|PSAC_ARATH	Photosystem I iron-sulfur center OS=Arabidopsis thaliana OX=3702 GN=psaC PE=3 SV=2

Accession	Description – *Aradopsis thaliana*
sp|P29511|TBA6_ARATH	Tubulin alpha-6 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA6 PE=1 SV=1
sp|Q0WV25|TBA4_ARATH	Tubulin alpha-4 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA4 PE=2 SV=2
sp|B9DGT7|TBA2_ARATH	Tubulin alpha-2 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA2 PE=2 SV=2
A0A1P8B4W3|A0A1P8B4W3_ARATH	1 2-dihydroxy-3-keto-5-methylthiopentene dioxygenase OS=Arabidopsis thaliana OX=3702 GN=ARD1 PE=3 SV=1
sp|Q8GXE2|MTND2_ARATH	1 2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 2 OS=Arabidopsis thaliana OX=3702 GN=ARD2 PE=2 SV=1
sp|P49040|SUS1_ARATH	Sucrose synthase 1 OS=Arabidopsis thaliana OX=3702 GN=SUS1 PE=1 SV=3 (ur c senate nept leav)
sp|P27323|HS901_ARATH	Heat shock protein 90-1 OS=Arabidopsis thaliana OX=3702 GN=HSP90-1 PE=1 SV=3 (ur c senate nept leav)
sp|Q9STX5|ENPL_ARATH	Endoplasmin homolog OS=Arabidopsis thaliana OX=3702 GN=HSP90-7 PE=1 SV=1
F4JQ55|F4JQ55_ARATH	Chaperone protein htpG family protein OS=Arabidopsis thaliana OX=3702 GN=SHD PE=1 SV=1 (ur c senate nept leav)

sp|Q8VYF1|RL152_ARATH	60S ribosomal protein L15-2 OS=Arabidopsis thaliana OX=3702 GN=RPL15B PE=2 SV=1
sp|O23515|RL151_ARATH	60S ribosomal protein L15-1 OS=Arabidopsis thaliana OX=3702 GN=RPL15A PE=2 SV=1
sp|Q56WH1|TBA3_ARATH	Tubulin alpha-3 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA3 PE=1 SV=2
sp|B9DHQ0|TBA5_ARATH	Tubulin alpha-5 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA5 PE=1 SV=2
sp|Q9LIK9|APS1_ARATH	ATP sulfurylase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=APS1 PE=1 SV=1 (dr c senate nept leav)
sp|Q9LHA8|MD37C_ARATH	Probable mediator of RNA polymerase II transcription subunit 37c OS=Arabidopsis thaliana OX=3702 GN=MED37C PE=1 SV=1
sp|Q9LZH9|RL7A2_ARATH	60S ribosomal protein L7a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL7AB PE=2 SV=1
sp|P93285|COX2_ARATH	Cytochrome c oxidase subunit 2 OS=Arabidopsis thaliana OX=3702 GN=COX2 PE=1 SV=2
sp|Q42564|APX3_ARATH	L-ascorbate peroxidase 3 OS=Arabidopsis thaliana OX=3702 GN=APX3 PE=1 SV=1 (dr c senate nept leav)
sp|Q9LIK0|PKP1_ARATH	Plastidial pyruvate kinase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PKP1 PE=1 SV=1
sp|P49693|RL193_ARATH	60S ribosomal protein L19-3 OS=Arabidopsis thaliana OX=3702 GN=RPL19C PE=2 SV=3
sp|Q04836|CP31A_ARATH	31 kDa ribonucleoprotein chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CP31A PE=1 SV=1
F4IVR2|F4IVR2_ARATH	Heat shock protein 60-2 OS=Arabidopsis thaliana OX=3702 GN=HSP60-2 PE=1 SV=1 (dr c senate nept leav)
sp|Q8L7B5|CH60B_ARATH	Chaperonin CPN60-like 1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At2g33210 PE=1 SV=1 (dr c senate nept leav)
sp|Q8RUF8|NILP3_ARATH	Omega-amidase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=NLP3 PE=1 SV=1
sp|Q9LXL5|SUS4_ARATH	Sucrose synthase 4 OS=Arabidopsis thaliana OX=3702 GN=SUS4 PE=1 SV=1 (ur c senate nept leav)
sp|Q8L7K9|MAO2_ARATH	NAD-dependent malic enzyme 2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=NAD-ME2 PE=1 SV=1
sp|Q39085|DIM_ARATH	Delta(24)-sterol reductase OS=Arabidopsis thaliana OX=3702 GN=DIM PE=1 SV=2
F4IS32|F4IS32_ARATH	Acyl-[acyl-carrier-protein] desaturase OS=Arabidopsis thaliana OX=3702 GN=SSI2 PE=1 SV=1
sp|O22832|STAD7_ARATH	Stearoyl-[acyl-carrier-protein] 9-desaturase 7 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=FAB2 PE=1 SV=1
sp|Q9LH76|RHM3_ARATH	Trifunctional UDP-glucose 4 6-dehydratase/UDP-4-keto-6-deoxy-D-glucose 3 5-epimerase/UDP-4-keto-L-rhamnose-reductase RHM3 OS=Arabidopsis thaliana OX=3702 GN=RHM3 PE=2 SV=1
A0A1I9LN83|A0A1I9LN83_ARATH	Rhamnose biosynthesis 3 OS=Arabidopsis thaliana OX=3702 GN=RHM3 PE=4 SV=1

sp|Q9M1B9|SCRK4_ARATH	Probable fructokinase-4 OS=Arabidopsis thaliana OX=3702 GN=At3g59480 PE=2 SV=1
sp|O82191|MAOP1_ARATH	NADP-dependent malic enzyme 1 OS=Arabidopsis thaliana OX=3702 GN=NADP-ME1 PE=1 SV=1
sp|Q9FLP6|SUMO2_ARATH	Small ubiquitin-related modifier 2 OS=Arabidopsis thaliana OX=3702 GN=SUMO2 PE=1 SV=1 (dr c senate nept leav)
sp|Q42560|ACO1_ARATH	Aconitate hydratase 1 OS=Arabidopsis thaliana OX=3702 GN=ACO1 PE=1 SV=2

Accession	Description
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
F4IVZ7|F4IVZ7_ARATH	Rubisco activase OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1

Accession	Description
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|Q8H103|G6PIP_ARATH	Glucose-6-phosphate isomerase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PGI1 PE=1 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|O23254|GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana OX=3702 GN=SHM4 PE=1 SV=1 (ur senite senate sun leav)
sp|Q05758|ILV5_ARATH	Ketol-acid reductoisomerase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At3g58610 PE=1 SV=2
sp|P55228|GLGS_ARATH	Glucose-1-phosphate adenylyltransferase small subunit chloroplastic OS=Arabidopsis thaliana OX=3702 GN=APS1 PE=2 SV=2
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3

sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|O23627|SYGM1_ARATH	Glycine--tRNA ligase mitochondrial 1 OS=Arabidopsis thaliana OX=3702 GN=At1g29880 PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur c senite nept root)
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|Q9LFN6|RH56_ARATH	DEAD-box ATP-dependent RNA helicase 56 OS=Arabidopsis thaliana OX=3702 GN=RH56 PE=1 SV=2
sp|Q56XG6|RH15_ARATH	DEAD-box ATP-dependent RNA helicase 15 OS=Arabidopsis thaliana OX=3702 GN=RH15 PE=1 SV=3
sp|P17745|EFTU_ARATH	Elongation factor Tu chloroplastic OS=Arabidopsis thaliana OX=3702 GN=TUFA PE=1 SV=1
A0A1P8B564|A0A1P8B564_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senite nept root)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (dr c senite nept root)
F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senite nept root)
sp|P51430|RS62_ARATH	40S ribosomal protein S6-2 OS=Arabidopsis thaliana OX=3702 GN=RPS6B PE=1 SV=3
F4K5C7|F4K5C7_ARATH	40S ribosomal protein S4 OS=Arabidopsis thaliana OX=3702 GN=At5g07090 PE=3 SV=1
sp|Q93VH9|RS41_ARATH	40S ribosomal protein S4-1 OS=Arabidopsis thaliana OX=3702 GN=RPS4A PE=2 SV=1
sp|Q8VYK6|RS43_ARATH	40S ribosomal protein S4-3 OS=Arabidopsis thaliana OX=3702 GN=RPS4D PE=2 SV=2
sp|P49204|RS42_ARATH	40S ribosomal protein S4-2 OS=Arabidopsis thaliana OX=3702 GN=RPS4B PE=2 SV=4
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1

A0A1P8B564|A0A1P8B564_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senate nept root)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (dr c senate nept root)
F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senate nept root)
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|Q9LPW0|G3PA2_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPA2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPA2 PE=2 SV=1
A0A1P8APR6|A0A1P8APR6_ARATH	Glyceraldehyde-3-phosphate dehydrogenase OS=Arabidopsis thaliana OX=3702 GN=GAPA-2 PE=3 SV=1
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9LFN6|RH56_ARATH	DEAD-box ATP-dependent RNA helicase 56 OS=Arabidopsis thaliana OX=3702 GN=RH56 PE=1 SV=2
sp|Q56XG6|RH15_ARATH	DEAD-box ATP-dependent RNA helicase 15 OS=Arabidopsis thaliana OX=3702 GN=RH15 PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur c senate nept root)
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2

sp|Q8H103|G6PIP_ARATH	Glucose-6-phosphate isomerase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PGI1 PE=1 SV=1
sp|Q9SIB9|ACO3M_ARATH	Aconitate hydratase 3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=ACO3 PE=1 SV=2
sp|Q9ZUT9|RS51_ARATH	40S ribosomal protein S5-1 OS=Arabidopsis thaliana OX=3702 GN=RPS5A PE=1 SV=1
sp|P51427|RS52_ARATH	40S ribosomal protein S5-2 OS=Arabidopsis thaliana OX=3702 GN=RPS5B PE=1 SV=2
sp|P31414|AVP1_ARATH	Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2

Accession	Description
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9ZUT9|RS51_ARATH	40S ribosomal protein S5-1 OS=Arabidopsis thaliana OX=3702 GN=RPS5A PE=1 SV=1
sp|P51427|RS52_ARATH	40S ribosomal protein S5-2 OS=Arabidopsis thaliana OX=3702 GN=RPS5B PE=1 SV=2
sp|Q9FNN5|NDUV1_ARATH	NADH dehydrogenase [ubiquinone] flavoprotein 1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08530 PE=1 SV=1
sp|Q9SIB9|ACO3M_ARATH	Aconitate hydratase 3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=ACO3 PE=1 SV=2
sp|P29197|CH60A_ARATH	Chaperonin CPN60 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=CPN60 PE=1 SV=2 (ur senite senate nept root)
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|Q9SSB5|PRS7A_ARATH	26S proteasome regulatory subunit 7 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT1A PE=1 SV=1

Accession	Description
sp|P25858|G3PC1_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPC1 cytosolic OS=Arabidopsis thaliana OX=3702 GN=GAPC1 PE=1 SV=2
sp|Q0WL56|EF1A3_ARATH	Elongation factor 1-alpha 3 OS=Arabidopsis thaliana OX=3702 GN=A3 PE=1 SV=2
sp|P0DH99|EF1A1_ARATH	Elongation factor 1-alpha 1 OS=Arabidopsis thaliana OX=3702 GN=A1 PE=1 SV=1
sp|Q8GTY0|EF1A4_ARATH	Elongation factor 1-alpha 4 OS=Arabidopsis thaliana OX=3702 GN=A4 PE=1 SV=2
sp|Q8W4H7|EF1A2_ARATH	Elongation factor 1-alpha 2 OS=Arabidopsis thaliana OX=3702 GN=A2 PE=1 SV=2

Accession	Description

sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P25858|G3PC1_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPC1 cytosolic OS=Arabidopsis thaliana OX=3702 GN=GAPC1 PE=1 SV=2
sp|Q0WL56|EF1A3_ARATH	Elongation factor 1-alpha 3 OS=Arabidopsis thaliana OX=3702 GN=A3 PE=1 SV=2
sp|P0DH99|EF1A1_ARATH	Elongation factor 1-alpha 1 OS=Arabidopsis thaliana OX=3702 GN=A1 PE=1 SV=1
sp|Q8GTY0|EF1A4_ARATH	Elongation factor 1-alpha 4 OS=Arabidopsis thaliana OX=3702 GN=A4 PE=1 SV=2
sp|Q8W4H7|EF1A2_ARATH	Elongation factor 1-alpha 2 OS=Arabidopsis thaliana OX=3702 GN=A2 PE=1 SV=2
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|Q9SRZ6|ICDHC_ARATH	Cytosolic isocitrate dehydrogenase [NADP] OS=Arabidopsis thaliana OX=3702 GN=CICDH PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senite senate sun root)
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1

sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1
sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|O23254|GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana OX=3702 GN=SHM4 PE=1 SV=1 (dr senite senate sun root)
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9SZD4|PRS4A_ARATH	26S proteasome regulatory subunit 4 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT2A PE=1 SV=1
Q9M1P5|Q9M1P5_ARATH	ADP-ribosylation factor A1E OS=Arabidopsis thaliana OX=3702 GN=T17J13.250 PE=2 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1
sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|Q9SZD4|PRS4A_ARATH	26S proteasome regulatory subunit 4 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT2A PE=1 SV=1
sp|P41917|RAN2_ARATH	GTP-binding nuclear protein Ran-2 OS=Arabidopsis thaliana OX=3702 GN=RAN2 PE=1 SV=3
sp|P41916|RAN1_ARATH	GTP-binding nuclear protein Ran-1 OS=Arabidopsis thaliana OX=3702 GN=RAN1 PE=1 SV=1
sp|Q8H156|RAN3_ARATH	GTP-binding nuclear protein Ran-3 OS=Arabidopsis thaliana OX=3702 GN=RAN3 PE=1 SV=2

sp|O23254|GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana OX=3702 GN=SHM4 PE=1 SV=1 (dr senite nept sun root)
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|Q0WL56|EF1A3_ARATH	Elongation factor 1-alpha 3 OS=Arabidopsis thaliana OX=3702 GN=A3 PE=1 SV=2
sp|P0DH99|EF1A1_ARATH	Elongation factor 1-alpha 1 OS=Arabidopsis thaliana OX=3702 GN=A1 PE=1 SV=1
sp|Q8GTY0|EF1A4_ARATH	Elongation factor 1-alpha 4 OS=Arabidopsis thaliana OX=3702 GN=A4 PE=1 SV=2
sp|Q8W4H7|EF1A2_ARATH	Elongation factor 1-alpha 2 OS=Arabidopsis thaliana OX=3702 GN=A2 PE=1 SV=2
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1

Accession	Description
sp|Q9SEI4|PRS6B_ARATH	26S proteasome regulatory subunit 6B homolog OS=Arabidopsis thaliana OX=3702 GN=RPT3 PE=1 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senate nept sun root)
sp|P29197|CH60A_ARATH	Chaperonin CPN60 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=CPN60 PE=1 SV=2 (ur senate nept sun root)
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1

Accession	Description
A0A1P8B564|A0A1P8B564 ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur senite nept leav root)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (ur senite nept leav root)
F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur senite nept leav root)
sp|Q93VR3|GME_ARATH	GDP-mannose 3 5-epimerase OS=Arabidopsis thaliana OX=3702 GN=At5g28840 PE=1 SV=1

sp|P25696|ENO2_ARATH	Bifunctional enolase 2/transcriptional activator OS=Arabidopsis thaliana OX=3702 GN=ENO2 PE=1 SV=1
sp|Q9SSB5|PRS7A_ARATH	26S proteasome regulatory subunit 7 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT1A PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senite nept leav root)
sp|Q9S7C0|HSP7O_ARATH	Heat shock 70 kDa protein 14 OS=Arabidopsis thaliana OX=3702 GN=HSP70-14 PE=1 SV=1 (dr senite nept leav root)
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P59224|RS132_ARATH	40S ribosomal protein S13-2 OS=Arabidopsis thaliana OX=3702 GN=RPS13B PE=2 SV=1
sp|P59223|RS131_ARATH	40S ribosomal protein S13-1 OS=Arabidopsis thaliana OX=3702 GN=RPS13A PE=2 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|Q9SRZ6|ICDHC_ARATH	Cytosolic isocitrate dehydrogenase [NADP] OS=Arabidopsis thaliana OX=3702 GN=CICDH PE=1 SV=1
sp|P51430|RS62_ARATH	40S ribosomal protein S6-2 OS=Arabidopsis thaliana OX=3702 GN=RPS6B PE=1 SV=3
sp|Q9SF16|TCPH_ARATH	T-complex protein 1 subunit eta OS=Arabidopsis thaliana OX=3702 GN=CCT7 PE=1 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
F4IVZ7|F4IVZ7_ARATH	Rubisco activase OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1

sp|Q9SIF2|HS905_ARATH	Heat shock protein 90-5 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=HSP90-5 PE=1 SV=1 (ur senate nept leave root)
sp|Q39102|FTSH1_ARATH	ATP-dependent zinc metalloprotease FTSH 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=FTSH1 PE=1 SV=2
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P17745|EFTU_ARATH	Elongation factor Tu chloroplastic OS=Arabidopsis thaliana OX=3702 GN=TUFA PE=1 SV=1
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
A0A2H1ZE23|A0A2H1ZE23_ARATH	Aldehyde dehydrogenase 11A3 OS=Arabidopsis thaliana OX=3702 GN=ALDH11A3 PE=1 SV=1
sp|Q1WIQ6|GAPN_ARATH	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase OS=Arabidopsis thaliana OX=3702 GN=ALDH11A3 PE=1 SV=2
F4INS6|F4INS6_ARATH	Aldehyde dehydrogenase 11A3 OS=Arabidopsis thaliana OX=3702 GN=ALDH11A3 PE=1 SV=1
sp|P62090|PSAC_ARATH	Photosystem I iron-sulfur center OS=Arabidopsis thaliana OX=3702 GN=psaC PE=3 SV=2
A0A1P8ANC0|A0A1P8ANC0_ARATH	Hydroxypyruvate reductase OS=Arabidopsis thaliana OX=3702 GN=HPR PE=3 SV=1
sp|Q9C9W5|HPR1_ARATH	Glycerate dehydrogenase HPR peroxisomal OS=Arabidopsis thaliana OX=3702 GN=HPR PE=1 SV=1
sp|O65396|GCST_ARATH	Aminomethyltransferase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GDCST PE=2 SV=1
A0A2H1ZEA9|A0A2H1ZEA9_ARATH	Aminomethyltransferase OS=Arabidopsis thaliana OX=3702 GN=At1g11860 PE=3 SV=1
sp|P31414|AVP1_ARATH	Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
sp|P21238|CPNA1_ARATH	Chaperonin 60 subunit alpha 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPN60A1 PE=1 SV=2 (dr senate nept leav root)

sp|Q9LV33|BGL44_ARATH	Beta-glucosidase 44 OS=Arabidopsis thaliana OX=3702 GN=BGLU44 PE=2 SV=1
sp|Q9FLP6|SUMO2_ARATH	Small ubiquitin-related modifier 2 OS=Arabidopsis thaliana OX=3702 GN=SUMO2 PE=1 SV=1 (ur senate nept leav root)
sp|Q9ZUT9|RS51_ARATH	40S ribosomal protein S5-1 OS=Arabidopsis thaliana OX=3702 GN=RPS5A PE=1 SV=1
sp|P51427|RS52_ARATH	40S ribosomal protein S5-2 OS=Arabidopsis thaliana OX=3702 GN=RPS5B PE=1 SV=2
sp|Q9SIB9|ACO3M_ARATH	Aconitate hydratase 3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=ACO3 PE=1 SV=2
sp|Q9LFN6|RH56_ARATH	DEAD-box ATP-dependent RNA helicase 56 OS=Arabidopsis thaliana OX=3702 GN=RH56 PE=1 SV=2
sp|Q56XG6|RH15_ARATH	DEAD-box ATP-dependent RNA helicase 15 OS=Arabidopsis thaliana OX=3702 GN=RH15 PE=1 SV=3

Accession	Description
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2

sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P25857|G3PB_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPB chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPB PE=1 SV=2
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|P25697|KPPR_ARATH	Phosphoribulokinase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g32060 PE=2 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur senate sun leav root)
sp|P56771|CYF_ARATH	Cytochrome f OS=Arabidopsis thaliana OX=3702 GN=petA PE=3 SV=1
sp|P31414|AVP1_ARATH	Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1

Accession	Description
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9LD57|PGKH1_ARATH	Phosphoglycerate kinase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PGK1 PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|Q5XF33|CHLI2_ARATH	Magnesium-chelatase subunit ChlI-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CHLI2 PE=1 SV=1
sp|P16127|CHLI1_ARATH	Magnesium-chelatase subunit ChlI-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CHLI1 PE=1 SV=1

sp|Q56YA5|SGAT_ARATH	Serine--glyoxylate aminotransferase OS=Arabidopsis thaliana OX=3702 GN=AGT1 PE=1 SV=2 (dr c senite sun leav)

Accession	Description
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|Q9SI75|EFGC_ARATH	Elongation factor G chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPEFG PE=1 SV=1
sp|P53492|ACT7_ARATH	Actin-7 OS=Arabidopsis thaliana OX=3702 GN=ACT7 PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P17745|EFTU_ARATH	Elongation factor Tu chloroplastic OS=Arabidopsis thaliana OX=3702 GN=TUFA PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2

Accession	Description
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P41916|RAN1_ARATH	GTP-binding nuclear protein Ran-1 OS=Arabidopsis thaliana OX=3702 GN=RAN1 PE=1 SV=1
sp|P41917|RAN2_ARATH	GTP-binding nuclear protein Ran-2 OS=Arabidopsis thaliana OX=3702 GN=RAN2 PE=1 SV=3
sp|Q8H156|RAN3_ARATH	GTP-binding nuclear protein Ran-3 OS=Arabidopsis thaliana OX=3702 GN=RAN3 PE=1 SV=2
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1
sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P55229|GLGL1_ARATH	Glucose-1-phosphate adenylyltransferase large subunit 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=ADG2 PE=1 SV=3

sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|Q8W593|LGUC_ARATH	Probable lactoylglutathione lyase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g67280 PE=1 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2
sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P25697|KPPR_ARATH	Phosphoribulokinase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g32060 PE=2 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2

Accession	Description
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1

sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P62090|PSAC_ARATH	Photosystem I iron-sulfur center OS=Arabidopsis thaliana OX=3702 GN=psaC PE=3 SV=2
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senite nept sun leav)
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9SEI4|PRS6B_ARATH	26S proteasome regulatory subunit 6B homolog OS=Arabidopsis thaliana OX=3702 GN=RPT3 PE=1 SV=1
sp|Q9S7E4|FDH_ARATH	Formate dehydrogenase chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=FDH1 PE=1 SV=1
A0A1P8B9N1|A0A1P8B9N1_ARATH	Formate dehydrogenase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=FDH PE=3 SV=1
A0A1P8B9L1|A0A1P8B9L1_ARATH	Formate dehydrogenase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=FDH PE=3 SV=1
sp|P25857|G3PB_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPB chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPB PE=1 SV=2
sp|P31414|AVP1_ARATH	Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1
sp|O23654|VATA_ARATH	V-type proton ATPase catalytic subunit A OS=Arabidopsis thaliana OX=3702 GN=VHA-A PE=1 SV=1
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
F4IVZ7|F4IVZ7_ARATH	Rubisco activase OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=1

sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P25857|G3PB_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPB chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPB PE=1 SV=2
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9SIF2|HS905_ARATH	Heat shock protein 90-5 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=HSP90-5 PE=1 SV=1 (ur c senite nept leav)
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P27323|HS901_ARATH	Heat shock protein 90-1 OS=Arabidopsis thaliana OX=3702 GN=HSP90-1 PE=1 SV=3 (ur c senite nept leav)
sp|Q9SI75|EFGC_ARATH	Elongation factor G chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPEFG PE=1 SV=1
sp|Q43127|GLNA2_ARATH	Glutamine synthetase chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLN2 PE=1 SV=1
A0A1P8ANC0|A0A1P8ANC0_ARATH	Hydroxypyruvate reductase OS=Arabidopsis thaliana OX=3702 GN=HPR PE=3 SV=1
sp|Q9C9W5|HPR1_ARATH	Glycerate dehydrogenase HPR peroxisomal OS=Arabidopsis thaliana OX=3702 GN=HPR PE=1 SV=1
sp|Q9SA52|CP41B_ARATH	Chloroplast stem-loop binding protein of 41 kDa b chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CSP41B PE=1 SV=1
A0A1P8ATL2|A0A1P8ATL2_ARATH	Chloroplast RNA binding protein OS=Arabidopsis thaliana OX=3702 GN=CRB PE=4 SV=1
sp|Q93VR3|GME_ARATH	GDP-mannose 3 5-epimerase OS=Arabidopsis thaliana OX=3702 GN=At5g28840 PE=1 SV=1

sp|Q56YA5|SGAT_ARATH	Serine--glyoxylate aminotransferase OS=Arabidopsis thaliana OX=3702 GN=AGT1 PE=1 SV=2 (ur c senite nept leav)
sp|P34788|RS18_ARATH	40S ribosomal protein S18 OS=Arabidopsis thaliana OX=3702 GN=RPS18A PE=1 SV=1
sp|Q93W22|RL103_ARATH	60S ribosomal protein L10-3 OS=Arabidopsis thaliana OX=3702 GN=RPL10C PE=2 SV=1
sp|Q9LZH9|RL7A2_ARATH	60S ribosomal protein L7a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL7AB PE=2 SV=1
sp|P56771|CYF_ARATH	Cytochrome f OS=Arabidopsis thaliana OX=3702 GN=petA PE=3 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2
sp|P42794|RL112_ARATH	60S ribosomal protein L11-2 OS=Arabidopsis thaliana OX=3702 GN=RPL11B PE=2 SV=2
sp|P42795|RL111_ARATH	60S ribosomal protein L11-1 OS=Arabidopsis thaliana OX=3702 GN=RPL11A PE=2 SV=2
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur c senite nept leav)
sp|Q9FLP6|SUMO2_ARATH	Small ubiquitin-related modifier 2 OS=Arabidopsis thaliana OX=3702 GN=SUMO2 PE=1 SV=1 (dr c senite nept leav)
sp|P21238|CPNA1_ARATH	Chaperonin 60 subunit alpha 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPN60A1 PE=1 SV=2 (ur c senite nept sun)
sp|O65396|GCST_ARATH	Aminomethyltransferase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GDCST PE=2 SV=1
A0A2H1ZEA9|A0A2H1ZEA9_ARATH	Aminomethyltransferase OS=Arabidopsis thaliana OX=3702 GN=At1g11860 PE=3 SV=1
sp|Q8W593|LGUC_ARATH	Probable lactoylglutathione lyase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g67280 PE=1 SV=1
sp|Q07473|CB4A_ARATH	Chlorophyll a-b binding protein CP29.1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=LHCB4.1 PE=1 SV=1
A0A1P8B564|A0A1P8B564_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur c senite nept leav)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (ur c senite nept leav)

F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur c senite nept leav)
sp|P41916|RAN1_ARATH	GTP-binding nuclear protein Ran-1 OS=Arabidopsis thaliana OX=3702 GN=RAN1 PE=1 SV=1
sp|P41917|RAN2_ARATH	GTP-binding nuclear protein Ran-2 OS=Arabidopsis thaliana OX=3702 GN=RAN2 PE=1 SV=3
sp|Q8H156|RAN3_ARATH	GTP-binding nuclear protein Ran-3 OS=Arabidopsis thaliana OX=3702 GN=RAN3 PE=1 SV=2
Q2V3Q0|Q2V3Q0_ARATH	Proteasome subunit alpha type OS=Arabidopsis thaliana OX=3702 GN=PAD1 PE=3 SV=1
sp|P30186|PSA7A_ARATH	Proteasome subunit alpha type-7-A OS=Arabidopsis thaliana OX=3702 GN=PAD1 PE=1 SV=1
sp|P62090|PSAC_ARATH	Photosystem I iron-sulfur center OS=Arabidopsis thaliana OX=3702 GN=psaC PE=3 SV=2

Accession	Description – *Aradopsis thaliana*
sp|P29511|TBA6_ARATH	Tubulin alpha-6 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA6 PE=1 SV=1
sp|Q0WV25|TBA4_ARATH	Tubulin alpha-4 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA4 PE=2 SV=2
sp|B9DGT7|TBA2_ARATH	Tubulin alpha-2 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA2 PE=2 SV=2
A0A1P8B4W3|A0A1P8B4W3_ARATH	1 2-dihydroxy-3-keto-5-methylthiopentene dioxygenase OS=Arabidopsis thaliana OX=3702 GN=ARD1 PE=3 SV=1
sp|Q8GXE2|MTND2_ARATH	1 2-dihydroxy-3-keto-5-methylthiopentene dioxygenase 2 OS=Arabidopsis thaliana OX=3702 GN=ARD2 PE=2 SV=1
sp|P49040|SUS1_ARATH	Sucrose synthase 1 OS=Arabidopsis thaliana OX=3702 GN=SUS1 PE=1 SV=3 (ur c senate nept leav)
sp|P27323|HS901_ARATH	Heat shock protein 90-1 OS=Arabidopsis thaliana OX=3702 GN=HSP90-1 PE=1 SV=3 (ur c senate nept leav)
sp|Q9STX5|ENPL_ARATH	Endoplasmin homolog OS=Arabidopsis thaliana OX=3702 GN=HSP90-7 PE=1 SV=1
F4JQ55|F4JQ55_ARATH	Chaperone protein htpG family protein OS=Arabidopsis thaliana OX=3702 GN=SHD PE=1 SV=1 (ur c senate nept leav)
sp|Q8VYF1|RL152_ARATH	60S ribosomal protein L15-2 OS=Arabidopsis thaliana OX=3702 GN=RPL15B PE=2 SV=1
sp|O23515|RL151_ARATH	60S ribosomal protein L15-1 OS=Arabidopsis thaliana OX=3702 GN=RPL15A PE=2 SV=1
sp|Q56WH1|TBA3_ARATH	Tubulin alpha-3 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA3 PE=1 SV=2
sp|B9DHQ0|TBA5_ARATH	Tubulin alpha-5 chain OS=Arabidopsis thaliana OX=3702 GN=TUBA5 PE=1 SV=2
sp|Q9LIK9|APS1_ARATH	ATP sulfurylase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=APS1 PE=1 SV=1 (dr c senate nept leav)

sp|Q9LHA8|MD37C_ARATH	Probable mediator of RNA polymerase II transcription subunit 37c OS=Arabidopsis thaliana OX=3702 GN=MED37C PE=1 SV=1
sp|Q9LZH9|RL7A2_ARATH	60S ribosomal protein L7a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL7AB PE=2 SV=1
sp|P93285|COX2_ARATH	Cytochrome c oxidase subunit 2 OS=Arabidopsis thaliana OX=3702 GN=COX2 PE=1 SV=2
sp|Q42564|APX3_ARATH	L-ascorbate peroxidase 3 OS=Arabidopsis thaliana OX=3702 GN=APX3 PE=1 SV=1 (dr c senate nept leav)
sp|Q9LIK0|PKP1_ARATH	Plastidial pyruvate kinase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PKP1 PE=1 SV=1
sp|P49693|RL193_ARATH	60S ribosomal protein L19-3 OS=Arabidopsis thaliana OX=3702 GN=RPL19C PE=2 SV=3
sp|Q04836|CP31A_ARATH	31 kDa ribonucleoprotein chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CP31A PE=1 SV=1
F4IVR2|F4IVR2_ARATH	Heat shock protein 60-2 OS=Arabidopsis thaliana OX=3702 GN=HSP60-2 PE=1 SV=1 (dr c senate nept leav)
sp|Q8L7B5|CH60B_ARATH	Chaperonin CPN60-like 1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At2g33210 PE=1 SV=1 (dr c senate nept leav)
sp|Q8RUF8|NILP3_ARATH	Omega-amidase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=NLP3 PE=1 SV=1
sp|Q9LXL5|SUS4_ARATH	Sucrose synthase 4 OS=Arabidopsis thaliana OX=3702 GN=SUS4 PE=1 SV=1 (ur c senate nept leav)
sp|Q8L7K9|MAO2_ARATH	NAD-dependent malic enzyme 2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=NAD-ME2 PE=1 SV=1
sp|Q39085|DIM_ARATH	Delta(24)-sterol reductase OS=Arabidopsis thaliana OX=3702 GN=DIM PE=1 SV=2
F4IS32|F4IS32_ARATH	Acyl-[acyl-carrier-protein] desaturase OS=Arabidopsis thaliana OX=3702 GN=SSI2 PE=1 SV=1
sp|O22832|STAD7_ARATH	Stearoyl-[acyl-carrier-protein] 9-desaturase 7 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=FAB2 PE=1 SV=1
sp|Q9LH76|RHM3_ARATH	Trifunctional UDP-glucose 4 6-dehydratase/UDP-4-keto-6-deoxy-D-glucose 3 5-epimerase/UDP-4-keto-L-rhamnose-reductase RHM3 OS=Arabidopsis thaliana OX=3702 GN=RHM3 PE=2 SV=1
A0A1I9LN83|A0A1I9LN83_ARATH	Rhamnose biosynthesis 3 OS=Arabidopsis thaliana OX=3702 GN=RHM3 PE=4 SV=1
sp|Q9M1B9|SCRK4_ARATH	Probable fructokinase-4 OS=Arabidopsis thaliana OX=3702 GN=At3g59480 PE=2 SV=1
sp|O82191|MAOP1_ARATH	NADP-dependent malic enzyme 1 OS=Arabidopsis thaliana OX=3702 GN=NADP-ME1 PE=1 SV=1
sp|Q9FLP6|SUMO2_ARATH	Small ubiquitin-related modifier 2 OS=Arabidopsis thaliana OX=3702 GN=SUMO2 PE=1 SV=1 (dr c senate nept leav)
sp|Q42560|ACO1_ARATH	Aconitate hydratase 1 OS=Arabidopsis thaliana OX=3702 GN=ACO1 PE=1 SV=2

Accession	Description
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
F4IVZ7|F4IVZ7_ARATH	Rubisco activase OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1

Accession	Description
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|Q8H103|G6PIP_ARATH	Glucose-6-phosphate isomerase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PGI1 PE=1 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|O23254|GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana OX=3702 GN=SHM4 PE=1 SV=1 (ur senite senate sun leav)
sp|Q05758|ILV5_ARATH	Ketol-acid reductoisomerase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At3g58610 PE=1 SV=2
sp|P55228|GLGS_ARATH	Glucose-1-phosphate adenylyltransferase small subunit chloroplastic OS=Arabidopsis thaliana OX=3702 GN=APS1 PE=2 SV=2
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1

sp|O23627|SYGM1_ARATH	Glycine--tRNA ligase mitochondrial 1 OS=Arabidopsis thaliana OX=3702 GN=At1g29880 PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur c senite nept root)
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|Q9LFN6|RH56_ARATH	DEAD-box ATP-dependent RNA helicase 56 OS=Arabidopsis thaliana OX=3702 GN=RH56 PE=1 SV=2
sp|Q56XG6|RH15_ARATH	DEAD-box ATP-dependent RNA helicase 15 OS=Arabidopsis thaliana OX=3702 GN=RH15 PE=1 SV=3
sp|P17745|EFTU_ARATH	Elongation factor Tu chloroplastic OS=Arabidopsis thaliana OX=3702 GN=TUFA PE=1 SV=1
A0A1P8B564|A0A1P8B564_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senite nept root)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (dr c senite nept root)
F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senite nept root)
sp|P51430|RS62_ARATH	40S ribosomal protein S6-2 OS=Arabidopsis thaliana OX=3702 GN=RPS6B PE=1 SV=3
F4K5C7|F4K5C7_ARATH	40S ribosomal protein S4 OS=Arabidopsis thaliana OX=3702 GN=At5g07090 PE=3 SV=1
sp|Q93VH9|RS41_ARATH	40S ribosomal protein S4-1 OS=Arabidopsis thaliana OX=3702 GN=RPS4A PE=2 SV=1
sp|Q8VYK6|RS43_ARATH	40S ribosomal protein S4-3 OS=Arabidopsis thaliana OX=3702 GN=RPS4D PE=2 SV=2
sp|P49204|RS42_ARATH	40S ribosomal protein S4-2 OS=Arabidopsis thaliana OX=3702 GN=RPS4B PE=2 SV=4
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
A0A1P8B564|A0A1P8B564_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senate nept root)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (dr c senate nept root)
F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (dr c senate nept root)
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1

sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|Q9LPW0|G3PA2_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPA2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPA2 PE=2 SV=1
A0A1P8APR6|A0A1P8APR6_ARATH	Glyceraldehyde-3-phosphate dehydrogenase OS=Arabidopsis thaliana OX=3702 GN=GAPA-2 PE=3 SV=1
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9LFN6|RH56_ARATH	DEAD-box ATP-dependent RNA helicase 56 OS=Arabidopsis thaliana OX=3702 GN=RH56 PE=1 SV=2
sp|Q56XG6|RH15_ARATH	DEAD-box ATP-dependent RNA helicase 15 OS=Arabidopsis thaliana OX=3702 GN=RH15 PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur c senate nept root)
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2
sp|Q8H103|G6PIP_ARATH	Glucose-6-phosphate isomerase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PGI1 PE=1 SV=1
sp|Q9SIB9|ACO3M_ARATH	Aconitate hydratase 3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=ACO3 PE=1 SV=2
sp|Q9ZUT9|RS51_ARATH	40S ribosomal protein S5-1 OS=Arabidopsis thaliana OX=3702 GN=RPS5A PE=1 SV=1
sp|P51427|RS52_ARATH	40S ribosomal protein S5-2 OS=Arabidopsis thaliana OX=3702 GN=RPS5B PE=1 SV=2
sp|P31414|AVP1_ARATH	Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1

sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
Accession	Description
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9ZUT9|RS51_ARATH	40S ribosomal protein S5-1 OS=Arabidopsis thaliana OX=3702 GN=RPS5A PE=1 SV=1
sp|P51427|RS52_ARATH	40S ribosomal protein S5-2 OS=Arabidopsis thaliana OX=3702 GN=RPS5B PE=1 SV=2
sp|Q9FNN5|NDUV1_ARATH	NADH dehydrogenase [ubiquinone] flavoprotein 1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08530 PE=1 SV=1
sp|Q9SIB9|ACO3M_ARATH	Aconitate hydratase 3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=ACO3 PE=1 SV=2
sp|P29197|CH60A_ARATH	Chaperonin CPN60 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=CPN60 PE=1 SV=2 (ur senite senate nept root)
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|Q9SSB5|PRS7A_ARATH	26S proteasome regulatory subunit 7 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT1A PE=1 SV=1

Accession	Description
sp|P25858|G3PC1_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPC1 cytosolic OS=Arabidopsis thaliana OX=3702 GN=GAPC1 PE=1 SV=2
sp|Q0WL56|EF1A3_ARATH	Elongation factor 1-alpha 3 OS=Arabidopsis thaliana OX=3702 GN=A3 PE=1 SV=2
sp|P0DH99|EF1A1_ARATH	Elongation factor 1-alpha 1 OS=Arabidopsis thaliana OX=3702 GN=A1 PE=1 SV=1
sp|Q8GTY0|EF1A4_ARATH	Elongation factor 1-alpha 4 OS=Arabidopsis thaliana OX=3702 GN=A4 PE=1 SV=2
sp|Q8W4H7|EF1A2_ARATH	Elongation factor 1-alpha 2 OS=Arabidopsis thaliana OX=3702 GN=A2 PE=1 SV=2

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1

sp|P25858|G3PC1_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPC1 cytosolic OS=Arabidopsis thaliana OX=3702 GN=GAPC1 PE=1 SV=2
sp|Q0WL56|EF1A3_ARATH	Elongation factor 1-alpha 3 OS=Arabidopsis thaliana OX=3702 GN=A3 PE=1 SV=2
sp|P0DH99|EF1A1_ARATH	Elongation factor 1-alpha 1 OS=Arabidopsis thaliana OX=3702 GN=A1 PE=1 SV=1
sp|Q8GTY0|EF1A4_ARATH	Elongation factor 1-alpha 4 OS=Arabidopsis thaliana OX=3702 GN=A4 PE=1 SV=2
sp|Q8W4H7|EF1A2_ARATH	Elongation factor 1-alpha 2 OS=Arabidopsis thaliana OX=3702 GN=A2 PE=1 SV=2
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|Q9SRZ6|ICDHC_ARATH	Cytosolic isocitrate dehydrogenase [NADP] OS=Arabidopsis thaliana OX=3702 GN=CICDH PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senite senate sun root)
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1

sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|O23254|GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana OX=3702 GN=SHM4 PE=1 SV=1 (dr senite senate sun root)
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9SZD4|PRS4A_ARATH	26S proteasome regulatory subunit 4 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT2A PE=1 SV=1
Q9M1P5|Q9M1P5_ARATH	ADP-ribosylation factor A1E OS=Arabidopsis thaliana OX=3702 GN=T17J13.250 PE=2 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1
sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|Q9SZD4|PRS4A_ARATH	26S proteasome regulatory subunit 4 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT2A PE=1 SV=1
sp|P41917|RAN2_ARATH	GTP-binding nuclear protein Ran-2 OS=Arabidopsis thaliana OX=3702 GN=RAN2 PE=1 SV=3
sp|P41916|RAN1_ARATH	GTP-binding nuclear protein Ran-1 OS=Arabidopsis thaliana OX=3702 GN=RAN1 PE=1 SV=1
sp|Q8H156|RAN3_ARATH	GTP-binding nuclear protein Ran-3 OS=Arabidopsis thaliana OX=3702 GN=RAN3 PE=1 SV=2
sp|O23254|GLYC4_ARATH	Serine hydroxymethyltransferase 4 OS=Arabidopsis thaliana OX=3702 GN=SHM4 PE=1 SV=1 (dr senite nept sun root)
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|Q0WL56|EF1A3_ARATH	Elongation factor 1-alpha 3 OS=Arabidopsis thaliana OX=3702 GN=A3 PE=1 SV=2

sp|P0DH99|EF1A1_ARATH	Elongation factor 1-alpha 1 OS=Arabidopsis thaliana OX=3702 GN=A1 PE=1 SV=1
sp|Q8GTY0|EF1A4_ARATH	Elongation factor 1-alpha 4 OS=Arabidopsis thaliana OX=3702 GN=A4 PE=1 SV=2
sp|Q8W4H7|EF1A2_ARATH	Elongation factor 1-alpha 2 OS=Arabidopsis thaliana OX=3702 GN=A2 PE=1 SV=2
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1

Accession	Description
sp|Q9SEI4|PRS6B_ARATH	26S proteasome regulatory subunit 6B homolog OS=Arabidopsis thaliana OX=3702 GN=RPT3 PE=1 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senate nept sun root)
sp|P29197|CH60A_ARATH	Chaperonin CPN60 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=CPN60 PE=1 SV=2 (ur senate nept sun root)
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1

Accession	Description
A0A1P8B564|A0A1P8B564 ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur senite nept leav root)
sp|P25819|CATA2_ARATH	Catalase-2 OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=1 SV=3 (ur senite nept leav root)
F4JM86|F4JM86_ARATH	Catalase OS=Arabidopsis thaliana OX=3702 GN=CAT2 PE=3 SV=1 (ur senite nept leav root)
sp|Q93VR3|GME_ARATH	GDP-mannose 3 5-epimerase OS=Arabidopsis thaliana OX=3702 GN=At5g28840 PE=1 SV=1
sp|P25696|ENO2_ARATH	Bifunctional enolase 2/transcriptional activator OS=Arabidopsis thaliana OX=3702 GN=ENO2 PE=1 SV=1
sp|Q9SSB5|PRS7A_ARATH	26S proteasome regulatory subunit 7 homolog A OS=Arabidopsis thaliana OX=3702 GN=RPT1A PE=1 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senite nept leav root)

sp|Q9S7C0|HSP7O_ARATH	Heat shock 70 kDa protein 14 OS=Arabidopsis thaliana OX=3702 GN=HSP70-14 PE=1 SV=1 (dr senite nept leav root)
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P59224|RS132_ARATH	40S ribosomal protein S13-2 OS=Arabidopsis thaliana OX=3702 GN=RPS13B PE=2 SV=1
sp|P59223|RS131_ARATH	40S ribosomal protein S13-1 OS=Arabidopsis thaliana OX=3702 GN=RPS13A PE=2 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|Q9SRZ6|ICDHC_ARATH	Cytosolic isocitrate dehydrogenase [NADP] OS=Arabidopsis thaliana OX=3702 GN=CICDH PE=1 SV=1
sp|P51430|RS62_ARATH	40S ribosomal protein S6-2 OS=Arabidopsis thaliana OX=3702 GN=RPS6B PE=1 SV=3
sp|Q9SF16|TCPH_ARATH	T-complex protein 1 subunit eta OS=Arabidopsis thaliana OX=3702 GN=CCT7 PE=1 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
F4IVZ7|F4IVZ7_ARATH	Rubisco activase OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|Q9SIF2|HS905_ARATH	Heat shock protein 90-5 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=HSP90-5 PE=1 SV=1 (ur senate nept leave root)
sp|Q39102|FTSH1_ARATH	ATP-dependent zinc metalloprotease FTSH 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=FTSH1 PE=1 SV=2
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2

sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P17745|EFTU_ARATH	Elongation factor Tu chloroplastic OS=Arabidopsis thaliana OX=3702 GN=TUFA PE=1 SV=1
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
A0A2H1ZE23|A0A2H1ZE23_ARATH	Aldehyde dehydrogenase 11A3 OS=Arabidopsis thaliana OX=3702 GN=ALDH11A3 PE=1 SV=1
sp|Q1WIQ6|GAPN_ARATH	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase OS=Arabidopsis thaliana OX=3702 GN=ALDH11A3 PE=1 SV=2
F4INS6|F4INS6_ARATH	Aldehyde dehydrogenase 11A3 OS=Arabidopsis thaliana OX=3702 GN=ALDH11A3 PE=1 SV=1
sp|P62090|PSAC_ARATH	Photosystem I iron-sulfur center OS=Arabidopsis thaliana OX=3702 GN=psaC PE=3 SV=2
A0A1P8ANC0|A0A1P8ANC0_ARATH	Hydroxypyruvate reductase OS=Arabidopsis thaliana OX=3702 GN=HPR PE=3 SV=1
sp|Q9C9W5|HPR1_ARATH	Glycerate dehydrogenase HPR peroxisomal OS=Arabidopsis thaliana OX=3702 GN=HPR PE=1 SV=1
sp|O65396|GCST_ARATH	Aminomethyltransferase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GDCST PE=2 SV=1
A0A2H1ZEA9|A0A2H1ZEA9_ARATH	Aminomethyltransferase OS=Arabidopsis thaliana OX=3702 GN=At1g11860 PE=3 SV=1
sp|P31414|AVP1_ARATH	Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
sp|P21238|CPNA1_ARATH	Chaperonin 60 subunit alpha 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPN60A1 PE=1 SV=2 (dr senate nept leav root)
sp|Q9LV33|BGL44_ARATH	Beta-glucosidase 44 OS=Arabidopsis thaliana OX=3702 GN=BGLU44 PE=2 SV=1
sp|Q9FLP6|SUMO2_ARATH	Small ubiquitin-related modifier 2 OS=Arabidopsis thaliana OX=3702 GN=SUMO2 PE=1 SV=1 (ur senate nept leav root)
sp|Q9ZUT9|RS51_ARATH	40S ribosomal protein S5-1 OS=Arabidopsis thaliana OX=3702 GN=RPS5A PE=1 SV=1
sp|P51427|RS52_ARATH	40S ribosomal protein S5-2 OS=Arabidopsis thaliana OX=3702 GN=RPS5B PE=1 SV=2

sp|Q9SIB9|ACO3M_ARATH	Aconitate hydratase 3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=ACO3 PE=1 SV=2
sp|Q9LFN6|RH56_ARATH	DEAD-box ATP-dependent RNA helicase 56 OS=Arabidopsis thaliana OX=3702 GN=RH56 PE=1 SV=2
sp|Q56XG6|RH15_ARATH	DEAD-box ATP-dependent RNA helicase 15 OS=Arabidopsis thaliana OX=3702 GN=RH15 PE=1 SV=3

Accession	Description
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1

Accession	Description
sp|O03042|RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana OX=3702 GN=rbcL PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P25857|G3PB_ARATH	Glyceraldehyde-3-phosphate dehydrogenase GAPB chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPB PE=1 SV=2
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3

sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|P25697|KPPR_ARATH	Phosphoribulokinase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g32060 PE=2 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P23321|PSBO1_ARATH	Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (ur senate sun leav root)
sp|P56771|CYF_ARATH	Cytochrome f OS=Arabidopsis thaliana OX=3702 GN=petA PE=3 SV=1
sp|P31414|AVP1_ARATH	Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1

Accession	Description
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9LD57|PGKH1_ARATH	Phosphoglycerate kinase 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PGK1 PE=1 SV=1
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|Q5XF33|CHLI2_ARATH	Magnesium-chelatase subunit ChlI-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CHLI2 PE=1 SV=1
sp|P16127|CHLI1_ARATH	Magnesium-chelatase subunit ChlI-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CHLI1 PE=1 SV=1
sp|Q56YA5|SGAT_ARATH	Serine--glyoxylate aminotransferase OS=Arabidopsis thaliana OX=3702 GN=AGT1 PE=1 SV=2 (dr c senite sun leav)

Accession	Description
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1

sp|Q9SI75|EFGC_ARATH	Elongation factor G chloroplastic OS=Arabidopsis thaliana OX=3702 GN=CPEFG PE=1 SV=1
sp|P53492|ACT7_ARATH	Actin-7 OS=Arabidopsis thaliana OX=3702 GN=ACT7 PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P17745|EFTU_ARATH	Elongation factor Tu chloroplastic OS=Arabidopsis thaliana OX=3702 GN=TUFA PE=1 SV=1
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2

Accession	Description
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P41916|RAN1_ARATH	GTP-binding nuclear protein Ran-1 OS=Arabidopsis thaliana OX=3702 GN=RAN1 PE=1 SV=1
sp|P41917|RAN2_ARATH	GTP-binding nuclear protein Ran-2 OS=Arabidopsis thaliana OX=3702 GN=RAN2 PE=1 SV=3
sp|Q8H156|RAN3_ARATH	GTP-binding nuclear protein Ran-3 OS=Arabidopsis thaliana OX=3702 GN=RAN3 PE=1 SV=2
sp|Q9C5A9|ATPBO_ARATH	ATP synthase subunit beta-3 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08680 PE=1 SV=1
sp|P83483|ATPBM_ARATH	ATP synthase subunit beta-1 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08670 PE=1 SV=1
sp|P83484|ATPBN_ARATH	ATP synthase subunit beta-2 mitochondrial OS=Arabidopsis thaliana OX=3702 GN=At5g08690 PE=1 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|P55229|GLGL1_ARATH	Glucose-1-phosphate adenylyltransferase large subunit 1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=ADG2 PE=1 SV=3
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|Q9ZNZ7|GLTB1_ARATH	Ferredoxin-dependent glutamate synthase 1 chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=GLU1 PE=1 SV=3
sp|Q8W593|LGUC_ARATH	Probable lactoylglutathione lyase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g67280 PE=1 SV=1
sp|Q8LD46|R23A1_ARATH	60S ribosomal protein L23a-1 OS=Arabidopsis thaliana OX=3702 GN=RPL23AA PE=2 SV=2

sp|Q9M3C3|R23A2_ARATH	60S ribosomal protein L23a-2 OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=2 SV=1
A8MS83|A8MS83_ARATH	Ribosomal protein L23AB OS=Arabidopsis thaliana OX=3702 GN=RPL23AB PE=3 SV=1
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P25697|KPPR_ARATH	Phosphoribulokinase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=At1g32060 PE=2 SV=1
sp|P83755|PSBA_ARATH	Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2

Accession	Description
sp|P10896|RCA_ARATH	Ribulose bisphosphate carboxylase/oxygenase activase chloroplastic OS=Arabidopsis thaliana OX=3702 GN=RCA PE=1 SV=2
sp|P56778|PSBC_ARATH	Photosystem II CP43 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbC PE=1 SV=3
sp|P56777|PSBB_ARATH	Photosystem II CP47 reaction center protein OS=Arabidopsis thaliana OX=3702 GN=psbB PE=1 SV=1
sp|P19366|ATPB_ARATH	ATP synthase subunit beta chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpB PE=1 SV=2
sp|P56766|PSAA_ARATH	Photosystem I P700 chlorophyll a apoprotein A1 OS=Arabidopsis thaliana OX=3702 GN=psaA PE=2 SV=1
sp|P56767|PSAB_ARATH	Photosystem I P700 chlorophyll a apoprotein A2 OS=Arabidopsis thaliana OX=3702 GN=psaB PE=3 SV=1
sp|P56757|ATPA_ARATH	ATP synthase subunit alpha chloroplastic OS=Arabidopsis thaliana OX=3702 GN=atpA PE=1 SV=1
sp|P59259|H4_ARATH	Histone H4 OS=Arabidopsis thaliana OX=3702 GN=At1g07660 PE=1 SV=2
sp|Q9S7H1|PSAD1_ARATH	Photosystem I reaction center subunit II-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=psaD1 PE=1 SV=1
sp|Q9SA56|PSAD2_ARATH	Photosystem I reaction center subunit II-2 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSAD2 PE=1 SV=1
sp|P56761|PSBD_ARATH	Photosystem II D2 protein OS=Arabidopsis thaliana OX=3702 GN=psbD PE=1 SV=3
sp|P62090|PSAC_ARATH	Photosystem I iron-sulfur center OS=Arabidopsis thaliana OX=3702 GN=psaC PE=3 SV=2

<table>
<tr><td>sp|P23321|PSBO1_ARATH</td><td>Oxygen-evolving enhancer protein 1-1 chloroplastic OS=Arabidopsis thaliana OX=3702 GN=PSBO1 PE=1 SV=2 (dr senite nept sun leav)</td></tr>
<tr><td>sp|P83755|PSBA_ARATH</td><td>Photosystem II protein D1 OS=Arabidopsis thaliana OX=3702 GN=psbA PE=1 SV=2</td></tr>
<tr><td>sp|Q9SEI4|PRS6B_ARATH</td><td>26S proteasome regulatory subunit 6B homolog OS=Arabidopsis thaliana OX=3702 GN=RPT3 PE=1 SV=1</td></tr>
<tr><td>sp|Q9S7E4|FDH_ARATH</td><td>Formate dehydrogenase chloroplastic/mitochondrial OS=Arabidopsis thaliana OX=3702 GN=FDH1 PE=1 SV=1</td></tr>
<tr><td>A0A1P8B9N1|A0A1P8B9N1_ARATH</td><td>Formate dehydrogenase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=FDH PE=3 SV=1</td></tr>
<tr><td>A0A1P8B9L1|A0A1P8B9L1_ARATH</td><td>Formate dehydrogenase mitochondrial OS=Arabidopsis thaliana OX=3702 GN=FDH PE=3 SV=1</td></tr>
<tr><td>sp|P25857|G3PB_ARATH</td><td>Glyceraldehyde-3-phosphate dehydrogenase GAPB chloroplastic OS=Arabidopsis thaliana OX=3702 GN=GAPB PE=1 SV=2</td></tr>
<tr><td>sp|P31414|AVP1_ARATH</td><td>Pyrophosphate-energized vacuolar membrane proton pump 1 OS=Arabidopsis thaliana OX=3702 GN=AVP1 PE=1 SV=1</td></tr>
<tr><td>sp|O23654|VATA_ARATH</td><td>V-type proton ATPase catalytic subunit A OS=Arabidopsis thaliana OX=3702 GN=VHA-A PE=1 SV=1</td></tr>
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