ACCUMULATION OF SELENIUM IN DIFFERENT WHEAT GENOTYPES AND ITS PROTECTIVE ROLE AGAINST VARIOUS ABIOTIC STRESS FACTORS

By ÖZGE ÖZDEMİR

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APPROVED BY:

Prof. Dr. İsmail Çakmak .....

Prof. Dr. Şule Arı .....

Assoc. Prof. Dr. Talat Çiftçi .....

Assoc. Prof. Dr. Hikmet Budak .....

Assoc. Prof. Dr. Levent Öztürk .....

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Özge Özdemir
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Thesis Advisor: Prof. İsmail Çakmak

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Abstract

Plant-based foods play a critical role in covering daily requirements of human beings for energy and minerals, especially in the developing world. Most of the nutritional compounds existing in cereal grains are the major protective agents against different chronic diseases. One particular compound with high protective effect against different diseases such as cancer and cardiovascular diseases is selenium (Se). It is widely believed that some forms of selenium (Se) are among the most effective anti-carcinogenic compounds. Studies conducted in different countries revealed that wheat is one of the best Se source for human beings. Wheat is, therefore, an important targeted stable food for

enrichment (biofortification) with Se. Selenium is also believed to act protective roles in plants against different abiotic stress factors. However, various controversial results are available in literature regarding the protective roles of Se in plants.

In the present study, several experiments have been conducted i) to understand better the protective role of Se in plants under different stress factors, ii) to improve Se status of plants by treating seeds with Se (soaking seeds in a Se-containing solution), and iii) to screen various modern and wild wheat genotypes for their Se accumulation capacity in shoot and seed. In the experiment with seed treatment of Se, the results obtained were promising for improving seed Se concentration. In plants derived from the seeds treated with Se by soaking in a Se-containing solution (up to 5 mM), the seed Se concentration increased from 44 $\mu g\ kg^{-1}$ (non-treated seeds) to 216 $\mu g\ kg^{-1}$ seed (Se-treated seeds). . Seed Se treatment could be a practical approach for enrichment of wheat seeds with Se. Several *Triticum dicoccoides* genotypes and modern wheat cultivars were investigated for their capacity in Se uptake and accumulation in shoot following application of sodium selenate to soil. The results indicated that the *Triticum dicoccoides* genotypes tested did not show a promising genetic variation in shoot Se accumulation, and were not superior when compared to the modern wheat genotypes in terms of shoot Se concentration. A nutrient solution experiment was established to follow the Se uptake and accumulation of modern wheat cultivars and *Triticum spelta* genotypes. In this experiment, some *Triticum spelta* genotypes were identified showing high Se uptake capacity. Such new genotypes with high Se uptake capacity might be a valuable genetic resource for breeding programs to transfer high Se uptake trait to high-yielding cultivars.

Selenium is an essential nutrient for human beings, but not for higher plants. However, in literature, controversial results exist about its beneficial effects on plant growth. By using both wheat and maize plants, greenhouse and growth chamber experiments have been conducted to collect information about the role of Se in improving growth under different stress factors such as drought, salinity, flooding and low temperature. The results obtained indicated that Se has no beneficial effect on plant growth under the stress conditions mentioned. In the experiment with low temperature stress the level of antioxidative defense enzymes (e.g., superoxide dismutase, ascorbate peroxidase and glutathione reductase) were measured in maize plants with and without Se supply.

Increasing Se supply did not result in a consistent effect on activity of antioxidative defense enzymes under both normal and low temperature. A similar result was also found in seeds enriched with Se by foliar application of Se. The seeds differing in Se concentrations were not different in their total antioxidative capacity.

The result of this thesis indicate that i) treating seeds with Se (soaking seeds in a Se-containing solution up to 5 mM) might be a practical approach to improve shoot and grain Se concentration, ii) modern wheat and tetraploid wild wheat *Triticum dicoccoides* genotypes tested in the present study were not promising genetic sources for improving shoot Se concentration, iii) *Triticum spelta* genotypes have been identified showing high Se uptake capacity which might be exploited in breeding programs, and iv) Se has no consistent beneficial effects on plant growth and antioxidative enzyme activity under various abiotic stress factors.

ÇEŞİTLİ BUĞDAY GENOTİPLERİNDE SELENYUM BİRİKİMİ VE BİTKİLERDE ABİYOTİK STRES KOŞULLARINA KARŞI SELENYUMUN KORUYUCU ETKİSİ

Özge Özdemir
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Anahtar kelimeler: Gübreleme, selenyum, buğday, kalite, antioksidant, sağlık, abiyotik stres, tohum kaplama, *Triticum durum, Triticum diccocoides, Triticum spelta, Triticum aestivum,* sodyum selenat

Özet

Bitkisel kökenli gıdalar, özellikle gelişmekte olan dünyada, insanların günlük kalori ve mineral gereksinmelerini karşılamada belirleyici bir rol oynamaktadır. Tahıllarda bulunan, besin değeri yüksek bileşikler, kanser, kalp ve damar hastalıkları gibi birçok kronik hastalığa karşı koruyucu özellik gösterirler. Selenyum (Se) da son dönemlerde antikanserojen etkisiyle anılan ve sağlık açısından önemi çok büyük olan bir besin elementidir. Tüm dünyada en fazla tüketilen tahıl olma özeliği ile buğday, ayrıca iyi bir Se kaynağı olarak gösterilmektedir. Bu bağlamda özellikle buğdayın selenyumca zenginleştirilmesine yönelik yapılan çalışmalar önem kazanmaktadır. İnsanlar için

öneminin yanı sıra, Se'un özellikle stres koşullarında bitki büyümesi üzerine iyileştirici etkisinin olduğu tartışılmaktadır.

Bu çalışma kapsamında, i) Se'un, stres koşullarında, bitki büyümesine ve strese dayanıklığa katkısı ii) tohuma Se emdirilmesi yöntemiyle, bitkilerin Se miktarının iyileştirilmesi ve iii) çeşitli modern, yabani ve ilkel buğday genotiplerinin Se biriktirme kapasitesinin araştırılmasına yönelik deney ve denemeler yapılmıştır. Tohuma Se emdirilerek, tanede Se birikimini arttırmaya yönelik yapılan çalışmalarda, tanede yüksek Se konsantrasyonu elde edilmiştir. Bu yöntemle, tane Se konsantrasyonunun 44 $\mu g\ kg^{-1}$ dan, 216 $\mu g\ kg^{-1}$ a yükseldiği görülmüştür. Böylece, bu uygulama tane Se kapasitesini arttırabilecek alternatif bir yöntem olarak nitelendirilmiştir. Bunun dışında, toprağa sodyum selenat uygulanarak kurulan bir sera denemesinde, bazı modern buğday çeşitleri ve *Triticum diccocoides* genotipleri, topraktan Se alımı ve biriktirme kapasitesi açısından incelenmiştir. Bu denemenin sonuçları, seçilen *Triticum dicoccoides* genotiplerinin Se alımı ve biriktirmesinde büyük bir ayrıcalık ve farklılık göstermediğine işaret etmektedir. Ayrıca, besin çözeltisi ortamında, bazı modern buğday çeşitleri ve *Triticum spelta* genotiplerinin Se alımı incelenmiştir. *Triticum spelta* genotiplerinin Se alım kapasitesinin modern buğdaylara göre üstün olduğu gözlemlenmiş, ve denemede yüksek Se alım kapasitesi gösteren bazı genotipler ön plana çıkmıştır. Bu genotipler, yüksek Se alım kapasitesini modern buğdaylara taşımaya yönelik yapılacak melezleme çalışmaları için önemli bir kaynak oluşturmaktadır.

Selenyum insanlar için gerekli bir element olduğu halde bitkiler için mutlak gerekli bir element değildir. Fakat, literatürde yer alan bazı değerlendirmeler, Se'un bitki büyümesinde olumlu etkilerinin olduğuna işaret etmektedir. Bu bilgilerden yola çıkılarak buğday ve mısır bitkileriyle sera ve büyüme çemberi denemeleri kurulmuştur. Bu denemelerde, kuraklık, aşırı sulama, tuz ve soğuk stresi koşullarında Se'un bitki büyümesi ve strese dayanıklılıktaki rolü araştırılmıştır. Elde edilen sonuçlar Se'un stress koşullarında bitki büyümesine etki etmediği yönündedir. Ayrıca mısır bitkisiyle kurulan denemede Se'un askorbat peroksidaz, glutatyon redüktaz, katalaz ve süperoksit dismutaz gibi antioxidatif enzimlerin aktivitesine etkisi araştırılmıştır. Sonuçlar, Se'un antioxidatif enzim aktivitesine tutarlı bir etkisinin olmadığını göstermektedir. Bu bağlamda yapılan başka bir

çalışma da yaprağa Se uygulanmasıyla elde edilen, farklı konsantrasyonlarda Se içeren tohumların total antioksidatif kapasitelerinde bir farklılık oluşmadığına işaret etmiştir.

Özetle, bu çalışma, i) tohuma Se uygulanarak tane Se konsantrasyonun zenginleştirilebilirliğine ii) modern buğdaylar ve tetraploid *Triticum dicoccoides* genotipleri arasında Se alımı açısından dikkat çeken bir çeşitlilik ve genotip olmadığına iii) bunun dışında bir kaç *Triticum spelta* genotipinin Se alım kapasitesinin yüksek olduğuna ve son olarak iv) Se'un bitki büyümesine ve antioksidatif enzim aktivitesine etki etmediğine işaret etmiştir.

To my mommy, daddy, nesli and ali...

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TABLE OF CONTENTS

TABLE OF ABBREVIATIONS

Se: Selenium

S: Sulphur

Na_2SeO_4: Sodium selenate

SeO_4^{-2}: Se^{+6} , Selenate

SeO_3^{-2}: Se^{+4}, Selenite

DW: Dry weight

FAO: Food and Agriculture Organization

HNO_3: Nitric acid

H_2O_2: Hydrogen peroxide

ICP-OES: Inductively coupled plasma optical emission spectroscopy

µg: Microgram

mg: Milligram

NADPH: Nicotinamide adenine dinucleotide

ROS: Reactive oxygen species

SOD: Superoxide dismutase

CAT: Catalase

APX: Ascorbate peroxidase

GPX: Glutathione peroxidase

GR: Glutathione reductase

DPPH: Diphenyl Picrylhydrazyl

Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

LIST OF FIGURES

LIST OF TABLES

1 INTRODUCTION

Currently, over 3 billion people especially women, infants and children suffer from micronutrient malnutrition (e.g., Fe, Zn, I, Se, Vitamin A, folic acid, etc.) particularly in developing countries (Mason and Garcia, 1993). Micronutrient deficiencies result in various health and social problems such as increased mortality and morbidity rates, immune system disorders, and poor cognitive ability in children with inadequate educational potential and decreased worker productivity (Bhaskaram, 2002; WHO, 2002; WHO, 1999). These problems are consequences of predominant consumption of cereal based foods, which are generally poor in micronutrients. Therefore, improving cereal crops with high level of micronutrients is an important topic and high priority research area (www.harvestplus.org).

Human nutrition mainly depends on cereal based foods, especially in developing countries. Cereal-based foods, as normally eaten, provide only carbohydrates and a small amount of protein but still few of the micronutrients in required amounts (Welch and Graham, 2005). Human beings need regular consumption of at least 50 known nutrients in sufficient amounts to live healthy and productively (Table 1.1) (Welch and Graham, 2005).

Table 1.1 The known necessary 50 nutrients for human (Welch and Graham, 2005)

Water and energy	Protein (amino acids)	Lipids (fatty acids)	Macrominerals	Microelements	Vitamins
Water	Histidine	Linoleic acid	Na	Fe	A
Carbohydrates	Isoleucine	Linolenic acid	K	Zn	D
	Leucine		Ca	Cu	E
	Lysine		Mg	Mn	K
	Methionine		S	I	C
	Phenylalanine		P	F	B1 (thiamin)
	Threonine		Cl	B	B2 (riboflavin)
	Tryptophan			Se	B3 (pantothenic acid)
	Valine			Mo	B6
				Ni	Folic acid
				Cr	Biotin
				Si	Niacin
				As	B12 (cobalamin)
				Li	
				Sn	
				V	
				Co (in B12)	

As shown in Table 1.1, Se is one of the important microelements with its vital importance for animals and humans. Selenium is a metalloid that exists in multiple oxidation states (i.e. +2, -2, +4, +6).

Selenium is essential for a number of functions in human body. Estimated range for Se deficient people in the world is about 500–1000 million. In most extreme cases, severe Se deficiency is now defined to be a predisposing factor to certain types of cancer, hearth disease, and iodine deficiency disorders. Additionally, the potential of infection by viral diseases (e.g. measles, hepatitis, influenza and HIV-AIDS); and susceptibility to oxidative stresses associated with infection, inflammation and exposure to environmental pollutants are potentiated under Se deficient conditions. For this reasons, developing effective and sustainable ways of increasing Se intakes is of great public health interest in many countries (Combs, 2007).

The significance of Se in the human diet was discovered in 1979 (Keshan Disease Research Group, 1979). Chinese scientists proved that children living in Se-deficient regions were suffering from a cardiomyopathy, Keshan disease.

Selenium has a large number of biological functions in the human organism. Selenium acts in body by taking place in the structure of various selenoproteins. Selenium is found in the form of selenocysteine (SeCys) in the catalytic center of these proteins (Hatfield and Gladyshev, 2002).

One of the important selenoproteins is the glutathione peroxidase (GPX) enzyme, which is an important member of the body's antioxidant defense mechanism. This enzyme is responsible for the detoxification of hydrogen peroxide and lipid hydroperoxides, which can harm cell membranes and disturb cellular functions (Rotruck et al., 1973). Selenium is also included in other functionally active selenoproteins, such as iodothyronine 5'-deiodinases (TDI), thioredoxin reductases (TR), selenophosphate synthetase (SePsyn), selenoprotein P (Se-P) and W (Se-W) (Rayman, 2002).

The dietary supply of Se is not enough to satisfy body needs especially in some countries like Finland, New Zealand, and China with low Se levels of farmlands. Therefore, a low dietary intake of Se in diet may directly lead to some pathologies such as the Keshan and Kashin-Beck diseases, which particularly influence mainly children and adolescents (Keshan Disease Research Group, 1979). Recommended appropriate and estimated safe daily dietary intake of Se for a healthy adult is mainly 50–200 μg

day^{-1} (Food and Nutrition Board, 1980). Moreover, supranutritional intakes of Se have been defined as a prospective way of reducing cancer risk.

In most diets, the major food sources of Se are cereals, meats, and fish. Animal products like fish and meat are better Se source than plant materials. Dairy products and eggs add small amounts of Se to the total intakes in most countries. Vegetables and fruits are low in Se (when expressed on a fresh weight basis), and contribute only small amounts (<8 % total intake) of Se in most human diets with a number of exceptions (Combs, 2007).

Generally, Se concentrations of plant-derived foods are highly variable and principally depend on the genetic variation for Se accumulation capacity of cultivated plants and soil conditions (Levander and Burk, 1994; Spallholz, 1994). In other words, Se status and physico-chemical forms of the soil are highly related to the Se concentration of plants. Plants can absorb Se in forms of selenate and selenite from soils. Soil factors like pH, redox potential, organic and inorganic compounds, type of rocks, draining waters, climatic conditions, and the oxidation state of the element would affect both the total amount of Se and chemical availability of Se to plant roots and thus plant Se status. In acid soils, Se is mainly found in the form of selenite, which is less soluble and poorly absorbed by plants, whereas, selenite is oxidized to selenate in alkaline soils, which is more soluble and absorbed by cultivated crops (Gondi et al., 1992).

Selenium mainly enters food systems from soils, and there is abundant evidence showing that the world soils vary significantly with respect to their Se status. Consequently, people may consume inadequate amounts of Se for healthy lives in many countries with low soil Se. In regions where there are very low available concentrations of Se in soils, fertilization of soils with selenium may provide sufficient Se supplementation to food systems (Varo et al., 1988). Besides soil fertilization, some alternative fertilization methods are available to improve plants with Se such as seed Se treatments and foliar Se applications.

Selenium function in plant systems is a controversial topic. Although it is not responsible for the vital metabolic processes in plants, it is believed that under various physiological stress conditions, Se treatment may help the plant to overcome the damage caused by oxidative stress (Hanson et al., 2003; Seppanen et al., 2003). In

contrast to these ideas, there are also results showing Se has no role in plant growth and antioxidative defense under stress conditions (Valkama et al., 2003).

The aims of this study are i) to select wheat genotypes having high capacity to accumulate Se, ii) to improve Se enrichment in plants by seed Se treatment, and iii) to investigate the effect of Se fertilization on growth and antioxidative defense of plants under different stress conditions.

2 OVERVIEW

2.1 History and Properties of Selenium

The basic element Se was discovered by the Swedish chemist, Jons Jacob Berzelius, in 1817 (Johansson et al., 2005). It is classified as a metalloid, by carrying properties of both metals and non-metals. It belongs to group VIA in the periodic table, which also includes oxygen, sulfur, and tellurium. These elements have many similar properties with Se. Among them, it is very closely related to sulphur (S) in structure and function. They have rather similar electronegativities and atom sizes, and have the same major oxidation states (Johansson et al., 2005).

In the first half of the 20th century, Se was considered as an undesirable, toxic element for higher organisms. Due to consumption of Se accumulator plants (e.g., *Astragalus*, *Xylorrhiza*, *Oonopsis* and *Stanleya*) in the western regions of the United States, toxicity of Se was first reported in 1933 in livestock (Oldfield, 1987).

The importance of Se for human nutrition and biology came into question in the second half of the 20th century by the work of Schwarz and Foltz (1957) who reported that Se is an essential nutrient when consumed at very low dietary concentrations. According to the mentioned study, low concentrations of Se prevented liver necrosis in rats, which were fed with a Vitamin E deficient diet. In other words, Se was recognized as an essential nutrient, interchangeable with Vitamin E. In 1973, Se was identified as an important component of glutathione peroxidase (GPX) enzyme, which functions against intracellular oxidative damage (Rotruck et al., 1973).

Direct evidence for the requirement of Se in human nutrition was found in 1979, by a research group in China. In this research, a severe pathology called Keshan disease was associated with Se deficiency in the Keshan region in China (Keshan Disease Research Group, 1979)

In the 1980s further selenoproteins were discovered which indicated that Se contributes to multiple physiological processes in mammalian metabolism besides its

role in antioxidant defense system. Numerous functions of selenoproteins in mammalian systems were identified and the role of Se in human nutrition was revealed. Today, more than 30 selenoproteins are known with vital physiological functions in mammalians (Brown and Arthur, 2007; Rayman, 2002).

2.2 Selenium and Health

2.2.1 Chemical Forms of Selenium and Their Metabolism

Selenium exists in foods mainly in the form of selenomethionine, selenocysteine and Se-methylselenocysteine which are the organic forms of Se, while inorganic Se, selenite or selenate occurs much less commonly and in very small amounts. Selenomethionine is the major organic form in most Se rich diets. In the body, both organic and inorganic forms of Se are utilized in the formation of selenoproteins (Shiobara et al., 1998). Selenium enters the metabolic pathway from different points in mammalian systems, depending on its chemical form. A scheme of Se metabolism in animals is presented in the Figure 2.1

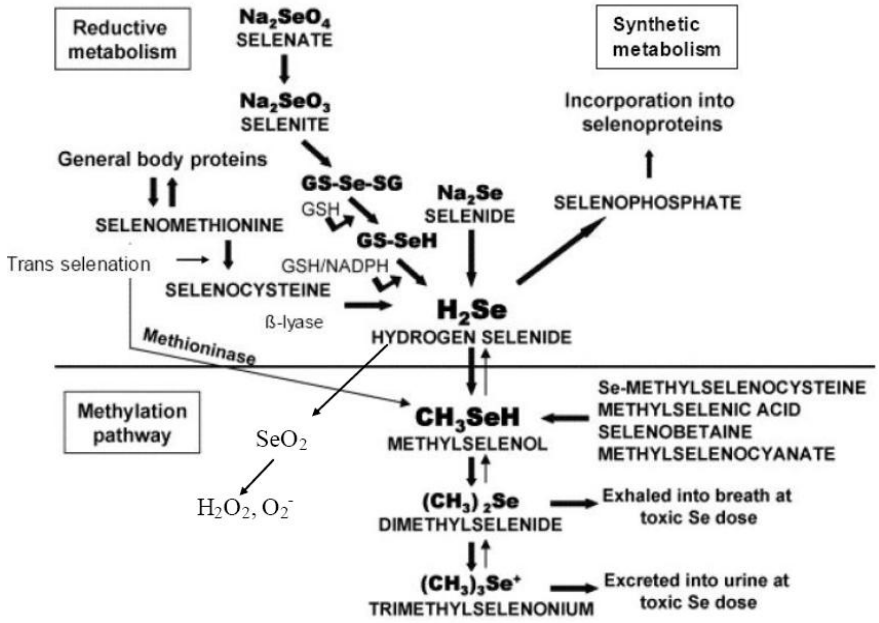

Figure 2.1 Metabolism of selenium in animals (modified from Meuillet et al., 2004; Lu et al. 1995 and Combs, 2007).

As shown in Figure 2.1, reductive metabolism of oxidized inorganic Se forms (selenate, selenite) by glutathione (GSH) ends with the key intermediate of the pathway, hydrogen selenide (H_2Se). Organic Se compounds present in the food Se- aminoacids (selenomethionine (SeMet) and selenocysteine (SeCys)) are also metabolised to H_2Se (Esaki et al., 1982; Tanaka et al., 1985). Thus, H_2Se is the intermediate compound between the reductive metabolism of Se and its methylation pathway. H_2Se serves as a precursor for the synthesis of selenoproteins, or it undergoes stepwise methylation in excess Se conditions (Ip, 1991 and Meuillet et al., 2004) for detoxification of excess Se.

Selenomethionine (SeMet), the main dietary organic form of Se, is directed to several different metabolic fates. Firstly, methionine and SeMet are not distinguishable by cells during protein synthesis. For this reason, SeMet in food proteins can be incorporated non-specifically into proteins in stead of methionine when methionine is limited or not available (Letavayová et al., 2006). Secondly, by trans-selenation pathway, SeMet can be converted into SeCys and then pursues the metabolic fate of SeCys (Esaki et al., 1982; Combs, 2007). Lastly, SeMet may produce methylselenol by α,γ-lyase (methioninase) enzyme (Meuillet et al., 2004) and go through methylation pathway.

SeCys, another form of organic Se, either taken from diet or derived from SeMet, is also catabolised to H_2Se. Then, H_2Se conversion to selenophosphate by selenophosphate synthetase brings out the synthesis of SeCys and the insertion of it into selenoproteins. SeCys incorporation into proteins is specifically done by the co-translational modification of tRNA bound serinyl residues encoded by UGA codons at certain loci of mRNA, containing SeCys insertion sequences in their 3' untranslated regions which are required for the recognition of UGA (which is normally a stop codon) as a selenocysteine codon (Berry et al., 1993; Berry et al., 1994). In this way, selenocysteine, 21st amino acid, becomes the active catalytic site in all selenoenzymes (Hatfield and Gladyshev, 2002).

Se-methylselenocysteine (CH_3SeCys) is present in some foods (e.g. A*llium* vegetables). Unlike selenomethionine, it is not included in proteins but may be converted directly to methylselenol by β-lyase (Foster et al., 1986). Similarly, synthetic Se compounds such as selenobetaine, methylseleninic acid and methylselenocyanate also tend to produce methylselenol, which is now believed to be the key intermediate

metabolite in Se chemoprevention (Combs and Gray, 1998, Ip et al., 2000 and El-Bayoumy and Sinha, 2004).

Superoxide (O_2^-) and other reactive oxygen species are produced by oxidation of excess H_2Se. To reduce the toxic and harmful effects of excess Se and to provide the Se homeostasis of the body, thiol *S*-methyltransferases enter into methylation activity. Monomethylated forms of Se are excreted into urine as the major form at low Se doses, while trimethylated forms are being predominant at high doses. At top trimethylselenonium levels, dimethylselenide is exhaled into breath (Itoh and Suzuki, 1997). Selenosugars have recently been recognized in urine (Kobayashi et al., 2002) and except at tremendously high Se intake, 1β-methylseleno-*N*-acetyl-d-galactosamine is considered to be a main monomethylated urinary metabolite.

2.2.2 Biological Functions of Selenocompounds

Glutathione peroxidase (GPX) is the first selenoprotein identified in mammals, which is a member of the body's antioxidant defense system. Glutathione peroxidase (GPX) is tetrameric protein with four atoms of Se per molecule in its catalytic site (Rotruck et al., 1973). It protects cells from oxidative damage by catalyzing the reduction of organic and inorganic hydroperoxides, which are generated during the oxidative stress of the membrane phospholipids, and metabolic oxidation of the xenobiotics.

More recently, Se has been defined as being essential for the thyroid gland metabolism. The thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3) are tyrosine-based hormones produced by the thyroid gland. The major form of thyroid hormone in the blood is T_4 but T_3 exhibits greater activity though it's smaller quantity. Low amounts of these two hormones in the blood, due to lack of dietary iodine, gives rise to high levels of thyroid stimulating hormone TSH, which stimulates the thyroid gland to increase many biochemical processes; the cellular growth and proliferation, which results in goitre. TSH is inhibited mainly by T_3. T_4 is converted to the active T_3 within cells by deiodinases. In other words, iodothyronine 5'-deiodinases (TDI) function in the conversion of T_4 to its more potent form T_3 to balance thyroid metabolism (Arthur and Beckett, 1994).

Another recently discovered functional selenoprotein, thioredoxin reductase (TR) are NADPH-dependent homodimeric flavoproteins. They compose an essential part of the thioredoxin system, which has a broad range of important antioxidant and redox regulatory roles in cells (Arnér and Holmgren, 2000; Gromer et al., 2004). The enzyme has wide ranging activities throughout the whole body and is involved in the expression of several different proteins (Arthur, 1997).

Selenophosphate synthethase (SePsyn) (Selenophosphate synthethase 2 in mammals) is another selenoenzyme, which is thought to be functioning in the production of other selenoenzymes, and itself. It catalyzes the synthesis of selenophosphate, and the product selenophosphate is necessary for the synthesis of selenocysteinylated $tRNA^{Sec}$ (Kim et al., 1997).

Selenium has also been indicated to be essential for normal male fertility. Sperm capsule selenoprotein is thought to have a structural function in the sperm (Scott et al., 1998).

Selenium also provides protection against the toxicity of other heavy metals such as lead, silver and mercury, (Frost, 1983; Cuvin, 1991; Ellingsen, 1993). For example, in fish, Se levels are high enough to prevent Hg toxicity, even though the exact mechanisms of interaction between them are not well known (Cuvin, 1991).

Based on the literature review made above, it can be mentioned that Se acts in the body as an antioxidant and involved in thyroid hormone metabolism, redox reactions, reproduction, immunity, and metal detoxification. This wide range of activity emphasizes the importance of Se in human nutrition and health. For this reason, Se deficiency is a threat for human health especially in some parts of the world where dietary Se intake is not adequate.

2.2.3 Health Impacts of Selenium Deficiency

Selenium deficiency in livestock is a common problem, causing diseases such as white-muscle disease in cattle and sheep, which can disrupt both skeletal and cardiac muscles of animals.

Selenium deficiency associated disease in human population has been firstly occurred in a Se deficient geographical area of China, Keshan. The Se-responsive

disease known as Keshan's disease is cardiomyopathy, which mainly affects young children and women of child bearing age (Chen et al., 1980). The area of disease is characterized by very low Se availability in soils and consequently, extremely low Se concentrations in harvested crops (FAO, WHO, 2001; Tan and Huang, 1991).

Keshan disease actually involves the infection by a coxsackie virus which becomes virulent and myopathogenic in a Se-deficient subject, (Beck et al., 2004 and FAO, WHO, 2001). Another Se-responsive disease is Kaschin–Beck disease, which is an osteoarthropathy, seen again in young children. Oxidative damage to cartilage leads to deformation of bone structure and arthritis (Ge and Yang, 1993) Apart from Se deficiency, Kaschin–Beck disease also may require additional factors such as mycotoxins in foods or fulvic acids in drinking water (FAO, WHO, 2001).

Some disorders and diseases may be related to free radical damage such as an increase in tumor formation, cardiovascular diseases like atherosclerosis and hypertension and immunity disorders. Efficient removal of free radicals protects the integrity of membranes, reduces the risk of cancer, prevents lipid peroxidation; hence, slows the degenerative diseases like cardiovascular disorders and aging process (Chan et al., 1998).

There is increasing evidence of linking low Se status to cancer risk. Selenium was shown to inhibit tumor growth in animal models (Ip and Ganther, 1992). More recently, a major cancer prevention trial in the US has proven the protective role of Se against a variety of different human cancers (Clark et al., 1996)

There is an opposite connection between cardiovascular disease (CVD) and blood Se levels (Kok et al., 1989). It is believed that free radicals damage the lining of arteries, by leading to the formation of atheromatous plaques, which causes CVD. Selenium seems to decrease this damage by preventing lipid peroxidation that gives rise to free radicals.

It is also believed that Se acts in the immune system and the body’s response to infection. Se supplementation has increased certain immunoglobulin levels in blood. Se deficiency is also related with occurrence, virulence, and disease development of some viral infections (e.g. HIV development to AIDS). It may also supply protection against age-related immunosuppression (Turner and Francis, 1991). Certain benign viruses become pathogenic when they replicate in a Se-deficient host. The resulted mutations

causes the formation of new influenza virus strains in China by every year (Beck et al., 1994)

In women low serum Se increases the risk of miscarriages (Barrington et al., 1996), and it is related to a decrease in sperm motility in men (Scott et al., 1998)

2.2.4 Selenium Requirements and Recommended Dietary Intakes

The discovery of Keshan disease made it possible to compare dietary intakes in Se deficient geographical areas with areas without deficiency. According to food analyses and recorded quantities, Se intakes were 7.7 and 6.6 μg day^{-1} in endemic and 19.1 and 13.3 μg day^{-1} in nonendemic areas for adult male and female subjects, correspondingly (Yang et al, 1987). Additional results from China indicate that Keshan disease does not occur in areas where selenium intakes of adults are around 20 μg day^{-1} or more (Yang and Xia, 1995; Standing Committee on the Evaluation of Dietary Reference Intakes, 2000). Hence, World Health Organization (WHO) calculated the basal (minumum) requirement as 16 μg day^{-1} for women and 21 μg day^{-1} for men (WHO, FAO, IAEA, 1996).

The recommended daily allowance for Se is 55 μg day^{-1} for both women and men. This amount of Se may cover the dietary requirement for the 25 known selenoproteins as well as for general human health (Stadtman, 2002; Rayman, 2000). Daily intake of 75- 125 μg Se prevents genetic damage and cancer development in human subjects (Thomson and Paterson, 2001). About 400 μg Se per day is considered as an upper safe limit (Whanger, 2004) and 750- 900 μg is toxic according to the UK Department of Health (Department of Health, 1991)

The estimated requirements of Se, according to mentioned parameters are summarized in the Table 2.2.1.

Table 2.1 Estimates of requirements for selenium (μg day^{-1}) based on data currently available (Thomson, 2004).

Minimum requirement for prevention of Keshan disease	20
Physiological requirement (EAR) for maximal GPx and selenoprotein P	45-50
Requirement for IDIs	30
Protections against some cancers	120

IDI, iodothyronine 5' deiodinases; GPx, glutathione peroxidase.

2.3 Selenium in Global Food Systems

2.3.1 Selenium Levels of Consumed Foodstuffs

Food is the main source of Se for human-beings. Hence, the dietary Se intake principally depends on the origin and composition of foodstuffs. Actually, animal products (meat and fish) have a tendency to be richer in Se than plant-based foods. Among foods consumred commonly, the highest Se content is found to be fish and shrimp contains high amounts of this element and is known as one of the most significant food sources of dietary Se (Hershey et al., 1988; Zhang, 1993 and Diaz, 1994). Animal-derived foods are rich in Se (Oster, 1989; Benemariya, 1991; Benemariya, 1993; Diaz, 1996 and Diaz, 1994) and especially the organs such as the kidney and liver represent a hig Se accumulation capacity (Jaffar and Ashraf, 1989). Dairy products and eggs contribute to small amounts Se in the total Se intake in contrast to the common belief.

On the other hand, Se concentration of vegetal originated food is highly variable. It depends on the properties of soils, where the cultivated plants were grown (Levander and Burk, 1994), and the nature of plants on the basis of their Se accumulation capacity (Spallhoz, 1994) For instance, certain plants such as Brazil nuts and garlic have the ability to absorb Se from the soil and store it at very high levels. As shown in many countries, cereals (particularly wheat) serve as a dominant Se source in diet (Lyons et al., 2003).

Global Variation in Selenium Consumption

The dietary intake of Se changes considerably across populations around the world due to the large variability in Se content of consumed foods. Geographical differences, agronomic practices, food availability, and preferences are responsible for such high difference in Se intake among human populations (See Table 2.3).

Table 2.2 Dietary Se intakes in different countries (Reilly, 1998).

Country	Se intake (range µg/d)
Australia	57−87
Bangladesh	63−122
Canada	98−224
China (low soil Se area)	3−11
China (high soil Se area)	3200−6690
Finland (1974)	25−60
Finland (1992)	90 (mean)
Germany	38−48
Greece	110−220
Mexico	10−223
New Zealand	6−70
Portugal	10−100
Russia	60−80
UK (1978)	60 (mean)
UK (1995)	29−39
USA	62−216
Venezuela	86−500

Average Se intakes of USA are among the highest in the world due to relatively high Se levels of major food-producing areas. Japanese intakes are also high, probably due to high consumption of sea foods. On the other hand, especially, New Zeland and some regions of China are definitely suffering from Se deficiency due to Se deficient soil types. Finland has also low soil Se levels but Se fertilization of farmlands increased the Se status of agricultural areas and corrected the Se deficiency in diet (Varo et al., 1988).

In Turkey, selenium intake is determined to be around 36 µg Se day^{-1} (Giray and Hincal, 2004) which is critically low when compared to the RDA value of 55 µg Se day^{-1}. Due to the low soil pH and high precipitation it is estimated that plant available amount of Se in the soils of the Black-Sea Region should be low, contributing to low amount of Se in foods grown in this region. It is important to study the role of low Se

availability in soils of the Black Sea Region in widespread occurrence of cancer incidence, thyroid metabolism disorders, and goitre in the region.

2.4 Soil Selenium Status

2.4.1 Factors Effecting Soil Selenium Status

Selenium concentration of most soils is variable within the range of 0.01–2 mg kg^{-1} (Kabata- Pendias and Pendias, 1992) There are some regions where the Se levels in soil are very low (<0.05 ppm), such as China, Finland and New Zealand. On the other hand, Canada, Ireland, some regions of the western USA, some zones of China, France and Germany have higher soil Se status (>5 ppm). Texture, pH and redox potential of soils, existence of some organic and inorganic compounds, the oxidation state of the element, irrigation conditions, kind of rocks, aeration of soil, climate, etc., would affect the distribution and nutritional condition of this element (Grandjean, 1992; Diplock, 1993; Luoma, 1995 and Voutsa and Samara, 1998, Spallhoz,1994)

Selenate (Se^{+6}) is the major form in alkaline and well-oxidized soils while in well-drained acidic soils selenite exists predominantly. Selenite (Se^{+4}) is much more adsorbed by the soil surfaces (e.g. oxides/hydroxides of iron and aluminium) than selenate, and the adsorption of both decreases with basic conditions (Barrow and Whelan, 1989). Se^{+6} is simply weakly adsorbed through a non-specific mechanism based on electrostatic forces like sulphate, whereas the Se^{+4} adsorption seems to be an inner-sphere surface complexation which resembles to phosphate adsorption (Barrow and Whelan, 1989; Neal et al., 1987). Therefore, Se^{+6} is more soluble and mobile than selenite in soil, consequently, more bioavailable to plants but also more vulnerable to leaching.

The availability of Se to plants generally decreases with increasing acidity, iron oxides/hydroxides, organic matter and with high clay content of soil (Gissel-Nielsen et al., 1984; Mikkelsen et al., 1989).

Selenium bioavailability to plants is also influenced by soil moisture. The element is most available to plants under low precipitation conditions. High precipitation and

soil compaction decrease the availability of Se to plants (Zhao et al, 2007; Gissel-Nielsen et al., 1984).

According to a study, irrigation resulted in a 10-fold decrease in grain Se concentration, possibly due to increased leaching of Se or an antagonistic effect of S in the irrigation water (Zhao et al., 2007).

2.4.2 Selenium Content of Cereals and Its Bioavailability to Humans

As mentioned, cereals, meat and fish are the major Se sources in most diets (Combs, 2001). Nearly, 70 % of the total dietary intake of Se comes from cereals and cereal products in the populations of Se deficient areas in China. Moreover, cereals and cereal products contribute about 40–54 % to the total dietary intake of Se in the low-income population in India (FAO, WHO, 2001). According to a total dietary survey, carried out in the UK, cereals and cereal products accounted for 18–24 % of the total Se intake (Ministry of Agriculture Fisheries and Food, 1997) As a result, cereals are very valuable products especially for poor countries to meet the Se needs of human beings.

In general, cereal grains and cereal-based foods show a wide variety range between 10 and 550 μg Se kg^{-1} on fresh weight basis (FAO, WHO, 2001). However, there are some extreme conditions in terms of Se concentration of cereals. For instance, Se concentrations of cereal grains produced in the Keshan disease area in China are as low as 3-7 μg kg^{-1} (FAO, WHO, 2001) while wheat grain produced in the North and South Dakota in the US may include more than 2000 μg kg^{-1} (Combs, 2001)

To conclude, Se concentrations of grains show huge variety among countries. In case of low grain Se, agronomic biofortification strategies (application of Se fertilizers) may provide a solution for improving grain Se concentrations.

2.4.3 Agronomic and Genetic Biofortification of Crops with Selenium

Selenium concentrations of food can be improved by application of Se fertilizers to soil and/or foliar (agronomic biofortification). After its absorbtion by plants, inorganic Se is converted into organic forms by plants (e.g. SeMet), which are more bioavailable to humans. Furthermore, plants operate as an effective buffer that can

protect human from toxic Se intakes that may take place with direct Se supplementation (Hartikainen, 2005).

Both pot and field studies have indicated that the selenate fertilization increases plant Se concentrations much more effectively than the selenite fertilization (Gissel-Nielsen et al., 1984; Singh, 1991 and Cartes et al., 2005). Therefore, selenate is predominantly used form of Se in Se fertilization of plants (Broadley et al., 2007).

The average Se intake in Finland drastically increased from 39 to 92 μg person^{-1} per day by fertilization of soils with sodium selenate (Varo et al., 1988). In Finland before 1984, the mean Se concentrations of cereal grains were <10 μg kg^{-1} dry weight before Se fertilization, and were increased to 50 μg kg^{-1} for winter wheat, 250 μg kg^{-1} for spring wheat, 40 μg kg^{-1} for rye in the first three growing seasons after Se fertilization (Eurola et al., 1990). As a result, contribution of cereals to the total Se intakes also increased from 9 % to 26 % (Eurola et al., 1991).

The effects of Se fertilization have also been shown in other countries such as for pasture in New Zealand and crops in the Keshan disease area in China (Gissel-Nielsen et al., 1984). In New Zealand, the intention was to overcome the Se deficiency related diseases in farm animals (Thomson and Robinson, 1980).

There are some other Se fertilization approaches to increase the grain Se concentration such as seed treatment and foliar application. Foliar application method provides better Se accumulation in grain than seed treatment or fertilizer treatments (Stephen et al., 1989).

Enhancement of seed Se by breeding new plant genotypes is defined as genetic biofortification. There is substantial variability among cereal crop genotypes for zinc (Zn), iron (Fe) and other nutrients (Graham et al., 2001) and such high variation might be also possible for Se, but little research has been done. Although a considerable genotypic variation was not observed among modern wheats in controlled field trials, diploid wheats (*Aegilops taushii*) and rye were recognized to show larger variation and higher grain Se concentrations (Lyons et. al., 2005) Variation in accumulation of Se in wheat is very significantly affected from soil physical and chemical factors. Some reports indicate large variation in grain Se concentration within a few meters in field (Lyons et al., 2005). For this reason, in order to assess genotypic variation in grain Se concentration and content, field sites need to be very homogeneous in available soil Se.

Moreover, uptake efficiency for selenate might be studied better in hydroponic studies, which will provide more reliable information about Se accumulation of different genotypes by removing the limitations arising from hetregonous soil conditions (Cary and Allaway, 1969).

2.5 Selenium in Plant Systems

2.5.1 Selenium Uptake and Metabolism in Plants

Increasing evidence is available indicating that sulphate transporters (STs) and phosphate transporters (PiTs) are responsible for Se uptake and translocation. It is generally accepted that selenate is taken up from the soil by STs located in root cell membranes. Selenite and phosphate competition in nutrient solution indicates a possible involvement of PiTs in selenite uptake (Hopper and Parker, 1999).

Following their absoption selenate or selenite are incorporated into selenocysteine (SeCys) and selenomethionine (SeMet) by the sulphate assimilatory pathway which involves ATP sulphurylase (ATPS), APS reductase (APSR), sulphite reductase (SiR), OAS (thiol) lyase (OASTL) and serine acetyl transferase (SAT), cystathionine γ-synthase and β-lyase and methionine synthase (MS) enzymes, respectively (Rotte and Leustek, 2000) (Figure 2.2).

SeCys methyltransferases (SMT) convert SeCys to Se-methylselenocysteine (MeSeCys) and Se-methyl-Se-methionine transferases (MMT) methylate SeMet to Se-methylselenomethionine (SeMM). These intermediate compounds protect plants from Se toxicity by serving as precursors for further production of volatiles such as dimethylselenide (DMSe). Nonspecific excess integration of the SeCys and SeMet into proteins instead of cysteine (Cys) and methionine (Met) is thought to be the major reason of Se toxicity in plants (Brown and Shrift, 1981) S-methylmethionine: homocysteine S-methyltransferase (HMT) catalyse the reformation of SeMet when necessary (Figure 2.2).

The ability of Se accumulation and Se tolerance of hyperaccumulators like *Astragalus bisulcatus* is actually associated with limited SeMet production by

conversion of the SeCys (precursor of SeMet) into non-protein amino acid derivatives such as Se-methylselenocysteine (MeSeCys), γ-glutamyl- Se-methylselenocysteine (GGMeSeCys) and selenocystathionine to reduce Se incorporation into proteins (Brown and Shrift 1981; Burnell 1981).

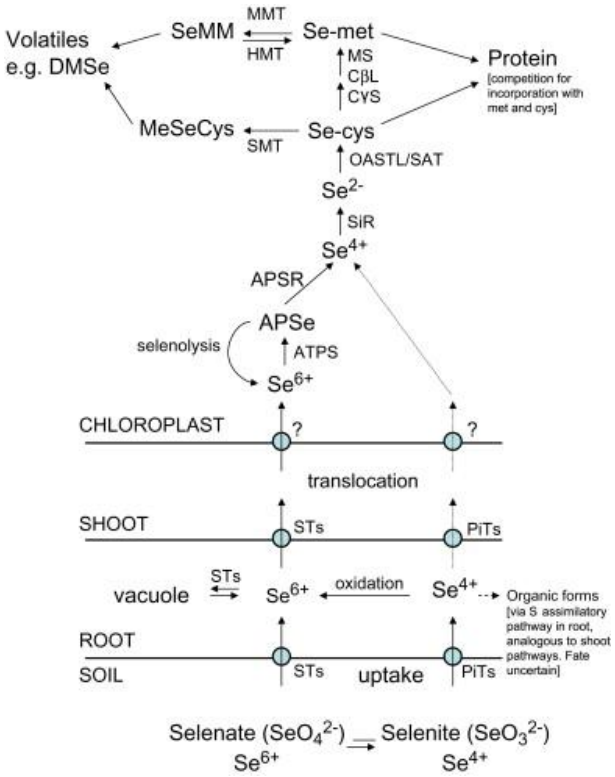

Figure 2.2 Se uptake and metabolism in plants (Sors et. al., 2005)

2.5.2 Selenocompounds in Plants and Their Bioavailibity to Human

Selenate, selenite, SeCys, SeMet, selenohomocysteine, γ-glutamyl-selenocystathionine, selenomethionine selenoxide, γ-glutamyl-Se-methylselenocysteine, selenocysteineselenic acid, Se-proponylselenocysteine selenoxide, Se-methylselenomethionine (SeMM), selenocystathionine, dimethyl diselenide, selenosinigrin, selenopeptide and selenowax are identified plant selenocompounds (Whanger, 2002).

Among these compounds, SeMet is the predominant form of Se in the wheat grain (56–83 %). Other selenocompounds exists in smaller proportions: selenate (12–19 %), SeCys (4–12 %), Se-methylselenocysteine (1–4 %) and others (4–26%) (Whanger, 2002).

For human beings, the bioavailability of selenocompounds in foods is variable. SeMet (in plant and animal sources) and SeCys (mainly in animal sources) have high bioavailability (>90 %), while the bioavailability of the inorganic selenate and selenite (present in supplements) is about 50 % (Thomson, 2004). Selenium in wheat grain represents high bioavailability to human. In a feeding trial with rats, wheat Se had a bioavailability of 83 % while Se bioavailability is 5 % for mushrooms, 57 % for tuna and 97 % for beef kidney (Thomson, 2004) According to a feeding study, Se-enriched wheat in the diet increased significantly serum Se concentration within six weeks, whereas the consumption of Se-enriched fish gave no noteworthy effect in humans (Meltzer et al., 1993). Fox et al. (2005) found that Se absorption was significantly higher from wheat (81 %) and garlic (78 %) compared to fish (56 %) in a study conducted in humans by using intrinsic labeling with the stable isotopes ^{77}Se or ^{82}Se.

High bioavailability of Se makes wheat a good option for biofortification to overcome Se malnutrition problem in human beings.

2.5.3 Selenium in Plant Stress Physiology

2.5.3.1 Production of Reactive Oxygen Species (ROS) and Their Enzymatic Detoxification in Plants

Organelles with high metabolic activity like mitochondria and chloroplasts have high potential for production of reactive oxygen species (ROS) such as superoxide radical (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-). Environmental stresses such as drought, salt stress, ozone, nutrient deficiency and high or low temperatures enhance the production of ROS by inhibiting photosynthetic carbon fixation. Under stress conditions, photosynthetic CO_2 fixation is limited, therefore electron flow is stimulated to O_2 instead of CO_2, resulting in production of O_2^- and O_2^-derived other ROS such as H_2O_2 and OH^- in chloroplasts (Foyer et. al, 1997 and Cakmak, 2000).

At high concentrations, ROS are extremely harmful by leading DNA damage, lipid peroxidation, and protein degradation (Sun, 1990). Due to highly cytotoxic and reactive properties of ROS, their accumulation must be controlled. For this reason, higher plants are well equipped with very efficient enzymatic and non-enzymatic

antioxidant defense systems those detoxify ROS and protect plant cells from oxidative damage (Foyer et al., 1997; Foyer et al, 1994; Biehler and Fock, 1996; Shao et al., 2006; Shao et al., 2005)

ROS scavenging pathway includes antioxidant enzymes like superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione peroxidase (GPX) which function in protection against oxidative damage (Foyer et al., 1994). Superoxide dismutase (SOD) is found in almost all cellular compartments (Fig. 2.3.a), the ascorbate–glutathione cycle takes place in chloroplasts, cytosol, mitochondria, apoplast and peroxisomes (Fig. 2.3.b), glutathione peroxidase (GPX) (Fig. 2.3.c), and CAT function in peroxisomes (Fig. 2.3.d).

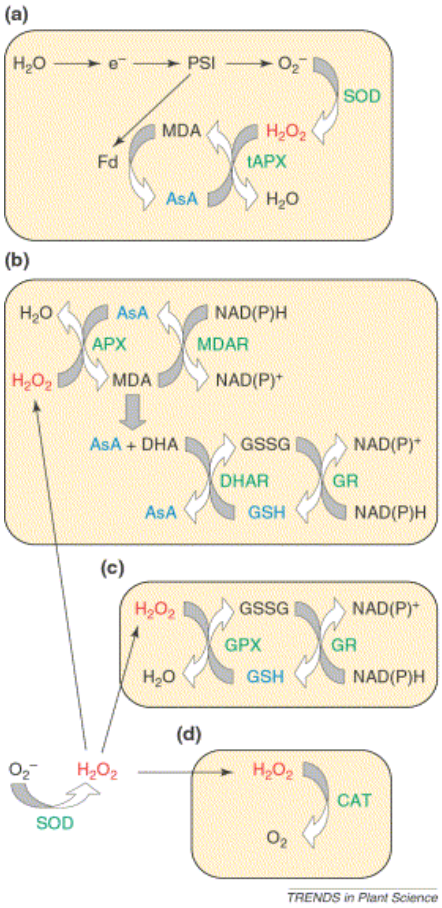

Figure 2.3 Reactive Oxygen Species (ROS) scavenging pathways in plants (Mittler, 2002). (a) The water–water cycle. (b) The ascorbate–glutathione cycle. (c) The glutathione peroxidase (GPX) cycle. (d) Catalase (CAT). Abbreviations: DHA, dehydroascorbate; DHAR, DHA reductase; Fd, ferredoxin; GR, glutathione reductase; GSSG, oxidized glutathione; MDA, monodehydroascorbate; MDAR, MDA reductase; PSI, photosystem I; tAPX, thylakoid-bound APX; SOD, superoxide dismutase.

In principle, superoxide dismutase (SOD) acts as the first step of defense by converting O_2^- into H_2O_2. H_2O_2 is reduced to H_2O by ascorbate peroxidase (APX) activity. In this reaction ascorbic acid is used as an electron donor while monodehydroascorbate (MDA) and dehydroascorbate (DHA) are products of the reaction. The regeneration of reduced ascorbate (AsA) from MDA or DHA can be catalyzed either by NADH-dependent monodehydroascorbate reductase (MDAR), or GSH dependent dehydroascorbate reductase (DHAR) coupled with glutathione reductase (GR) activity. Glutathione (GSSG) that was oxidized during the regeneration of ascorbic acid is again converted to the reduced form (GSH) through GR activity by a NADPH-dependent reaction (Cakmak, 1994; Foyer et. al., 1994) (Fig 2.3).

The balance between SOD and APX or CAT activities in cells is critical to keep a low level of superoxide radicals and hydrogen peroxide (Bowler, 1991). This balance is important to prevent the formation of highly toxic hydroxyl radical (OH^-) from O_2^- via the metal-dependent Haber–Weiss or the Fenton reactions (Asada and Takahashi, 1987). The finding of the ascorbate–glutathione cycle in almost all cellular compartments and the high affinity of APX for H_2O_2, suggests that this cycle plays a significant role in controlling the level of ROS in cell. On the other hand, CAT is only present in peroxisomes, but it is obligatory for ROS detoxification during stress, when high levels of ROS are produced in peroxisomes (Willekens et al., 1997).

A fundamental role of AsA (Vitamin C) in the plant defense system is to protect metabolic processes against H_2O_2 toxicity through its contribution to the activity of APX. AsA also can react non-enzymatically with superoxide and singlet oxygen. It can also function indirectly in regeneration α-tocopherol (Vitamin E) or in the synthesis of zeaxanthin in the xanthophyll cycle. Therefore, AsA influences many antioxidative processes directly or indirectly in plants (Shao et al., 2006; Shao et al., 2005 ; Li and Jin, 2007 and Shao et al., 2008).

α -tocopherols (vitamin E) are lipophilic antioxidants synthesized by all plants. α -tocopherols interact with the polyunsaturated acyl groups of lipids, stabilize membranes, and scavenge various reactive oxygen species (ROS) and lipid soluble by-products of oxidative stress (Cvetkovska et al., 2005). Scavenging of singlet oxygen by tocopherols is highly efficient mechanism (Wu and Tang, 2004)

As indicated above, glutathione takes part in the control of H_2O_2 levels (Shao et al., 2007). Additionally, glutathione is also used in the protection against heavy metal toxicity and certain exogenous and endogenous organic chemicals via participating in phytochelatin synthesis. Phytochelatins are responsible from heavy metal detoxification (Foyer and Noctor, 2005).

As discussed below, recent studies show that Se may also contribute to some of these defense mechanisms in plant cells.

2.5.3.2 Defensive Role of Selenium under Abiotic Stress Conditions

Selenium is an essential micronutrient for human and animals. According to current knowledge, higher plants do not need Se. However, recent findings indicate that despite its toxicity in high concentrations, it can be beneficial for plants at low concentrations. It may promote the antioxidant defense system by modifying the activities of antioxidant enzymes and reducing the detrimental effects of oxidative damage (Xue et. al, 2001). Hence, Se may be an antioxidative factor, which elevates defense capacity of plants, and therefore improving Se status of plants may be an approach to enhance plant tolerance to environmental stresses.

GPX is one of the most important defensive enzymes in eukaryotic systems other than plants. This enzyme catalyzes the reduction of organic peroxides and hydrogen peroxide to protect cells from ROS damage. In organisms with Se requirements, a SeCys residue in the catalytic site of this enzyme is necessary for proper functioning (Stadtman, 1990). As mentioned in 2.2.1, a specific SeCys-charged $tRNA^{ser/sec}$ which recognizes a UGA codon in combination with a SeCys insertion sequence (SECIS) provides the incorporation of SeCys residue into selenoproteins. Although Se containing enzymes such as GPX (Fu et al., 2002) and selenocysteyl-tRNAs (Novoselov et al., 2002) have been recognized in the single celled green alga *Chlamydomonas reinhardtii*, molecular evidence for SeCys incorporation in higher plants has not been found. GPX encoding gene isolation from several plant species has demonstrated that all the isolates contain Cys insted of SeCys residues in their catalytic sites (Terry et al., 2000). Interestingly, a plastidic cysteine desulfurase enzyme, which catalyzes an intermediate step in the synthesis of selenocysteyl-tRNA in *A. thaliana*, indicates more affinity for SeCys than Cys (Pilon-Smits et al., 2002). Additionally, a selenocysteyl-tRNA has been

identified in sugar beet (Hatfield et al., 1992). These results provide some evidence about the function and existence of selenoproteins in higher plants.

According to some recent studies, low levels of Se can promote plant growth and alleviate oxidative stress. For instance, applying 0.1 mg Se per kg soil Se can stimulate the growth of senescing lettuce seedlings by 14% (Xue et al., 2001). In the same study, Xue et al. (2001) also revealed that 1.0 mg kg^{-1} soil Se was toxic and reduced the yield of young plants. In another study, foliar Se application to soybean also promoted plant growth during senescence (Djanaguiraman et al., 2005). In both studies, Se application was associated with increased superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities. Selenium seems to activate the protective mechanisms against oxidative stress. In the senescing plants, increase in growth by Se may be explained by enhanced glutathione peroxidase (GPX) activity that inhibits lipid peroxidation and the percent injury of cell membranes. In a similar study conducted with ryegrass (*Lolium perenne*), the antioxidative effect of Se was associated with an increased activity of glutathione peroxidase (GPX), but superoxide dismutase (SOD) activity was not affected (Hartikainen et al., 2000). It is important to underline that the reported positive effects of Se on plant growth are rather very little and biological significance of these increases is a discussing matter.

In a study conducted with sorrel (*Rumex patientia* × *R. tianshanicus*) seedlings under varied salt treatments, low concentrations (1–5 μM) of Se improved the growth, the activities of superoxide dismutase and peroxidase enzymes and additionally the accumulation of water-soluble sugar in sorrel leaves (Kong et al., 2005). In this experiment, CAT activity was not altered by Se addition (1–30 μM). With 5 μM Se treatment the membrane damage was reduced (Kong et al., 2005).

In a study conducted with potato plant (*Solanum tuberosum* L.), treatments of plants witth Se improved tolerance to paraquat damage (an herbicide causing O_2^- formation in chloroplasts), probably due to increased activity of SOD (Seppänen et al. 2003). Moreover, Se supplementation improved the recovery of chlorophyll content under high light intensity stress. Increases in GPX activity and chloroplast-CuZnSOD transcripts were further explanation for elevated expression levels of antioxidative enzymes as a result of the Se treatment. These results with potato suggest that Se treatment can counteract the effects of oxidative stresses caused by light intensity and herbicide treatment in potato (Seppänen et al., 2003).

In this MSc thesis, experiments have been conducted with wheat and maize plants to evaluate role of Se in improving plant growth under different abiotic stress factors such as low temperature and drought stress by paying special attention to antioxidative defense mechanisms. Additionally, further experiments were established to collect information about the effectiveness of seed treatment with Se in increasing shoot and grain Se concentration of wheat. As indicated above, enrichment of cereals with Se is a high priority research area and will greatly contribute to human health. In the present study, by using various genotypes of wild tetraploid wheat *Triticum dicoccoides*, primitive (less-cultivated) wheat *Triticum spelta* and modern wheats screening studies have been realized to identify new genotypes showing high genetic capacity for root uptake and shoot accumulation of Se.

3 MATERIAL AND METHODS

3.1 Greenhouse Experiments

In the experiments various plant materials have been used, and these plant materials have been described below for each experiment.

Greenhouse experiments were conducted under natural light conditions. The soil used in the experiments was obtained from the Central Anatolia region, and has clay texture and high alkalinity (pH: 8.0). This soil is low in plant available Zn concentration (approximately 0.1 mg extractable Zn kg^{-1}), rich in $CaCO_3$ (14.9 %) and poor in organic matter content (0.69 %). For further properties of this soil see Cakmak et al.,(1998).

In the experiments, a basal fertilizer treatment was applied principally which consists of 200 mg N kg^{-1} soil as $Ca(NO_3)_2$, 100 mg P kg^{-1} soil as KH_2PO_4, 125 mg K kg^{-1} soil as KH_2PO_4, 25 mg S kg^{-1} soil as $CaSO_4.2H_2O$, and 2.5 mg F kg^{-1} soil as FeEDTA ($C_{10}H_{12}FeN_2NaO_8$) and 2.0 mg Zn kg^{-1} soil as $ZnSO_4.7H_2O$. These fertilizers were applied to soil by mixing thoroughly. In the experiment which was continued until seed formation (3.1.1), additionally 100 mg N kg^{-1} as $Ca(NO_3)_2$ has been applied to soil. Selenium was applied in the form of Na_2SeO_4 (Sodium selenate) in all greenhouse experiments.

All harvested plant material at each experiment was dried at 70ºC for determination of shoot dry matter production and Se concentration. Only, seeds obtained from experiment 3.1.1 were not dried at 70 ºC conditions.

3.1.1 Selenium Seed Treatment Experiment

The bread wheat (*Triticum aestivum*) cultivar, Bezostaya has been used in most of the experiments. The seeds of this cultivar were treated by Se by soaking them in a Se-contaning solution at increasing amounts (e.g., 0, 1, 10, 50, 100, 500, 1000, 5000 µM

Se) for about 30 minutes. Then, the seeds treated by Se were dried out on a plastic stage at room temperature (25ºC). Nearly 15 seeds treated by Se were sown in plastic pots filled with 1700 g soil. In this experiment, basal fertilizers excluding 25 mg S kg^{-1} ($CaSO_4.2H_2O$) were applied to soil. Plants were irrigated once or twice a day by deionized water depending on demand, and the pots were randomized once in a week. After development of seedlings, number of seedlings in each pot was reduced to have 10 plants. Firstly, 5 plants were harvested from each pot after 23 days of growth and they were dried at 70 º C for determination of shoot dry matter production, and Se concentrations. Then, remaining 5 plants were grown until seed formation. At harvest seeds were collected for measurement of grain yield and grain Se concentrations.

3.1.2 Shoot Selenium Concentrations of Various Wheat Genotypes under Varied Soil Selenium Treatments

Effect of Se application on growth and shoot concentration of Se was examined in 21 wild tetraploid wheat and 9 modern wheat genotypes. Plants were grown under 0.05 and 0.5 mg Se kg^{-1} soil Se applications with 3 replicates. The genotypes tested are given below (Table 3.1):

Table 3.1 Wheat genotypes used in the greenhouse experiment.

Genotypes				
Triticum dicoccoides				Modern wheat cultivars
Amirim	24-39	Ma'ale Merar	MM 5/2	Svevo
Gitit	18-39	Ma'ale Merar	MM 5/4	Inbar
Gitit	18-60	Mt. Gilbboa	16-34	Meram
Givat Koach	33-8	Mt. Gilbboa	16-40	Zenit
Givat Koach	33-48	Rosh Pinna	9-72	Selçuklu
Givat Koach	33-58	Tabigha Bas.	13-B-89	Alpu 01
Kokhav Hashahar	19-1	Tabigha TR.	15-T-6	Çetinel 2000
Kokhav Hashahar	19-36	Yehudiya Sun	12-2	İzmir 85
Kokhav Hayarden	KH 5/1	Yehudiya Sun	12-3	Bezostaya
Kokhav Hayarden	KH 5/3	Yehudiya Sun	12-4	
Kokhav Hayarden	P 2/3			

Ten seeds were sown to the pots containing 1700 g soil. After development of seedlings, number of plants per pot was reduced to 6. In order to determine dry matter production and Se concentration of shoots, plants were harvested at the end of 33 days of growth period under greenhouse conditions.

3.1.3 Effect of Selenium on Plant Growth under Various Stress Conditions

3.1.3.1 Selenium Effect on Plant Growth under Salt, Drought, Flooding Stress

The bread wheat (*Triticum aestivum*) cultivar Bezostaya was used in the experiments. Twenty seeds were sown to each pot, containing 1700 g soil, which was treated with the basal fertilizers. After development of seedlings, number of seedlings in each pot was reduced to have 10 plants. Three groups of treatments were established with respect to the Se application rates as shown in the legends of the relevant figures and tables. Soil Se application group was realized at the beginning before seed sowing, while foliar Se treatment to plants was conducted before the start of the stress applications. Foliar application of Se was realized at the rate of 0.125g Na_2SeO_4 per liter.

Four groups of treatments with four replicates were established with respect to stress conditions: i) control,ii) salinity stress,iii) drought stress, and iv) flooding stress. Stress applications were started when plants were one week old. Salt stress was established by 3 times application of 1000 mg NaCl (sodium chloride) per kg soil with 2 days interval. Drought stress was created by daily irrigating pots with the water amount of 5 % of dry soil weight. These treatments with salt and drougt stress were chosen based on our previous experiencies under same grerenhouse condsitions. Flooding stress was created by keeping soils always saturated with water. In order to determine the dry matter production and Se concentration of shoot, 10 plants were harvested at the end of 24 days of growth.

3.1.3.2 Germination of Selenium-Enriched Seeds under Salt Stress

Seeds of bread wheat (*Triticum aestivum*) cultivar Bezostaya were treated with Se by soaking tem in solutions which had 0, 1, 10, 50, 100, 500, 1000, 5000 μM Se concentrations for about 1 day then, seeds were dried at room temperature and used in the experiment. Ten seeds were sown in pots containing 400 g experimental soil, which was treated with basal fertilizers, and increasing NaCl applications (0, 1000 and 3000 mg NaCl kg^{-1} soil). Germination ability of the Se enriched seeds under 3 salt treatments were studied after 14 days of growth period.

3.2 Growth Chamber Experiments

3.2.1 Effect of Selenium on Growth of Maize under Low Temperatures Stress

The effect of Se application on growth of maize plants under low temperature stress was studied in nutrient solution under growth chamber conditions. Firstly, seeds of maize (*Zea mays*) genotype Şimal were germinated in a perlite containing saturated $CaSO_4$ solution in dark for 4 days at 25ºC. Germinated seeds were transferred into 2.8 L black plastic pots containing continuously aerated nutrient solution. The nutrient solution consisted of 2mM $Ca(NO_3)_2$, 1mM $MgSO_4.7H_2O$, 2mM K_2SO_4, 0.2mM KH_2PO_4, 10^{-6}M H_3BO_3, 10^{-6} M $MnSO_4.H_2O$, 10^{-6} M $ZnSO_4.7H_2O$, $2x10^{-7}$ M $CuSO_4.5H_2O$, $2x10^{-8}$M $(NH_4)_6Mo_7O_{24}.4H_2O$ and 10^{-4}M FeEDTA ($C_{10}H_{12}FeN_2NaO_8$). Plants were grown under controlled growth chamber conditions (light/dark regime: 16/8 h at 26-24ºC, relative humidity: 60-70 %, photon flux density: 700 μE m^{-2} s^{-1}). Nutrient solution in pots was renewed every 2 days. After plants were grown for 3 days in nutrient solution, Se applications (each group consisted of 4 replicates) started, as indicated in the legends of the relevant tables and figures.

Different temperature treatments (control and low temperature) were started two days after the beginning of Se applications. The control plants were grown at 26-24ºC, and the plants exposed to low temperature were grown at 18-16 ºC for 11days before the harvest. Each experiment has been replicated 4 times. Roots and shoots of 3 plants from each pot were harvested separately and dried at 70 ºC for determination of dry

matter production and Se concentrations. The shoot of remaining 1 plant was also used for measurement of antioxidative enzyme activities. Harvested leaf samples were treated with liquid nitrogen and stored at -80 ºC until enzyme analysis.

3.2.1.1 Determination of Soluble Protein Content

1 g of fresh leaf samples was homogenized in 5 ml of ice-cold 50 mM phosphate extraction buffer (pH 7.6) containing 0.1 mM Na-EDTA by using a mortar and kuvartz sand. The homogenized samples were first centrifuged at 4600 *g* for 15 min. Collected supernatant was centrifuged again at 15000 *g* for 15 min. Final supernatant was used for Bradford assay and enzyme analysis. All operations until analysis were carried out at +4 ºC.

Bradford assay was applied to determine total protein content of shoots by using bovine serum albumin as a standard (Bradford, 1976). The Bradford assay reagent was prepared as follows: 100 mg coomassie brillant blue G 250 was dissolved in 50 ml ethyl alcohol (99.5 %) and added with 100 ml of 85 % *ortho*-phosphoric acid. The mixture was filled up to 600 ml with deionised water and filtered. After filtration, 100 ml of glycerol (about 87 %) was added and completed to 1000 ml with deionised water. The reagent prepared was used for protein assay after 24 hours. For protein assay, 100 µl shoot sample solution and 5 ml Bradford reagent were mixed. After vortexing the mixture, the occurred color was measured at 595 nm versus albumin standards, which were prepared in the range of 0 to 800 µg ml^{-1}.

3.2.1.2 Ascorbate Peroxiase Activity

Ascorbate peroxidase (APX) activity was measured by monitoring the decrease in absorbance of ascorbic acid at 290 nm (extinction coefficient 2.8 mM cm^{-1}) in a 1 ml reaction mixture containing 50 mM phosphate buffer (pH 7.6), 0.1 mM Na-EDTA, 12 mM H_2O_2, 0.25 mM ascorbic acid and the sample extract as described by Cakmak, (1994).

3.2.1.3 Glutathione Reductase Activity

Activity of glutathione reductase (GR) was measured by following the oxidation of NADPH at 340 nm (extinction coefficent 6.2 mM cm^{-1}) in a 1 ml reaction mixture, containing 50 mM phosphate buffer (pH 7.6), 0.1 mM Na-EDTA, 0.5 mM oxidized glutathione (GSSG), 0.12 mM NADPH, and the sample extract as described by Cakmak and Marschner (1992).

3.2.1.4 Superoxide Dismutase Activity

Activity of superoxide dismutase (SOD) was assayed by a photochemical method which is based on a SOD-inhibiting reduction of nitro blue tetrazolium chloride (NBT) by superoxide radicals in a 5 ml reaction mixture, containing 50 mM phosphate buffer (pH 7.6), 0.1 mM Na-EDTA, sample extracts (50-150 µl), 50 mM Na_2CO_3 (pH 10.2), 12mM L-methionine, 75 µM p-nitro blue tetrazolium chloride (NBT) and 2µM riboflavin, respectively. The reaction occured in glass vials under intensive light (700 µE m^{-2} s^{-1}) during 15 min. The amount of enzyme extract that brought about a 50 % decrease in the SOD-inhibitable NBT reduction was defined as 1 unit, at 560 nm (Cakmak and Marschner, 1992).

3.2.1.5 Catalase Activity

Catalase (CAT) activity was measured by monitoring the decrease in the H_2O_2 absorbance at 240 nm (extinction coefficient 39.4 mM cm^{-1}). The reaction medium (1 ml) contained 50 mM phosphate buffer (pH 7.6), 0.1 mM Na-EDTA, 100 mM H_2O_2 and the enzyme extract (Cakmak and Marschner, 1992).

3.2.2 Root Uptake and Shoot Accumulation of Selenium in Various Wheat Genotypes in Nutrient Solution

Root uptake of Se was measured in 9 *Triticum spelta* genotypes which numbered as *T. spelta* 207, 247, 38, 19, 31, 94, 33, 2, 34; 2 modern wheat cultivars (Balcalı 2000, Bezostaya) and 1 *Triticum dicoccoides* (Kokhav Hashahar19-1) in nutrient solution.

The seeds of the selected genotypes were germinated in perlite moistened with saturated $CaSO_4$ solution in dark for 5 days at 25ºC. Germinated seeds were transferred into 2.8 L black plastic pots containing continuously aerated nutrient solution for growth. The composition of the solution was described above under 3.2.1. Plants were grown under growth chamber conditions (light/dark regime: 16/8 h at 25/22ºC, relative humidity: 60-70 %, photon flux density: 700 µE m^{-2} s^{-1}) for 14 days. For the Se uptake experiment, plants were transferred to a nutrient solution, which merely consists of 2µM Na_2SeO_4 ve 0.5 mM $CaCl_2$ for 12 hours. During the Se uptake process, nutrient solution had been sampled at different time intervals to measure depletion of Se in nutrient solution. Finally, plants were harvested after 12 hours of Se absorption period.

3.3 Assay of DPPH (Diphenyl Picrylhydrazyl) Radical Scavenging Activity

By using DPPH ((Diphenyl Picrylhydrazyl) method, the total antioxidant capacity of selected wheat samples differing in Se concentrations has been measured (Sanchez-Moreno, 1999). The principle of the assay is based on the reduction of DPPH radicals by antioxidants. The wheat extracts were prepared from the grains of the wheat plants treated with foliar Se applications under field conditions in Eskişehir. The variation in Se concentration of the grain samples were presented in the legends of relevant figure.

For each sample, 0.5 g of ground wheat was extracted with 100 ml distilled water at 65ºC for 4 hours. Then the extract was filtered. The filtrate was evaporated to dryness in vacuum lyophilizator and kept frozen for the assay. 0.05 g of the dry frozen sample was solubilized in 50 ml distilled water, and 1 ml of extract solution was added to 3 ml of 5. 10^{-5}M DMSO (Dimethyl sulfoxide) solution of DPPH in a cuvette and the absorbance measurements started immediately. Same procedure was applied for the

control group and the reference antioxidant molecules (e.g. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid: Trolox) and ascorbic acid: Vitamin C). The decrease in absorbance was measured at 517 nm, continuously at every 15 min. intervals by using a spectrophotometer (Varian, Cary 300 BIO, UV Visible).

3.4 Selenium Analysis in Plant Samples

The dried and ground plant samples were prepared for Se determination by wet digestion in a microwave oven with 5 ml concentrated HNO_3 and 2 ml 30% H_2O_2 by using a digesting programs, which have been developed for the plant samples. After digestion, the total volume was completed up to 20 ml, and Se concentration of the samples were measured by atomic absorption spectroscopy (Varian, Cary 300 BIO, UV Visible) equipped with VGA 77 (vapor generation accessories) and ETC-60 (elektro termal temperature controller).

All Se measurement in plant materials were checked against certificated Se values in different reference plant materials obtained from the National Institute of Standards and Technology (Gaithersburg, USA). The Se content (total amount of Se per plant or per shoot) was calculated by multiplying the dry weight values of roots or shoots with their Se concentration values.

The concentration of Se in the collected solution samples of the Se uptake experiment (3.2.2) was measured by ICP-OES (inductively coupled plasma optical emission spectroscopy) (Varian, Cary 300 BIO, UV Visible, AUSTRALIA). The results of the Se uptake experiment were presented as Se uptake rate (μmol Se g^{-1} root DW h^{-1}).

4 RESULTS

4.1 Effect of Seed Selenium Treatments on Growth and Plant Selenium Accumulation

Soaking seeds in Se containing solution up to 5000 µM did not result in any toxicity effect on germination or growth of seedlings (Table 4.1). The germination ability and seedling development were not affected by increasing Se concentration from 0 to 5000 µM (Table 4.1). These results indicate that 5000 µM Se containing solutions can be used for enrichment of plants (seeds) with Se without causing any toxicity symptoms and decrease in dry matter production. As expected, treatment of seeds by increasing Se concentrations also increased shoot Se concentration of seedlings (Table 4.1). As shown in Table 4.1, application of Se up to 50 µM remained ineffective in increasing shoot Se concentration. Following the 100 µM Se treatments, there was a progressive increase in shoot Se concentration (Table 4.1). By increasing Se treatment from 100 µM to 5000 µM, shoot Se concentration was increased nearly by 55 fold (e.g. form 139 µg Se kg^{-1} to 7837 µg Se kg^{-1}).

Table 4.1 Germination percentage, shoot dry matter production and shoot Se concentration of 23 days old bread wheat plants (cultivar: Bezostaya) derived from seeds which were treated by soaking seeds in a solution containing increasing concentrations of Se (from 0 to 5000 µM) for 30 min.

Se seed treatment (µM)	Germination Percentage (%)	Dry matter production (mg $plant^{-1}$)	Shoot Se concentration (µg kg^{-1} DW)
0	100	226 ± 12	98 ± 8
1	100	207 ± 13	101 ± 14
10	99	226 ± 8	91 ± 7
50	99	226 ± 11	103 ± 9
100	98	226 ± 12	139 ± 16
250	99	215 ± 15	238 ± 30
500	100	208 ± 4	518 ± 88
1000	98	214 ± 6	1595 ± 210
5000	100	199 ± 10	7837 ± 1355

Part of the plants described in Table 4.1 has been grown until seed formation in order to measure biomass production and seed yield under given conditions. As found, at early growth stage (Table 4.1), soaking seeds in solutions containing up to 5000 µM Se also did not effect shoot biomass production and seed yield at maturation (Table 4.2). At the highest Se treatment, seed yield tended to decrease, but based on ±SD values of the results it can be concluded that the decrease in seed yield at 5000 µM Se treatment seems to be statistically insignificant (Table 4.2).

Table 4.2 Shoot dry matter production and seed yield of bread wheat Bezostaya at maturation, as affected from the seed Se treatments before sowing. Seed treatment with Se has been realized by soaking seeds in solutions containing increasing Se concentrations (from 0 to 5000 µM) for 30 min.

Se seed treatment	Total matter production	Dry matter production	Seed production
(µM)		(g plant^{-1})	
0	4.26 ± 0.29	2.84 ± 0.32	1.42 ± 0.13
1	4.26 ± 0.28	2.87 ± 0.19	1.39 ± 0.10
10	4.47 ± 0.29	3.11 ± 0.10	1.35 ± 0.25
50	4.72 ± 0.26	3.31 ± 0.17	1.41 ± 0.19
100	4.52 ± 0.29	3.17 ± 0.24	1.34 ± 0.18
250	4.91 ± 0.36	3.47 ± 0.14	1.44 ± 0.30
500	4.81 ± 0.41	3.27 ± 0.42	1.54 ± 0.23
1000	4.66 ± 0.11	3.13 ± 0.14	1.53 ± 0.24
5000	4.45 ± 0.66	3.16 ± 0.44	1.28 ± 0.33

Increases in seed Se concentration by soaking seeds in Se containing solution were found at the Se concentrations higher than 100 µM (Table 4.3). At the 1000 µM and 5000 µM Se treatments seed Se concentrations were nearly 100 µg Se kg^{-1} and 200 µg Se kg^{-1}, respectively. These seed Se concentrations area very close or higher than the lowest optimum Se concentrations of wheat seeds required for better human nutrition with Se (e.g., 100 µg Se kg^{-1} seed).

Table 4.3 Seed Se concentration of bread wheat Bezostaya at maturation, as affected from seed Se treatments before sowing. Seed treatment with Se has been realized by soaking seeds in solutions containing increasing Se concentrations (from 0 to 5000 μM) for 30 min.

Se seed treatment (μM)	Seed Se concentration (μg kg^{-1})
0	44 ± 3
1	44 ± 1
10	49 ± 4
50	50 ± 2
100	63 ± 7
250	71 ± 3
500	82 ± 17
1000	91 ± 8
5000	216 ± 65

4.2 Selenium Uptake and Accumulation of Various Wheat Genotypes

4.2.1 Selenium Accumulation in Various Wheat Genotypes under Varying Soil Selenium Treatments

Plant growth and Se uptake of modern wheat cultivars (*Triticum durum* ve *Triticum aestivum*) and tetraploid wild wheat *Triticum dicoccoides* genotypes were investigated under two different soil Se treatments (Table 4.4). The aim of this experiment was to identify modern and wild wheat genotypes having high genetic capacity for Se uptake and accumulation. When compared to the 0.05 mg Se kg^{-1}soil treatment, the Se treatment of 0.5 mg Se kg^{-1}soil tended to decrease shoot growth of the wheat genotypes tested. Based on the mean values this decrease was pronounced more in the case of modern wheat genotypes (Table 4.4).

Table 4.4 Shoot dry matter production of 21 tetraploid wild wheat genotypes (*Triticum dicoccoides*) and 9 modern wheat cultivars grown at 0.05 and 0.5 mg Se kg^{-1} soil treatments for 33 days under greenhouse conditions. The data represents for mean ± SD of three independent replications.

Genotypes		Dry matter production (mg $plant^{-1}$)	
Triticum dicoccoides		0.05 mg Se kg^{-1}	0.5 mg Se kg^{-1}
Amirim	24-39	439 ± 38	436 ± 77
Gitit	18-39	366 ± 11	387 ± 21
Gitit	18-60	444 ± 21	445 ± 46
Givat Koach	33-8	348 ± 65	286 ± 27
Givat Koach	33-48	348 ± 33	359 ± 17
Givat Koach	33-58	426 ± 25	441 ± 62
Kokhav Hashahar	19-1	304 ± 13	264 ± 20
Kokhav Hashahar	19-36	300 ± 28	331 ± 79
Kokhav Hayarden	KH 5/1	332 ± 16	351 ± 49
Kokhav Hayarden	KH 5/3	396 ± 13	389 ± 7
Kokhav Hayarden	P 2/3	390 ± 20	402 ± 46
Ma'ale Merar	MM 5/2	417 ± 26	413 ± 63
Ma'ale Merar	MM 5/4	428 ± 21	407 ± 25
Mt. Gilbboa	16-34	436 ± 39	424 ± 46
Mt. Gilbboa	16-40	420 ± 27	429 ± 45
Rosh Pinna	9-72	468 ± 31	489 ± 14
Tabigha Bas.	13-B-89	340 ± 31	226 ± 68
Tabigha TR.	15-T-6	450 ± 9	395 ± 26
Yehudiya Sun	12-2	465 ± 55	328 ± 35
Yehudiya Sun	12-3	430 ± 31	365 ± 112
Yehudiya Sun	12-4	487 ± 39	482 ± 115
Mean		402 ± 28	383 ± 48
Modern wheat cultivars			
Svevo		453 ± 14	412 ± 43
Inbar		514 ± 18	462 ± 19
Meram		531 ± 7	463 ± 30
Zenit		441 ± 13	374 ± 17
Selçuklu		430 ± 32	315 ± 43
Alpu 01		526 ± 1	471 ± 20
Çetinel 2000		531 ± 58	453 ± 45
İzmir 85		501 ± 14	403 ± 9
Bezostaya		540 ± 12	520 ± 19
Mean		496 ± 19	430 ± 27
General mean		449 ± 23	407 ± 37

Modern and wild wheat genotypes were not clearly different in their shoot concentrations of Se under each Se treatment (Table 4.5). There was however, a genotypic variation in shoot Se concentrations, especially in the case of *Triticum dicoccoides* genotypes. At the lowest Se application, shoot Se concentrations varied from 3.61 to 6.15 mg Se kg^{-1} dry weight (Table 4.5). A similar genotypic variation was also found at the highest Se application (e.g., from 53 to 86 mg Se kg^{-1} dry weight). In the case of modern wheat cultivars, genotypic variation in shoot Se concentration was minimal. For example, at the highest Se application, shoot Se concentration of the genotypes showed a 1.6 fold variation among the *Triticum dicoccoides* genotypes and only 1.1 fold among modern wheat cultivars (Table 4.5). These results indicate existence of a useful genetic variation in shoot Se accumulation among the *Triticum dicoccoides* genotypes that can be exploited in breeding programs. Among the genotypes tested, the *Triticum dicoccoides* lines 19-1, 9-72 and 13-B-89 seem to be promising.

Like in shoot Se concentration *Triticum dicoccoides* genotypes also showed nearly 2-fold variation in grain Se concentration, (Table 4.6). The grain Se concentration of *Triticum dicoccoides* genotypes ranged between 66 to 113 μg kg^{-1} to 113 μg kg^{-1} with an average value of around 87.2 μg kg^{-1}. In the case of modern wheat cultivars, grain Se concentration was around 46 μg kg^{-1} indicating that Triticum *dicoccoides* genotypes have higher capacity to accumulate more Se than the modern wheat cultivars (Table 4.6). However, due to limited number of modern wheat cultivars such a comparison may not be reliable. According to Table 4.6, the *Triticum dicoccoides* genotypes 18-39, 33-48, 5-1, 5-3, and 19-1 seem to be promising genetic sources for high Se trait in grain, and the genotype 19-1 had also high shoot Se concentration (Table 4.5).

Table 4.5 Shoot Se concentration of 21 tetraploid wild wheat genotypes (*Triticum dicoccoides*) and 9 modern wheat cultivars grown at 0.05 and 0.5 mg Se kg^{-1} soil treatments for 33 days under greenhouse conditions. The data represents for mean ± SD of three independent replications.

Genotypes		0.05 mg Se kg^{-1}	0.5 mg Se kg^{-1}
Triticum dicoccoides		Shoot Se concentration (mg kg^{-1} DW)	
Amirim	24-39	4.26 ± 0.15	53 ± 4
Gitit	18-39	4.49 ± 0.47	61 ± 1
Gitit	18-60	4.43 ± 0.26	63 ± 1
Givat Koach	33-8	5.40 ± 0.40	71 ± 2
Givat Koach	33-48	5.47 ± 0.45	70 ± 1
Givat Koach	33-58	4.41 ± 0.63	66 ± 2
Kokhav Hashahar	19-1	6.15 ± 0.49	86 ± 2
Kokhav Hashahar	19-36	4.61 ± 0.32	68 ± 1
Kokhav Hayarden	KH 5/1	5.22 ± 0.24	76 ± 2
Kokhav Hayarden	KH 5/3	4.72 ± 0.48	73 ± 2
Kokhav Hayarden	P 2/3	4.91 ± 0.17	83 ± 8
Ma'ale Merar	MM 5/2	3.64 ± 0.81	70 ± 3
Ma'ale Merar	MM 5/4	4.15 ± 0.46	71 ± 7
Mt. Gilbboa	16-34	4.95 ± 0.23	73 ± 4
Mt. Gilbboa	16-40	4.85 ± 0.58	72 ± 2
Rosh Pinna	9-72	5.68 ± 0.58	80 ± 4
Tabigha Bas.	13-B-89	5.41 ± 0.54	85 ± 2
Tabigha TR.	15-T-6	5.08 ± 0.46	84 ± 17
Yehudiya Sun	12-2	4.03 ± 0.25	73 ± 4
Yehudiya Sun	12-3	4.16 ± 0.07	79 ± 4
Yehudiya Sun	12-4	3.61 ± 0.51	71 ± 1
Mean		4.74 ± 0.18	73 ± 4
Modern wheat cultivars			
Svevo		3.92 ± 0.26	88 ± 1
Inbar		3.75 ± 0.24	83 ± 3
Meram		3.56 ± 0.35	82 ± 1
Zenit		3.70 ± 0.17	87 ± 7
Selçuklu		4.76 ± 0.29	93 ± 4
Alpu 01		4.75 ± 0.12	89 ± 1
Çetinel 2000		4.77 ± 0.20	89 ± 2
İzmir 85		4.68 ± 0.07	88 ± 1
Bezostaya		4.79 ± 0.32	86 ± 4
Mean		4.30 ± 0.09	87 ± 2

Table 4.6 Grain Se concentration and content of 21 tetraploid wild wheat genotypes (*Triticum dicoccoides*) and 2 modern wheat cultivars. The data represents for mean ± SD of three independent replications.

		Concentration	Content
		Se	Se
Triticum dicoccoides genotypes		($\mu g\ kg^{-1}$)	($ng\ tane^{-1}$)
Amirim	24-39	91.1 ± 0.3	4.5 ± 0.2
Gitit	18-39	112.7 ± 1.0	4.9 ± 0.0
Gitit	18-60	74.5 ± 0.9	3.4 ± 0.0
Givat Koach	33-8	80.3 ± 11.2	1.8 ± 0.2
Givat Koach	33-48	113.0 ± 12.0	1.5 ± 0.4
Givat Koach	33-58	92.1 ± 2.2	2.4 ± 0.0
Kokhav Hashahar	19-1	104.1 ± 5.4	4.4 ± 0.1
Kokhav Hashahar	19-36	75.4 ± 3.8	2.2 ± 0.2
Kokhav Hayarden	KH 5/1	109.1 ± 22.3	3.3 ± 0.7
Kokhav Hayarden	KH 5/3	108.6 ± 8.0	4.6 ± 0.3
Kokhav Hayarden	P 2/3	87.5 ± 5.5	3.2 ± 0.0
Ma'ale Merar	MM 5/2	74.1 ± 6.3	3.5 ± 0.2
Ma'ale Merar	MM 5/4	89.2 ± 0.7	4.2 ± 0.1
Mt. Gilbboa	16-34	74.2 ± 3.4	3.3 ± 0.1
Mt. Gilbboa	16-40	70.9 ± 4.1	3.6 ± 0.3
Rosh Pinna	9-72	66.6 ± 4.1	2.8 ± 0.2
Tabigha Bas	13-B-89	79.2 ± 4.3	2.9 ± 0.1
Tabigha TR	15-T-6	95.9 ± 9.1	3.6 ± 0.4
Yehudiya Sun	12-2	86.9 ± 2.6	4.3 ± 0.2
Yehudiya Sun	12-3	71.6 ± 7.0	3.6 ± 0.3
Yehudiya Sun	12-4	74.9 ± 5.7	3.4 ± 0.4
Mean		87.2 ± 5.0	3.4 ± 0.2
Modern wheat cultivars			
Svevo		43.1 ± 0.3	2.3 ± 0.1
Inbar		48.0 ± 1.6	2.2 ± 0.2
Mean		46 ± 1	2.2 ± 0.07

4.2.2 Selenium Uptake and Accumulation in Various Wheat Genotypes Grown in Nutrient Solution

Selenium uptake capacity of 2 modern wheat cultivars, 1 *Triticum dicoccoides,* and 9 *Triticum spelta* genotypes has been studied in a growth chamber experiment by growing plants in nutrient solution culture. The *Triticum dicoccoides* genotype tested was the genotype, which showed highest Se accumulation in shoot in the greenhouse experiment described in Table 4.5. In this greenhouse experiment *Triticum dicoccoides* genotypes were not greatly different in their shoot Se concentrations when compared to the modern wheat cultivars (Table 4.5). Therefore, in the nutrient solution experiment *Triticum spelta* genotypes were considered in order to study both genotypic variation in the Se uptake and to compare their Se uptake capacity with those of modern wheat cultivars. *Triticum spelta* is a traditional European cereal species and ancient wheat. Due to its high nutritious content, in recent years an increasing attention is being paid to this wheat. The *Triticum spelta* genotypes tested in this study were selected from a *Triticum spelta* germplasm containing 766 genotypes, which are being used in the PhD thesis of Halil Erdem under a joint program between Sabanci University and Cukurova University.

Generally, *Triticum spelta* genotypes showed higher Se uptake rate when compared to modern wheat cultivars and the *Triticum dicoccoides* genotype (Table 4.7). It is important to notice that for a reliable comparison the number of modern wheat and *Triticum dicoccoides* genotypes should have been similar to the number of spelta genotypes tested. The important question in this nutrient solution experiment was the magnitude of the genetic variation in root uptake of Se among the 9 spelta genotypes. As shown in Table 4.7, spelta genotypes differed markedly in their capacity for Se uptake by roots. The genotypic variation in Se uptake among the spelta genotypes was nearly 2 fold, ranging from 874 to 1795 nmol Se g^{-1} root DW h^{-1} within the first 4 h of the uptake experiment (Table 4.7). A similar genetic variation in the Se uptake was also found at the end of 12 h. The spelta genotype with the number 19 seems to be promising in terms of Se uptake. Due to the limited number of seeds, this experiment could not be repeated. When the number of seeds would be sufficient, this uptake experiment will be repeated and the seed Se concentrations will be measured.

Table 4.7 Se uptake of 14-days-old wheat genotypes in nutrient solution containing 2 µM Na_2SeO_4 and 0.5 µM $CaCl_2$. Nutrient solution samples were collected at 4th and 12th hours of the uptake experiment. Uptake rate is expressed as nmol Se g^{-1} root DW h^{-1}. The data represents for mean ± SD of four independent replications.

Genotypes	Uptake rate (nmol Se g^{-1} root DW h^{-1})	
Modern cultivars	4h	12h
Bezostaya	716 ± 74	519 ± 71
Balcalı 2000	951 ± 208	598 ± 74
mean	834 ± 95	558 ± 3
Triticum dicoccoides		
Kokhav Hashahar 19-1	1033 ± 156	686 ± 32
Triticum spelta		
Spelta 94	926 ± 154	591 ± 53
Spelta 207	893 ± 192	528 ± 68
Spelta 246	1079 ± 272	629 ± 50
Spelta 19	1795 ± 230	906 ± 89
Spelta 31	1593 ± 500	706 ± 153
Spelta 38	874 ± 183	511 ± 29
Spelta 33	1379 ± 161	691 ± 63
Spelta 2	1491 ± 202	690 ± 95
Spelta 34	1247 ± 368	928 ± 111
mean	1253 ± 114	687 ± 38

Dry matter production of 2 µM Se treated plants was represented in Table 4.8. In general, *Triticum spelt*a genotypes showed higher shoot and root dry matter production than the modern wheat cultivars. However, dry matter production of *Triticum spelt*a genotypes did not show a great variation when the ± SD values are considered (Table 4.8). The spelta genotype 31 showed the highest shoot matter production among all genotypes while the spelta genotype 38 had the highest root matter production. *Triticum dicoccoides* genotype, KH 19-1 produced the lowest root and shoot dry matter among all genotypes (Table 4.8). However, Se uptake of the genotype KH 19-1 seems to be higher than many other genotypes tested (Table 4.7).

Table 4.8 Dry matter production of 14-days-old wheat genotypes used in the Se uptake experiment. The data represents for mean ± SD of four independent replications.

Genotypes	Dry matter production (mg 3 plants^{-1}) Shoot	Root
Modern cultivars		
Bezostaya	628 ± 32	299 ± 39
Balcalı 2000	472 ± 62	234 ± 65
mean	550 ± 47	267 ± 52
Triticum dicoccoides		
Kokhav Hashahar 19-1	232 ± 52	156 ± 58
Triticum spelta		
Spelta33	753 ± 80	278 ± 41
Spelta 2	808 ± 80	289 ± 44
Spelta 34	766 ± 131	306 ± 63
Spelta 207	554 ± 62	340 ± 33
Spelta 246	603 ± 52	261 ± 59
Spelta 19	576 ± 140	215 ± 24
Spelta 31	860 ± 113	297 ± 65
Spelta 38	791 ± 17	328 ± 34
Speelta 94	724 ± 80	310 ± 51
mean	715 ± 84	292 ± 46

4.3 Effect of Selenium on Plant Growth and Antioxidative Enzyme Activities under Various Abiotic Stress Conditions

The effect of the Se treatment on growth of plants under various abiotic stress conditions was studied in three different experiments. Two experiments were realized under greenhouse with wheat and one experiment under growth chamber conditions with maize by using nutrient solution culture. In nutrient solution experiment, besides plant growth also changes in activities of antioxidative enzymes were studied under various Se treatments.

4.3.1 Effect of Selenium on Plant Growth under Drought, Salt and Flooding Stress

In this greenhouse experiment, the effects of both soil and foliar Se application of Se on plant growth were studied in wheat exposed to 3 stress situations: salinity, water deficiency, and flooding. As indicated in Table 4.9, foliar application of Se was realized at the rate of 0.125g Na_2SeO_4 per liter and soil application at the rate of 0.5 mg Se kg^{-1} soil. Salt, drought and flooding stresses were created by applying 1000 mg NaCl (sodium chloride) kg^{-1} soil (3 times with 2 days interval), irrigating pots with water amount of 5 % of dry soil weight and keeping soils always saturated with water, respectively.

As expected, irrespective of the Se application, 3 stress treatments resulted in very distinct decreases in shoot dry matter production, particularly in the case of drought stress condition (Table 4.9; Figure 4.1). The Se treatments caused a slight decrease on shoot dry matter production when compared to the control treatment. However, this adverse effect of Se on growth seems to be statistically insignificant when ±SD values are considered (Table 4.9). Table 4.9 and Figure 4.1 indicate that both type of Se treatments remained ineffective to alleviate stress related decreases in shoot growth.

Table 4.9 Effect of soil and foliar Se applications[a, b] on shoot dry matter production of 24-days-old bread wheat cultivar Bezostaya plants, which were grown under drought, salinity, and flooding stress treatments. Stress conditions were explained in the text and in more detail in 3.1.3.1.

	Foliar Se application[a]	Soil Se application[b]	Dry matter production (mg $plant^{-1}$)
Control	-	-	420 ± 28
Salt	-	-	188 ± 19
Drought	-	-	114 ± 9
Flooding	-	-	195 ± 17
Control	-	+	383 ± 36
Salt	-	+	236 ± 25
Drought	-	+	138 ± 32
Flooding	-	+	177 ± 20
Control	+	-	357 ± 32
Salt	+	-	186 ± 2
Drought	+	-	120 ± 38
Flooding	+	-	168 ± 14

[a] Foliar Se application was realized by spraying Na_2SeO_4 solution with a rate of 0.125g L^{-1} to leaves 3 times with 5 days of interval.

[b] Soil Se application was carried out by treatment of Na_2SeO_4 solution to soil at a rate of 0.5 mg Se kg^{-1} soil before seed sowing.

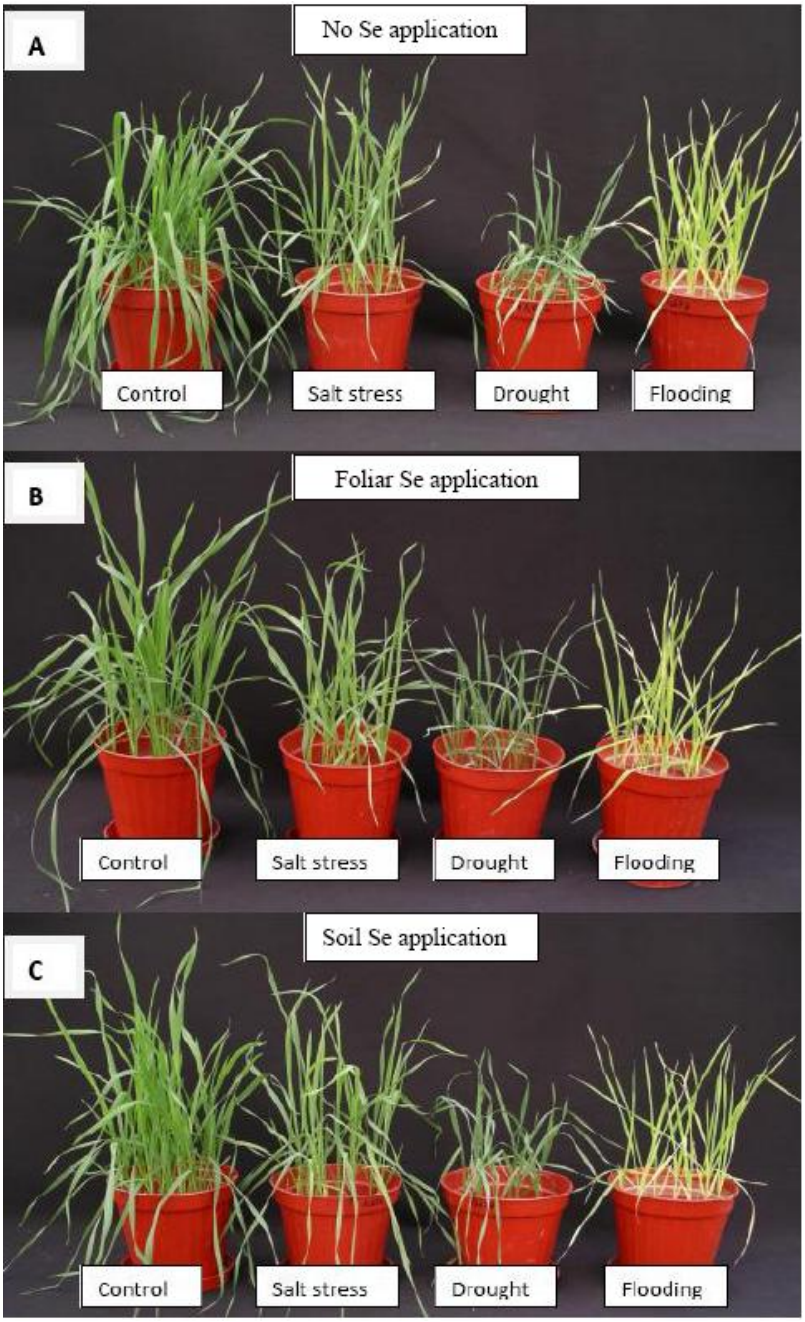

Figure 4.1 Shoot growth of 24 days old bread wheat cultivar Bezostaya plants, which were grown under drought, salinity, and flooding stress treatments. Detailed explanation for stress conditions is given in 3.1.3.1. A) Control (No Se application), B) Foliar Se application, and C) 0.5 ppm soil Se application. Foliar Se application was realized by spraying Na_2SeO_4 solution at a rate of 0.125g L^{-1} to leaves 3 times with 5 days of interval; soil Se application was carried out by treatment of Na_2SeO_4 solution to soil at a rate of 0.5 mg Se kg^{-1} soil before seed sowing. Stress treatments were initiated when the plants were 7 days old.

As expected, soil and foliar Se applications increased the shoot Se concentration of plants (Table 4.10). In contrast to other stress treatments, the flooding stress treatment resulted in a severe decrease in Se concentration of plants when Se applied to the soil. In the case of foliar Se application, the magnitude of the Se adhered to the leaf surface or existing in leaf apoplast is not clear. Therefore, the shoot Se concentration of the plants treated with foliar Se spray should be carefully interpreted.

Table 4.10 Effect of soil and foliar Se applications [a, b] on shoot Se concentration of 24-days-old bread wheat cultivar Bezostaya plants, which were grown under drought, salinity, and flooding stress treatments. Stress conditions were explained in the text and in more detail in 3.1.3.1. The data represent mean ± SD of four independent replications.

	Foliar Se application[a]	Soil Se application[b]	Shoot Se concentration (mg kg^{-1}DW)
Control	-	-	0.05 ± 0.01
Salt	-	-	0.05 ± 0.02
Drought	-	-	0.10 ± 0.03
Flooding	-	-	0.23 ± 0.08
Control	-	+	23 ± 2
Salt	-	+	32 ± 4
Drought	-	+	43 ± 2
Flooding	-	+	6 ± 1
Control	+	-	78 ± 16
Salt	+	-	69 ± 7
Drought	+	-	52 ± 5
Flooding	+	-	49 ± 3

[a] Foliar Se application was realized by spraying Na_2SeO_4 solution with a rate of 0.125g L^{-1} to leaves 3 times with 5 days of interval.

[b] Soil Se application was carried out by treatment of Na_2SeO_4 solution to soil at a rate of 0.5 mg Se kg^{-1} soil before seed sowing.

4.3.2 Germination and Growth of Se-enriched Seeds under Salt Stress

In the second experiment effect of salt stress was studied on germination and growth of plants derived from seeds enriched by increasing Se concentrations as

described in 4.1. Four group of seeds were used which have been soaked in solutions containing 0, 50, 500 and 5000 µM Se. The results in 4.11 shows that increasies in Se enrichment of seeds did not adversely effect on seed germination. However, increasing NaCl application reduced germination ability of seeds, especially at the 3000 ppm NaCl treatment.

Differences in seed Se did not result in a consistent effect on germination ability of seeds under various NaCl treatments (Table 4.11). Similarly, dry matter production of seedlings derived from seeds with differential Se treatments was not changed at each Se treatment (Table 4.12). These results indicate that variations in seed Se has no positive influence on salt stress-indıced decreases in growth of plants. As expected, dry matter production of seedlings was declined by increasing salt treatment.

Table 4.11 Effect of increasing NaCl concentrations (0, 1000, 3000 mg per kg soil) on germination of seeds which were soaked in solutions containing increasing amounts of Se (0 µM, 50 µM, 500 µM, 5000 µM) before sowing. The data represent mean ± SD of three independent replications.

	Germination percentage (%)		
Se seed treatment	NaCl (ppm)		
(µM)	0	1000	3000
0	100	97	73
50	97	100	83
500	100	97	83
5000	100	90	63

Table 4.12 Effect of increasing NaCl concentrations (0, 1000, 3000 mg per kg soil) on dry matter production of seeds which were soaked in solutions containing increasing amounts of Se (0 µM, 50 µM, 500 µM, 5000 µM) before sowing. The data represent mean ± SD of three independent replications.

Se seed treatment	Dry matter production (mg plant^{-1})		
(µM)	Control	1000 ppm NaCl	3000 ppm NaCl
0	28.15 ± 1.17	24.55 ± 1.99	10.68 ± 0.11
50	25.71 ± 0.48	25.74 ± 2.31	8.16 ± 1.45
500	27.40 ± 2.68	23.09 ± 3.29	10.52 ± 1.46
5000	27.63 ± 2.90	22.27 ± 1.11	9.62 ± 0.38

Increasing the Se concentration of seeds enhanced Se concentration of seedlings (Table 4.13). These increases in Se concentrations were less at higher NaCl treatments, possibly due to reduced root uptake and transport to shoot of Se.

Table 4.13 Effect of increasing NaCl concentrations (0, 1000, 3000 mg per kg soil) on shoot Se concentration of seeds which were soaked in solutions containing increasing amounts of Se (0 µM, 50 µM, 500 µM, 5000 µM) before sowing. The data represent mean ± SD of three independent replications.

Se seed treatment	Shoot Se concentration (mg kg^{-1}DW)		
(µM)	Control	1000 ppm NaCl	3000 ppm NaCl
0	0.08 ± 0.01	0.15 ± 0.08	0.25 ± 0.09
50	0.09 ± 0.02	0.09 ± 0.01	0.19 ± 0.06
500	0.24 ± 0.05	0.24 ± 0.01	0.35 ± 0.05
5000	4.46 ± 0.28	2.03 ± 0.77	2.80 ± 0.63

4.3.3 Effect of Selenium on Plant Growth and Antioxidative Enzyme Activities under Low Temperature Stress

4.3.3.1 Dry Matter Production and Selenium Concentration of Plants

In order to collect information on the effects of Se on plant growth under low temperature stress, maize plants (a cold sensitive crop) have been grown in a growth chamber with controlled climatic conditions. Plants were first grown at air temperature at 24 ºC (night) and 26 ºC (day) for 6 days. Then, part of the plants transferred to a second growth chamber with lower temperature, i.e., 16 ºC (night) and 18 ºC (day) for 5 days. The experimental plants were treated by increasing Se concentrations in nutrient solution (0 µM, 0.5 µM, 2.5 µM Se) and also sprayed with Se at a rate of 0.084 g Na_2SeO_4 per liter.

Irrespective of the Se treatments, exposure of plants to low temperature substantially reduced shoot growth (Table 4.14). In contrast to the shoot growth, low temperature treatment was not effective to decline root growth. The temperature effect

on root growth was very minimal, indicating that shoot growth is more sensitive to low temperature stress than the root growth under given conditions.

At each temperature treatment, Se treatments did not affect root and shoot growth. In the case of low temperature treatment, increasing Se applications tended to improve growth; but this effect is very minimal and insignificant when the SD values are considered.(Table 4.14). It can be concluded that Se has no effect on growth of plants at two temperatures considered in the experiment (Table 4.14).

Table 4.14 Shoot and root dry matter production of 11 days old maize plants (cv. Şimal) grown in nutrient solution with varying Se (0.5 µM, 2.5 µM) concentrations and foliar Se application. All plants were first grown at 24 (night) -26ºC (day) for 6 days, then part of plants were transported to 16 (night)-18 ºC (day) and grown for 5 days before harvest. The data represent mean ± SD of four independent replications.

	Shoot dry matter production (mg plant^{-1})	
Se supply	24-26ºC	16-18ºC
0µM	598 ± 49	372 ± 52
0.5µM	586 ± 41	397 ± 17
2.5µM	697 ± 84	408 ± 28
Foliar appl.*	629 ± 73	398 ± 51

	Root dry matter production (mg plant^{-1})	
Se supply	24-26ºC	16-18ºC
0µM	193 ± 16	183 ± 15
0.5µM	178 ± 4	191 ± 15
2.5µM	219 ± 41	196 ± 24
Foliar appl.*	204 ± 28	192 ± 26

*Foliar Se application was carried out by spraying Na2SeO4 solution at a rate of 0.084 g L^{-1} every other day

As expected, the root and shoot Se concentrations were increased by increasing Se applications (Table 4.15). Moreover, it was observed that foliar Se application also increased the root Se concentration, indicating that Se is easily transported via phloem into roots after foliar absorption of Se (Table 4.15). Differential temperature treatments

did not result in any consistent effect on root and shoot concentrations of Se at each Se treatment (Table 4.15).

Table 4.15 Shoot and root Se concentrations of 11 days old maize plants (cv. Şimal) grown in nutrient solution with varying Se (0.5 µM, 2.5 µM) concentrations and foliar Se application. All plants were first grown under 24-26ºC for 6 days, then part of plants were transported to 16-18 ºC and grown for 5 days before harvest. The data represent mean ± SD of four independent replications.

	Shoot Se concentration (µg kg^{-1}DW)	
Se supply	24-26ºC	16-18ºC
0µM	29 ± 11	14 ± 4
0.5µM	941 ± 67	1253 ± 146
2.5µM	5738 ± 371	5597 ± 372
Foliar appl.*	4446 ± 1148	3935 ± 1123

	Root Se concentration (µg kg^{-1}DW)	
Se supply	24-26ºC	16-18ºC
0µM	43 ± 6	31 ± 12
0.5µM	688 ± 143	742 ± 217
2.5µM	3894 ± 300	3873 ± 451
Foliar appl.*	1329 ± 459	975 ± 304

*Foliar Se application was carried out by spraying Na2SeO4 solution at a rate of 0.084 g L^{-1} every other day

4.3.3.2 Shoot Soluble Protein Concentration

The leaf samples collected from plants, which were exposed to varied temperature treatments were analyzed for protein concentrations and activities of antioxidative enzymes. At the low temperature treatment (16-18ºC), shoots of plants contained higher amount of soluble protein concentration than the plants under normal temperature treatment (Table 4.16). However, soluble shoot protein concentrations did not show any consistent change by increasing Se levels or different Se applications at both temperature groups (Table 4.16).

Table 4.16 Shoot soluble protein concentration of 11-days-old maize plants (cv. Şimal) grown in nutrient solution with varying Se (0.5 µM, 2.5 µM) concentrations and foliar Se application. All plants were first grown under 24-26ºC for 6 days, then part of plants were transported to 16-18 ºC and grown for 5 days before harvest. The data represent mean ± SD of four independent replications.

	Shoot soluble protein concentration (mg g^{-1} FW)	
Se supply	24-26ºC	16-18ºC
0µM	7.68 ± 1.18	11.28 ± 1.43
0.5µM	6.48 ± 0.89	11.21 ± 1.81
2.5µM	5.74 ± 1.54	10.42 ± 1.61
Foliar appl.*	8.63 ± 1.58	10.60 ± 1.12

*Foliar Se application was carried out by spraying Na2SeO4 solution at a rate of 0.084 g L^{-1} every other day

The activities of the antioxidant enzymes have been expressed only per gram of fresh weight; because Se applications did not influence protein concentration of leaves (see below).

4.3.3.3 Glutathione Reductase Activity

Glutathione reductase (GR) activity of shoots exhibited an increase in plants exposed to low temperature stress (Table 4.17). Under normal temperature conditions, activity of GR tended to be decreased by increasing Se levels of nutrient solution. In contrast to the effect of the Se added in nutrient solution, foliar applied Se increased GR activity under normal temperature conditions. However, GR activity was not affected by foliar Se application under cold conditions (Table 4.17).

Table 4.17 Glutathion reductase (GR) activity of 11 days old maize plants (cv. Şimal) grown in nutrient solution with varying Se (0.5 µM, 2.5 µM) concentrations and foliar Se application. All plants were first grown under 24-26ºC for 6 days, then part of plants were transported to 16-18 ºC and grown for 5 days before harvest. The data represent mean ± SD of four independent replications.

	GR (nmol g^{-1} FW min^{-1})	
Se supply	24-26ºC	16-18 ºC
0µM	755 ± 85	1197 ± 140
0.5µM	682 ± 101	1293 ± 184
2.5µM	529 ± 112	1359 ± 115
Foliar appl.*	947 ± 114	1028 ± 259

*Foliar Se application was carried out by spraying Na2SeO4 solution at a rate of 0.084 g L^{-1} every other day

4.3.3.4 Ascorbate Peroxidase Activity

As found with GR, there was also no consistent relationship between Se treatments and ascorbate peroxidase (APX) both under normal and low temperature conditions (Table 4.18).

Table 4.18 Ascorbate peroxidase (APX) activity of 11 days old maize plants (cv. Şimal) grown in nutrient solution with varying Se (0.5 µM, 2.5 µM) concentrations and foliar Se application. All plants were first grown under 24-26ºC for 6 days, then part of plants were transported to 16-18 ºC and grown for 5 days before harvest. The data represent mean ± SD of four independent replications.

	APX (nmol g^{-1} FW min^{-1})	
Se supply	24-26ºC	16-18ºC
0µM	2.62 ± 0.01	2.46 ± 0.47
0.5µM	2.32 ± 0.23	2.96 ± 0.36
2.5µM	2.35 ± 0.43	2.68 ± 0.37
Foliar appl.*	2.95 ± 0.28	3.01 ± 0.36

*Foliar Se application was carried out by spraying Na2SeO4 solution at a rate of 0.084 g L^{-1} every other day

4.3.3.5 Catalase Activity

There was a clear decrease in catalase (CAT) activity of shoots by adding Se concentration in nutrient solution when compared to the nil Se treatment under normal temperature conditions (Table 4.19). By contrast, catalase activity was not affected by Se treatments in nutrient solution at low temperature. Irrespective of the Se treatment, low temperature stress stimulated catalase activity under the Se treatments in nutrient solution. This effect was very similar to the effect on GR (Table 4.19). Foliar Se application was also ineffective on catalase activity under both temperature conditions when compared to the nil Se treatment (Table 4.19).

Table 4.19 Catalase (CAT) activity of 11 days old maize plants (cv. Şimal) grown in nutrient solution with varying Se (0.5 µM, 2.5 µM) concentrations and foliar Se application. All plants were first grown under 24-26ºC for 6 days, then part of plants were transported to 16-18 ºC and grown for 5 days before harvest. The data represent mean ± SD of four independent replications.

	CAT (nmol g^{-1} FW min^{-1})	
Se supply	24-26ºC	16-18ºC
0µM	120 ± 14	149 ± 23
0.5µM	79 ± 15	186 ± 25
2.5µM	80 ± 27	159 ± 12
Foliar app.*	140 ± 13	140 ± 19

*Foliar Se application was carried out by spraying Na2SeO4 solution at a rate of 0.084 g L^{-1} every other day

4.3.3.6 Superoxide Dismutase Activity

Shoot superoxide dismutase (SOD) activity was also increased under low temperature conditions. Like GR and CAT activity, superoxide dismutase (SOD) activity also tended to decrease by increasing Se applications to nutrient solution under normal temperature conditions (Table 4.20).

Table 4.20 Superoxide dismutase (SOD) activity of 11 days old maize plants (cv. Şimal) grown in nutrient solution with varying Se (0.5 µM, 2.5 µM) concentrations and foliar Se application. All plants were first grown under 24-26ºC for 6 days, then part of plants were transported to 16-18 ºC and grown for 5 days before harvest. The data represent mean ± SD of four independent replications.

	SOD (nmol g^{-1} FW min^{-1})	
Se supply	24-26ºC	16-18ºC
0µM	55 ± 6	85 ± 3
0.5µM	50 ± 4	84 ± 10
2.5µM	43 ± 8	80 ± 15
Foliar appl.*	70 ± 1	81 ± 7

*Foliar Se application was carried out by spraying Na2SeO4 solution at a rate of 0.084 g L^{-1} every other day

4.4 Assay of DPPH (Diphenyl Picrylhydrazyl) Radical Scavenging Activity

In this part of the thesis, the effect of high seed Se concentration on total antioxidant activity of seeds was measured by using the DPPH method. Ascorbic acid, which is a well known antioxidant, was found to be the best radical scavenging activity among all antioxidants Tested. Trolox is further excellent antioxidant also showed high antioxidant capacity like ascorbic acid (Figure 4.2). Water extracts of seeds containing 124 ppb and 2249 ppb Se concentrations did not have differential effect on DPPH scavenging capacity. However, the extract of the wheat seed with 5871 ppb Se concentration indicated slightly higher scavenging activity than the seeds with lower Se concentration (Figure 4.2).

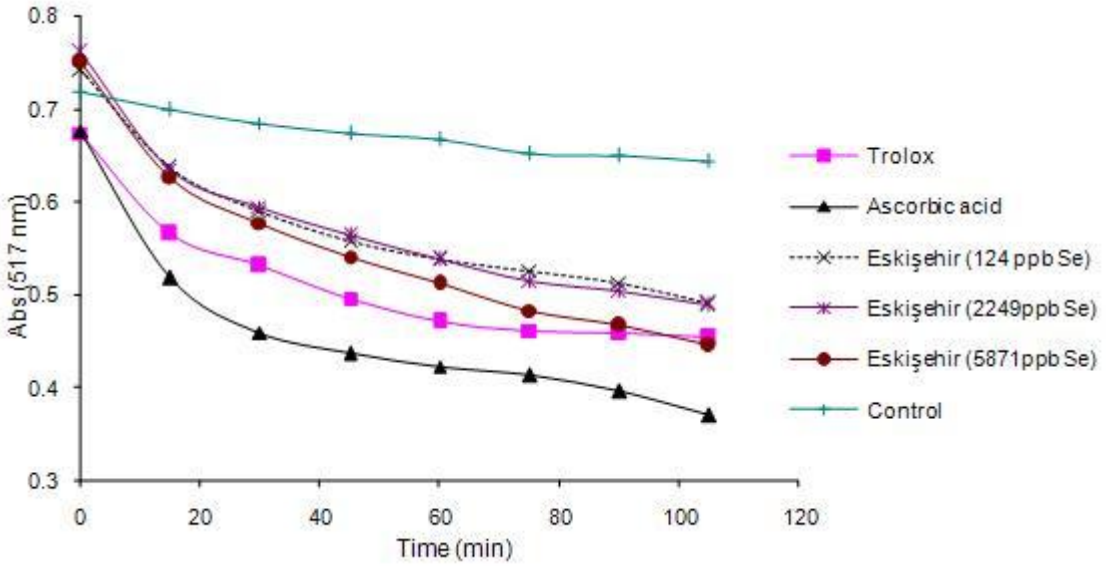

Figure 4.2 DPPH scavenging activity of aqueous extracts of wheat grains and reference antioxidants (Trolox and ascorbic acid). Wheat grains with varying Se concentrations (124 ppb, 2249 ppb, and 5871 ppb) were obtained after foliar Se applications in a field trial conducted in Eskişehir. Trolox and Ascorbic acid were used to compare their radical scavenging activity with those of wheat seeds with different Se concentrations. Control group contained neither wheat extract nor other antioxidants.

5 DISCUSSION

5.1 Seed Selenium Treatments

There are different options to improve edible parts of stable food crops with Se such as soil and foliar applications of Se-containing fertilizers. In some countries such as Denmark, Finland, and China, cultivated soils contain very low amounts of Se (< 0.05 ppm), and hence the edible parts of plants also have low Se concentrations (Oldfield, 1999). Application of Se-containing fertilizers on such Se-low regions is very important to meet daily Se requirement of human populations. For example, nearly 25 years ago, the Finnish government initiated a nationwide Se application program by enrichment regular fertilizers by Se. Selenium was added into fertilizers at the rate of 10 mg Se per kg regular NP or NPK fertilizers. This fertilization program was highly affective to improve Se concentrations of cereal grains and dietary Se intake by human-beings in Finland (Varo et al., 1988) (Table 2.2). The soil Se applications improved Se concentrations of grains from 10 μg kg^{-1} dry weight up to 250 μg kg^{-1} in spring wheat (Eurola et al., 1990).

Finnish Se-enrichment program is a good example for fertilizer strategy to improve Se concentrations of food crops and to contribute to human nutrition. However, in many regions, there are several soil chemical and physical factors affecting the solubility and root uptake of soil Se. As discussed before (see 2.4.1), solubility and root uptake of Se is adversely affected soil pH, precipitation, organic matter content, soil sulfur (S) status and soil texture (Grandjean, 1992; Diplock, 1993; Tato, 1994; Luoma, 1995 and Voutsa and Samara, 1998; Spallhoz, 1994). For this reason, foliar application of Se or seed treatment of Se just before sowing are being considered as further fertilization strategies to increase Se concentration of food crops. According to a study conducted by Curtin et al. (2006), foliar Se application in form of Na_2SeO_4 at flowering stage increased significantly grain Se concentrations in wheat. The increases in grain Se

by foliar Se application were even much greater than the increases found with soil Se application (Curtin et al., 2006).

Since Se has a high potential to be toxic at relatively low concentrations to plants ((Brown and Shrift, 1981), it is important to define Se application rates which are not toxic to plants. In the current study, seed treatment with Se (soaking seeds in Se-containing solution) has been used as a practical approach to contribute seed Se concentration. The concentrations tested (up to 5000 µM Se) were not toxic to wheat plants. Seed germination, development of seedlings and grain yield were not affected (Table 4.1, 4.2). Treatment of seeds with 1000 and 5000 µM Se markedly enhanced seed Se concentration of the bread wheat Bezostaya from 44 µg kg^{-1} seed to 91 and 216 µg kg^{-1} seed, respectively (Table 4.3). These increases in seed Se concentrations are very important in terms of human nutrition. As reported by Lyons et al. (2003), for a better human nutrition seed Se concentration in wheat should be between 100 to 1000 µg kg^{-1} seed. Concerning the effects of seed Se-treatments on seed Se concentration there is very rare published data in literature. According to Curtin et al (2006), treatment of seeds with a Se-fertilizer (e.g., *AgSel*) at the rates of 4 and 8 gram Se per hectare (by spraying Se on seeds) in three field experiments could enhance seed Se concentration, as average of 3 field experiments, from 31 µg kg^{-1} to 42 µg kg^{-1} and 68 µg kg^{-1} seed, respectively. Although the method for the seed treatment with Se was different, the results found by Curtin et al. (2006) seem to be agreement with the results presented in the current study. Similarly, also Stephen et al. (1989) showed positive effects of the seed treatment with Se on increasing seed-Se concentration of wheat plants.

Based on these results it can be suggested that seed treatment with Se might be a practical approach for improving seed Se concentration to meet daily Se requirement of human beings. The tested concentrations for the seed treatment with Se (up to 5 mM) did not lead to any toxicity in plants and can be recommended to those who are interested in enrichment seeds with Se. It is important to mention that in enrichment seeds with Se, a special attention should be paid to S status of soils. Selenium and S interact during root uptake. This interaction takes place between selenate and sulfate. Increasing soluble sulfate concentrations of soils or nutrient solution significantly reduce root uptake of Se (Adams et al. 2002; Lyons et al. 2004). Results from field trials in the United Kingdom showed that grain Se concentration of wheat decreased

from 0.09 mg kg^{-1} to 0.04 mg kg^{-1} when sulfate was applied at 100 kg S/ha (Adams et al. 2002).

Selenium enrichment program could be also very important for Turkey, especially in the Black Sea Region where soils should have very low chemical availability of Se due to low soil pH. It is well-documented that low soil pH (soil acidity) reduces chemical availability of Se to plant roots by stimulating the reduction from Se^{+6} (more bioavailable for plants) to Se^{+4} (less bioavailable for plants) (Barrow and Whelan, 1989). The Black Sea Region is known as an area where a high cancer incidence and thyroid metabolism disorder problems are increasingly observed (Giray and Hincal, 1999). Therefore, Se enrichment of common food crops grown in this region becomes an important issue. The Se concentration of various corn samples collected in the farmer fields and the wheat grains obtained from the Trabzon Office of Turkish Grain Board had an average Se concentration of 52 μg kg^{-1} (with a range of 29- 81 μg kg^{-1}) for corn and 81 μg kg^{-1} (with a range of 63- 97 μg kg^{-1}) for wheat (Giray and Hincal, 1999). In this screening study by Giray and Hincal (1999) the number of corn and wheat genotypes tested were not given in the paper; but, these results indicate that the Se status of cereal grains in the Black Sea region seem to be low and not sufficient to meet daily Se requirement of human beings. These Se values in the cereal grains from the Black Sea region are also in good agreement with the reported daily Se intake in Turkey which is around 36 μg Se day^{-1} (Giray and Hincal, 2004) and much lower than the recommended value of 55 μg day^{-1} (Stadtman, 2002). According to Thomson and Paterson (2001), the daily Se intake values between 75- 125 μg Se day^{-1} prevent genetic damage and cancer development in human beings.

5.2 Selenium Accumulation in Various Wheat Genotypes

Enrichment of food crops with Se can be also achieved by applying breeding strategy (genetic biofortification) besides fertilizer strategy (agronomic biofortification). A critical issue in a successful breeding program is the identification of new parental lines showing large genetic variation for Se concentration in seeds. Identification of new genotypes with high Se concentration in shoot or seed can be exploited in breeding programs aiming at development of high-yielding cultivars with high seed Se.

In order to determine new cereal genotypes with high Se concentrations, a greenhouse and a nutrient solution experiment were conducted by using various genotypes of tetraploid wild wheat (*Triticum dicoccoides*), primitive (less-cultivated) wheat *Triticum spelta* and modern (cultivated) wheat (*Triticum aestivum* or *Triticum turgidum*). In the greenhouse experiment, plant growth and Se uptake of both modern wheat cultivars and tetraploid wild wheat (*Triticum dicoccoides*) genotypes were investigated in a soil treated by 2 rates of Se application (0.05 and 0.5 mg Se kg^{-1} soil). Applying 0.5 mg Se kg^{-1} soil tended to result in a general decrease in shoot dry matter production of modern wheat cultivars while the shoot dry matter production of *Triticum dicoccoides* genotypes was not affected (Table 4.4). This result indicates that *Triticum dicoccoides* genotypes are more tolerant to high Se treatments than the modern wheat cultivars. This result might be related to generally high concentrations of protein (and S) in *Triticum dicoccoides* than the modern wheat cultivars (Nevo, 2001). High S nutritional status of plants may interact with use of Se at cellular level leading to alleviation of possible Se toxicity in plants (Brown and Shrift, 1981). Although *Triticum dicoccoides* genotypes had higher tolerance to high Se supply, they contained slightly less Se than the modern wheat cultivars (Table 4.5). There was, however, no significant genetic variation for the shoot Se concentration within the genotypes of modern wheat cultivars or *Triticum dicoccoides*. Only the *Triticum dicoccoides* genotype Kokhav Hashahar 19-1 exhibited a higher Se accumulation when compared to other genotypes at both low and high Se applications (Table 4.5). The *Triticum dicoccoides* genotype Kokhav Hashahar 19-1 has been included in the nutrient solution experiment to compare its capacity for root Se uptake with the modern wheat cultivars and *Triticum spelta* genotypes.

The *Triticum dicoccoides* genotypes tested for their shoot Se accumulation under greenhouse conditions were also analysed for Se concentrations in their seeds. As presented in Table 4.6, the seed Se concentration of *Triticum dicoccoides* genotypes varied from 66 ± 4.1 to 113 ± 12 μg Se kg^{-1} seed. This genetic variation and the concentrations for Se seem to be low and not promising to be exploited in breeding programs. Among the *Triticum dicoccoides* genotypes, the genotype 19-1 showed relatively higher Se concentration in shoot and also in seed. The modern cultivars included in the uptake experiment exhibited lower Se concentrations than the *Triticum dicoccoides* genotypes (Table 4.5).

To our knowledge, in literature there is no published data about the genetic variation for seed Se in *Triticum dicoccoides.* According to a survey study conducted by Lyons et al. (2005) by using diverse of wheat, triticale, and barley germplasm, seed Se concentrations were detected to be in the range of 5–720 μg kg^{-1}. However, much of this variation has been found to be resulted from spatial variation in soil Se, and was not ascribed to genetic factors. Only in the case of the diploid wheat *Aegilops tauschii* and rye the variation in seed Se concentrations was attributable to genetic factors, not to the soil factors (Lyons et al., 2005).

Due to high heterogeneity distribution of Se in soils, a uniform supply of Se into growth medium is very important for a reliable screening of genotypes for their capacity to absorb and accumulate Se.. Therefore, more uniform greenhouse or growth chamber conditions are considered for screening genotypes for measurement of Se uptake by roots. In this MSc study, a nutrient solution experiment has been conducted to study the Se uptake and Se accumulation capacity of different wheat genotypes. In this experiment, primitive (less-cultivated) *Triticum spelta* genotypes were used and compared with the modern wheat cultivars. The Se uptake capacity of genotypes was expressed on the basis of Se uptake per root weight and time (e.g., nmol Se g^{-1} root DW h^{-1}). As expected, uptake rate of all genotypes were higher in the first hours and then it declined over time (Table 4.7). In general, *Triticum spelta* genotypes showed higher Se uptake capacity than the modern wheat cultivars. The *spelta* genotypes SP 19 and SP 31 and the *Triticum dicoccoides* genotype, Kokhav Hashahar 19-1 showed higher Se uptake rate and may be considered as promising genotypes for future studies (Table 4.7). To our knowledge, this study is the first that investigated capacity of various *Triticum spelta* genotypes for Se uptake by roots. In future studies, attention should be given to higher number of *Triticum spelta* genotypes for Se uptake experiments and physiological tests.

5.3 Effect of Selenium on Growth of Plants under Abiotic Stress Conditions

According to the current knowledge, Se does not have any known structural and functional role in plant metabolism. However, beneficial effect of Se in plants under stress conditions is a controversial topic in scientific literature. In recent years

increasing number of evidence has been published indicating positive effects of Se in plants exposed to both biotic and abiotic stress conditions. For example, Se can stimulate plant growth during senescence (Djanaguiraman et al., 2005; Xue et al., 2001). Applying 0.1 ppm Se in soil was effective to improve the growth of senescing lettuce seedlings by 14% (Xue et al. 2001). In another study, foliar Se application promoted growth of soybean during senescence (Djanaguiraman, 2005). There were also published reports showing spectacular effects of Se on growth under Cd toxicity (Filek et al., 2007) and under salt stress (Kong et al., 2005).

Hanson et al. (2004) investigated the advantages of Se hyperaccumulation in Indian mustard (*Brassica juncea*) in protection of plants from phloem-feeding herbivores. In a choice feeding experiment, the aphids were able to detect and avoid Se-containing leaves as low as 10 mg Se kg^{-1} DW. In the no-choice feeding experiment, leaves containing 1.5 mg Se kg^{-1} DW leaf caused a 50% reduction in aphid population growth, and the leaves containing more than 10 mg Se kg^{-1} DW were lethal for aphids (Hanson et al. 2004). In a further test, Indian mustard (*Brassica juncea*) plants grown with or without Se were subjected to herbivory caterpillars (*Pieris rapae*) or to fungal infection by a root/stem pathogen (*Fusarium sp*.) and a leaf pathogen (*Alternaria brassicicola*). When given a choice between leaves with or without Se (0.1% Se of leaf DW), the caterpillars strongly preferred leaves without Se, and the Se leaves were lethal to the caterpillars. In addition, Se-containing plants were less susceptible to infection by both fungi (Hanson et al., 2004).

In the present study, there was no clear effect of Se supply on plant growth under various abiotic stress conditions. As expected, drought stress caused severe decreases in shoot growth under greenhouse conditions (Table 4.9, Fig. 4.1); but Se applications remained without effective on these decreases. Similarly also salinity- (Table 4.9) and flooding-dependent (Table 4.9) decreases were not influenced by either soil or foliar application of Se. Similarly, also Valkama et al. (2003) did not see any clear effect of Se on growth of barley under UV-B radiation stress despite the accumulation of Se up to 6 mg kg^{-1} in plants at 1 mg Se kg^{-1}soil conditions. In the same study, supplying 1 mg Se kg^{-1} soil caused a decrease in runner biomass of strawberry plants, while 0.1 mg Se kg^{-1} remained nearly ineffective on growth under UV radiation stress. As discussed below, Se was also not effective to alleviate low temperature-dependent decreases in root or shoot growth of maize plants (Table 4.14). In some studies Se applications

resulted in adverse effects on plant growth as shown in potatoes (Germ et al., 2007). Based on the results presented here and the results published,, it can be suggested that the Se has no consistent effect on growth under stress or normal conditions.

As expected, increasing Se treatments increased the shoot Se concentrations of plants (Table 4.10, 4.15). However, flooding stress caused a clear decrease in shoot Se concentration (Table 4.10). As mentioned before (see 2.4.1), bioavailability of Se to plants show a marked decline in soils with heavy irrigation or poor aeration (Barrow and Whelan, 1989). Selenium is easily reduced to less available forms under saturated conditions (Gissel-Nielsen et al., 1984). Most probably, due to poor availability of Se to plant roots (caused by low redox potential of soil as a result of oxygen deficiency) flooding stress reduced shoot Se accumulation of plants. Since such soil factors decreasing Se availability (e.g., poor aeration, low pH, low organic matter etc.) are very common in cultivated soils, applying Se to foliar could be a good option to contribute to plant Se concentrations.

One of the widely discussed effects of Se in plants is its effect on activities of the antioxidative enzymes. There are several papers investigated effects of Se on activities of superoxide (O_2^{-1})-scavenging (e.g., superoxide dismutase, SOD) and hydrogen peroxide (H_2O_2)-scavenging enzymes such as catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR). The published results related to the effects of Se on antioxidative enzymes seem to be controversial. In our results presented in this thesis, application of Se into nutrient solution or to foliar did not result in a clear effect on activities of GR, APX, CAT and SOD either under normal or low temperature treatment. The slight changes found in the activities of these antioxidative enzymes by the Se treatments are not significant and biologically unimportant. The only clear effect was the effect of low temperature stress on the activities of the enzymes measured. As shown in the Tables (4.17, 4.18, 4.19, 4.20), low temperature stress caused a general increase in GR, APX, CAT and SOD activities. This effect is an expected effect and well described before in literature (Pastori et al., 2000; Foyer et al., 2002; Hu et al., 2006).

Seppänen et al. (2003) showed that Se application alleviated photooxidative damage caused low temperature or paraquat treatments and this positive effect of Se was attributed to enhanced activities of SOD, and elevated expression levels of chloroplast-CuZnSOD transcripts. In contrast to this result, Filek et al.(2007) found that

the positive effects of Se application against Cd-induced cell damage was not related to the activities of antioxidative enzymes. Similarly, Hartikainen et al. (2000) found that the antioxidative effect of Se was associated with a-tocopherol synthesis, but not with SOD activity. The results shown in Tables (4.17, 4.18, 4.19, 4.20) are in good agreement with the results presented by Hartikainen et al. (2000) and Filek et al.(2007). Based on these results it can be concluded that Se has no significant effect on activities of SOD, CAT, APX and GR. According to an experiment conducted by Hajiboland and Amjand (2007), Se effect on antioxidative capacity and plant growth shows high dependency on S status of plants. Under S sufficient conditions, Se showed a growth promoting effect in cabbage, kohlrabi, and alfalfa plants; but, at lower S supply, Se had toxic effects on plants. Selenium application to the S-deficient plants reduced the activities of APX, GPX, and SOD while increasing the CAT and GR activity. Selenium application to S sufficient plants also resulted in similar effects as found under S deficient conditions (Hajiboland and Amjad, 2007).

5.4 Total Antioxidant Capacity of Selenium Enriched Grains

In this part of the thesis, the aim was to test the total antioxidative capacity of seeds differing in Se concentrations by using *in vitro* DPPH radical scavenging assay (Bors et. al., 1992). In this assay the DPPH radical is considered to be a model of a stable lipophilic radical. The wheat seeds used in this assay have been obtained in the framework of a TUBITAK-Selenium Project (No: 105 O 637), and were different in Se concentrations. Differences in seed Se concentration (between 124 to 5871 ug S kg^{-1} seed) have been obtained by applying Se at different times to foliar in Eskisehir.

The total antioxidant activity of seeds differing in Se concentration has been shown in Fig. 4.2. In this assay, the control group contained neither antioxidants nor wheat extracts. Therefore, radical scavenging activity of the control group remained nearly constant. On the other hand, the antioxidants added in the assay medium, especially ascorbic acid showed high scavenging activity (Fig. 4.2). In the case of seeds differing in Se concentrations there was no clear difference in their capacity to show high antioxidant activity on the basis of DPPH scavenging capacity. Only the extract of

the wheat grain containing 5871 ppb Se tended to exhibit a high scavenging activity (Fig. 4.2); but, this effect seems to be statistically not important. In a previous study conducted by Xu and Hu (2004), aqueous extracts of rice grains showed some increases in antioxidant capacity by increasing seed Se concentration; but these effects were not remarkable. To our knowledge there is no other studies dealing with antioxidative effects of Se in seeds. It is important to highlight that such poor effectiveness of high Se in seeds on total antioxidant activity does not necessarily mean that Se has no antioxidant activities in biological systems. As reported previously, major biological effects of Se are related to its organic forms (e.g., selenoproteins), such as selenomethionins (Hatfield and Gladyshev, 2002). The assays described above should be conducted by isolated active seleno-compounds in future studies.

6 CONCLUSION

The present study covers various experiments which investigated the i) role of seed treatment with Se in improving grain Se concentration, ii) genetic variation for grain and shoot concentrations of Se and Se uptake capacity in various modern and wild wheat genotypes and iii) the protective role of Se in plants under different stress factors.

It was important to determine that soaking seeds in a solution containing 5000 µM Se for about 30 min was effective to increase grain Se concentration up to 200 ug Se kg^{-1} without causing any adverse effect on plant growth and development. This approach could be considered in practical agriculture by farmers to enhance grain Se concentration. In Turkey, daily Se intake is found to be around 36 µg Se day^{-1} (Giray and Hincal, 2004) which is very low when compared to the RDA value of 75-125 µg Se day^{-1} (Food and Nutrition Board, 1980; Thomson and Paterson, 2001). It has been shown that daily intake of 75- 125 µg Se prevents genetic damage and cancer development in human subjects (Thomson and Paterson, 2001). An alternative approach to seed treatment is the soil and/or foliar application of Se-containing fertilizer (agronomic biofortification strategy). Agronomic biofortification strategy should be considered and implemented in Turkey as a quick and short solution to the low daily Se intakes.

A further approach to improving grain Se concentration is the breeding strategy (e.g., genetic biofortification): developement of new genotypes with high grain Se concentration (Lyons et al., 2003; Cakmak, 2008). In order to conduct a successful breeding program, the parental lines used in the breeding programs should have a promising genetic variation for the targeted traits. Therefore, to start a breeding program for high grain Se, first contrasting and promising parental lines are needed. In the second part of this thesis, attention has been paid to identification of new genotypes with high Se concentration in plant tissue or high Se uptake capacity by roots. Several *Triticum dicoccoides* genotypes, modern wheat cultivars and *Triticum spelta* genotypes

have been investigated to find out new genotypes for high Se uptake and accumulation capacity. The results indicated that *Triticum dicoccoides* genotypes and modern wheat cultivars did not show a promising and useful genetic variation to be exploited in breeding programs. Only in the case of *Triticum spelta*, a few genotypes have been identified showing relatively high root uptake capacity for Se. It was concluded that further screening programs are needed by using greater number of genotypes and new genetic sources to identify more promising genotypes for high grain Se. Since breeding strategy is a long-term process, as a short term solution to low daily Se intake in human beings, agronomic biofortification strategy (applying Se-containing fertilizer) should be considered and widely implemented in Turkey like in Finland (Varo et al., 1988; Eurola et al., 1991; Cakmak, 2008).

Role of Se in growth and development of plants is a controversial topic. There are reports indicating a role of Se in growth of plants, especially under stress conditions, while some reports are available indicating that positive roles of Se in growth and protection of cells from stress is only confined to mammalian systems, and could not be found in plant systems. The positive effects of Se in plant cells have been ascribed to its contribution to antioxidative defense system of plant cells (Seppänen et al., 2003; Djanaguiraman et al., 2005; Kong et al., 2005). In the present study, several greenhouse and growth chamber experiments have been conducted to investigate the role of Se in growth of plants under various stress conditions. From 3 different experiments conducted in this thesis, there was no any evidence that Se stimulates growth and affects the activities of enzymes, which are responsible for detoxification of reactive oxygen species. Similarly, the seeds having diverse Se concentrations did not affect the total antioxidative capacity. Thus, the results of this thesis disagree with the results showing that Se improves growth of plants under stress conditions.

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APPENDIX

Chemicals

All chemicals and standart solutions were supplied by Merck (Germany), SIGMA (USA), Fluka (Switzerland), Applichem (Germany) and Riedel de Häen (Germany).

Equipment

Centrifuge:	Kendro Lab. Prod., Heraeus Multifuge 3 S-R, GERMANY
Distilled water:	Millipore, Elix-S, FRANCE
	Millipore, MilliQ, Academic, FRANCE
Inductively coupled plasma-optical emission spectroscopy (ICP-OES):	Varian, Vista-Pro ccd, AUSTRALIA
Magnetic stirrer:	IKA®-WERKE, GERMANY
	VELP Scientifica, Microstirrer, ITALY

Microliter Pipette:	Gilson, Pipetman, FRANCE
	Eppendorf, GERMANY
pH meter:	Hanna Instrument, p213, Microprocessier pH meter, ROMANIA
Spectrophometer:	Varian, Cary 300 BIO, UV Visible, AUSTRALIA
Ultrafreezer:	-80 ºC Thermo, GERMANY
Ice machine:	Scotsman Inc., AF20, USA
Incubator:	Memmert, ULE 700, GERMANY ULE 600, GERMANY