Antioxidant profile of selenium enriched wheatgrass cultivated under different environmental conditions

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Degree of
Master of Science in Biotechnology

Submitted by

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Candidate's Declaration

I hereby declare that the work which is being presented in the dissertation entitled "**Antioxidant profile of selenium enriched wheatgrass cultivated under different environmental conditions**" in the partial fulfillment of the requirements for the award of the degree of Master in Science in Biotechnology, Department of Biotechnology and Environment Sciences, Thapar University, Patiala is an authentic record of my own work during a period of 6 months from January 2012 to June 2012, under the supervision of Dr. N. Tejo Prakash, Department of Biotechnology and Environmental Sciences, Thapar University, Patiala. I have not submitted the matter embodied in this dissertation for the award of any other degree or diploma.

Patiala ***Shruti Sharma***

Date:

……

This is to certify that the above statement given by the above candidate is correct and true to the best of my knowledge.

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Certificate

This is to certify that the project entitled "**Antioxidant profile of selenium enriched Wheatgrass cultivated under different environmental conditions**" being submitted by Ms.Shruti Sharma in partial fulfillment of the requirement for the award of degree for the Master of Science in the Department of Biotechnology and Environmental Sciences, Thapar University, Patiala, is a bonafide work carried out under omy guidance and supervision and that no part of this project has been submitted for the award of any other degree.

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Needless to say errors and omissions are solely mine.

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Summary

The present study was carried out to determine the effected of various environmental conditions such as cultivation in dark, sunlight and under UV light on the selenium induced variations in anti-oxidant profile of wheat grass. Various assays like DPPH, TBARS, total phenolic content and total protein content were used to monitor the antioxidant profile of the wheatgrass. The results indicated that the wheatgrass with high and moderate selenium exhibited potential antioxidant properties as compared to control.

Contents

1.0 Introduction

Selenium is a naturally occurring element and widely but unevenly distributed on Earth. It is present in igneous rocks and fossil fuels. Selenium is a metalloid having chemical and physical properties between metal and non- metal and located in the oxygen group of this periodic table. Selenium is a chemical element with atomic number 34, chemical symbol Se, and an atomic mass of 78.96. Selenium shares many similar chemical properties with sulfur (S). Se can exist in four valence states in nature: elemental Se (Se^{0}), Selenide (Se^{-2}), Selenite (Se^{+4}) and Selenate (Se^{+6}). Selenium also exists in volatile forms e.g. dimethylselenide (DMSe) dimethyldiselenide (DMDSe), and probably dimethyl selenone, dimethyl selenylsulfide, and methylselenol. The oxidation state of Se is influenced by the pH and redox conditions of the environment. Selenate is a major species in aerobic and neutral to alkaline environments, whereas selenite and elemental Se dominate in anaerobic environments. Elemental Se is stable in a reduced environment, but it can be oxidized to selenite and to selenate by a diverse group of microorganisms. The oxidized forms of Se (i.e. SeO_3^{-2} and SeO_4^{-2}) are highly soluble in water and therefore more bioavailable and potentially toxic. These highly toxic forms easily leach from the soil and reach the ground or surface water. Plants can easily absorb and accumulate selenium compounds from water. Bioaccumulation of selenium in fodder and cereal crops can result in biomagnification. Agricultural practices significantly contribute to the bioavailability of selenium to plants through irrigation. Se phytoaccessibility is dependent on a diverse variety of soil and climatic conditions such as soil pH, redox conditions, organic matter, competing ionic species, microbial activity, level of rainfall during the growing season, etc. (Dhillon and Dhillon, 2003a; Spadoni *et al*. 2007).

Selenium is not equally distributed in the earth crust. Consequently there are widespread areas of the world that have selenium deficient soils and smaller areas that have very high levels of se in the soil. These latter areas can have Se concentrations high enough to be potentially toxic. Selenium content in soil is highly

variable. It varies from 0.1 to 2 µg Se g^{-1} in most soils, but it is most frequently between 0.2 and 0.4 µg g^{-1} (McNeal and Balistrieri, 1989). Some soils are low in selenium, from 0.03 to 0.08 µg g^{-1} (NAS, 1976). Toxic concentrations occur in arid and semiarid parts of China, in Hawaii, Mexico, Columbia and western parts of the USA and Canada (McNeal and Balistrieri, 1989). In central US, for example, there are regions in which plants contain Se levels 10 times higher than the toxic level, while Se levels in plants in eastern and western US are low (Kubota *et al*.1967). In eastern Canada, Se concentrations in plant dry matter are much below 0.1 ppm (Winter and Gupta, 1979), while they are 10 times higher in western Canada. Selenium deficiency was observed in some parts of South America (Jaffe, 1973). Selenium deficiency was noted in western and southern parts of Australia and in New Zealand (Welsh *et al*. 1981). Severe selenium deficiency registered in southeastern China has been associated with Keshan disease, a disease that occurs almost exclusively in children.

Pockets of seleniferous soils have also been identified in India especially in the Northeastern parts of Punjab (Dhillon and Dhillon, 2003b), Haryana (Singh and Kumar, 1976) and some locations in North-eastern states of Assam and Meghalaya (Dey *et al*. 1999). The soils containing as low as 0.1-0.5 mg Se kg^{-1} (Ravikovitch and Margolin, 1957; Dhillon *et al*. 1992) are considered as seleniferous because the forages grown on such soils contain 0.4mg Se kg^{-1}, i.e., the maximum permissible level for animal consumption. In the Nawanshahar–Hoshiarpur region in Punjab, Se in the soil ranged from 0.25 to 4.55 mg kg^{-1} with a mean value of 3.63 mg kg^{-1} (Dhillon and Dhillon, 2003b; Srivastava *et al*. 2002). In this region, the irrigation water source being exclusively ground water, Se concentration is as high as 479 µg L^{-1} with a mean value of 170 µg L^{-1} (Srivastava *et al*. 2006). Preliminary report on chronic Se toxicity as a cause of hoof and horn diseases in livestock in seleniferous regions of Punjab was given by Gupta *et al*. (1982). Limited observations on human population in Se-rich regions of Punjab state had also shown signs of toxicity with selenosis in humans, mainly due to consumption of grains and vegetables harvested from Se-rich agricultural soils (Hira *et al*. 2003). Toxicity symptoms in humans include anorexia, watery diarrhea, labored breathing, elevated temperature and pulse rate, prostration and often death from respiratory failure. Toxicity to plants was seen

in wheat, sugarcane that exhibited papery snow white chlorosis of leaves with pinkish coloration on the sheath and lower surface of leaves (Dhillon *et al.* 1992).

Selenium is an essential micronutrient for animals and humans that is obtained from dietary sources including cereals, grains and vegetables. Plants convert Se mainly into Se-methionine (Se-Met) and incorporate it into protein in place of methionine (Met) while Selenocystine (Se-Cys), methyl-Se-Cys and γ-glutamyl-Se-methyl-Cys are not significantly incorporated into plant protein. In humans, selenium is a trace element nutrient which functions as cofactor for reduction of antioxidant enzymes such as glutathione peroxidases (Rotruck *et al*. 1972) and certain forms of thioredoxin reductase found in animals and some plants. Selenium is active immunomodulator, much more potent antioxidant than vitamins E, C and A, beta-carotene (Baraboi and Shestakova, 2004) but much more toxic. It takes part in thyroxine conversion to tri-iodethyronine in thyroid hormone biosynthesis. As sperm antioxidant, selenium protected its motility and fertility. Selenium is a serious factor of biological and antioxidant protection of vascular endothelium, of low-density lipoproteins, protection of DNA, chromosomes. As food component, selenium is an exceptional agent of protection from atherosclerosis, coronary ischaemic disease and cancer. Se has no known physiological function in plants and is metabolized via the S-assimilation pathway, which involves biosynthesis of selenoamino acids, i.e., SeCys and selenomethionine (SeMet), which are nonspecifically incorporated into proteins in place of cysteine and methionine, respectively (Terry *et al*. 2000). Se-accumulators, which can contain from hundreds to several thousands of micrograms of Se per gram in their tissues, differ from nonaccumulators in that they biosynthesize primarily nonprotein selenoamino acids, such as Se-methyl-selenocysteine (MeSeCys) and γ-glutamyl-Se-methyl-selenocysteine (γ-Glu-MeSeCys), thereby preventing the damaging effects on plant functions resulting from incorporation of SeCys and SeMet in proteins.

The content of Se in plants can be increased in different ways; by addition of Se to soil, soaking seeds in Se solution before sowing, hydroponic and aeroponic cultivation in a nutrient solution containing Se, and foliar application of plants with Se solution. Uptake and accumulation of Se by plants is determined by the chemical form and concentration, by soil factors such as pH, salinity and $CaCO_3$ content, the

identity and concentration of competing ions, and by the ability of the plant to absorb and metabolize Se (Kabata Pendias, 2001; Wu ,2004). Se sorption is highest at lower pH values, with Se (IV) sorption decreasing at pH values above 6, whereas Se (VI) decreased over the entire pH range (2.5-10). Se sorption increases with increasing Ca^{+2} concentrations while SO_4^{-2} suppresses sorption well above enhancements by Ca^{+2} (Hyun et al. 2006).

Wheatgrass (*Triticum aestivum*) a cereal grass of the Gramineae (Poaceae) family is the world's largest edible grain cereal-grass crop. Wheat has been a food crop for mankind since the beginning of agriculture. The wheat plant is an annual grass. In early growth stages the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear leaves. For over fifty years, researchers have known that the cereal plant, at their young green stage, is many times richer in levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant (Schnabel, 1940). The young germinated plant is a factory of enzyme and growth activity. In early stages of growth they store large amounts of vitamins and proteins in young blades. After jointing stage, nutritional level in leaves drops rapidly while fiber content increases rapidly. The jointing stage is that point at which the internodal tissue in grass leaf begins to elongate, forming a stem. This stage represents peak of cereal plant's vegetative development (Kohler, 1944). Dr. Ann Wigmore, U.S.A. founder director of the Hippocrates Health Institute, Boston, U.S.A. was one of proponents of 'Wheatgrass Therapy'. Dr. Wigmore reported that "wheatgrass" used in her program contain abscisic acid and laetrile, both of which may have anti-cancer activity. It was also reported that young grasses and other chlorophyll-rich plants are safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, pancreas and liver problems, fatigue, anemia, asthma, eczema, hemorrhoids, skin problems, halitosis, body odor and constipation (Wigmore, 1985). The reports and chemical analysis undertaken reveal that wheatgrass is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, calcium, phosphorus, sodium, sulphur and potassium, antioxidants like beta-carotene (pro-vitamin A), vitamin E, vitamin C, antianemic factors like vitamin B_{12}, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value (Hamilton,

1988). Clinically it was proved that different varieties of wheatgrass extracts are therapeutically used in treatment of anemia, thalassemia (major), cancer and bacterial diseases.

In the light of foregoing discussion the present study is planned for Investigation of Selenium rich *Triticum aestivum* (Wheat) grass for their antioxidant profile, grown under different environmental conditions.

2.0 Review of Literature

Reactive oxygen species, at moderate concentrations, nitric oxide (NO), superoxide anion play an important role as regulatory mediators in signaling processes. However, at high concentrations these reactive molecules are hazardous for living organisms and damage all major cellular constituents (Droge, 2002). Oxidative modifications of DNA, proteins, lipids and small cellular molecules caused by reactive oxygen species (ROS) play important role in a variety of common diseases and age-related degenerative conditions (Seifried *et al*. 2007). Under normal circumstances, 1-5% of the molecular oxygen in the cells creates active free radicals in many different ways, but the human bodies possess enzymatic and non-enzymatic antioxidative mechanisms and minimize the generation of reactive oxygen species to levels that are not harmful to the cells (Reuter *et al*. 2010). When the generation of the reactive oxygen free radical (O_2^-, $OH^\bullet$) is overgrown or the free radical scavenging capability is weakened for some reason, many degenerative diseases, such as brain dysfunction, cancer, heart diseases and declination of the immune system, could be caused by the excessive of free radicals (Aruoma, 1998). With the aging, the biosynthesis of enzymes which scavenge free radicals starts decreasing (Thompson *et al*. 1987). Thus, excessive free radicals react with biologically active substances such as lipids, protein and DNA to cause cell membrane injury, protein denaturation and wrong DNA replications.

Selenium is an essential element for antioxidant reactions in humans and animals. Biochemically, Se is a component of the enzyme glutathione peroxidase (GSH-Px), which along with superoxide dismutase (SOD), catalase (Cat) and vitamin E protects against damage to cellular components by preventing the accumulation of peroxides in the tissue. In plants, selenium exerted dual effects (Helina *et al*. 2000). At low concentrations it acted as an antioxidant, inhibiting lipid peroxidation, whereas at higher concentrations, it acts as a pro-oxidant, enhancing the accumulation of lipid peroxidation products. Se has not been classified as an essential element for plants, although its role has been considered to be beneficial in plants capable of accumulating large amounts of the element (Shanker, 2006). It

acted as an antioxidant, inhibiting lipid peroxidation in ryegrass (*Lolium perenne*) in concentrations 0.1 and 1.0 mg Se kg^{-1} (Hartikainen *et al*. 2000). Se increased yield in pumpkins (*Cucurbita pepo*) at a concentration 1.5 mg L^{-1} (Germ *et al*. 2005). Studies on ryegrass (*Lolium perenne*) and lettuce (*Lactuca sativa*) show that Se is harmful for plants at high concentrations >10 mg kg^{-1} and can exert beneficial effects at 1.0 mg kg^{-1} (Hartikainen *et al*. 2000; Xue *et al*. 2001). The toxicity of Se can be attributed to its pro-oxidative effects, as well as to metabolic disturbance (Hartikainen *et al*. 2000). Pro-oxidative effect of Se possibly increased the demand for counteracting antioxidative capacity. The accumulation of harmful lipid peroxide radicals ($LOO^{\bullet}$) could be counteracted by increasing the concentration of α-tocopherol scavenging them to LOOH which, in turn, were transformed to less toxic LOH through increased GPx activity. The activity of SOD increased to counteract a possible abnormal accumulation of anion radicals ($O_2^{-\bullet}$). It has been shown to promote the growth of plants subjected to UV-induced oxidative stress (Xue and Hartikainen, 2000); the UV intensity at the top of the pots was 0.177 UV-B and 0.077 UV-A mW cm^{-2}, plant were illuminated for one or three minutes daily (Xue and Hartikainen, 2000). Se can also delay senescence and promote the growth of ageing seedlings (Hartikainen and Xue, 1999; Xue *et al*. 2001). In the senescing plants, the addition of Se strengthens the antioxidative capacity by preventing the reduction of tocopherol concentration and by enhancing superoxide dismutase (SOD) activity (Xue *et al*. 2001). Senescence processes are partly delayed due to enhanced antioxidation, which is associated with an increase of glutathione peroxidase (GPx) activity (Hartikainen *et al*. 2000). In ryegrass (*Lolium perenne*) up to Se addition of 1.0 mg kg^{-1}, the decreased lipid peroxidation (decreased TBARS-thiobarbituric acid reactive substances) is connected with Se-induced increase in GPx activity. The positive relationship between the Se concentration and GPx activity suggests the expression and presence of Se-dependent GPx in plant systems (Hartikainen *et al*. 2000).

In the past two decades, an increasing interest focusing on the study of natural products with antioxidant activity has been observed through many reports appearing in scientific and popular literature. Many authors have described beneficial effects of fruits and vegetables, such as protection against aging and several human

diseases. According to Eicholzer *et al.* (2001) diets rich in fruit and vegetables may be powerful in the prevention of cardiovascular disease. Natural antioxidants also have positive effects on cataract and ocular diseases as demonstrated by Anderson *et al.* (1994), Taylor *et al.* (1995), Niwa *et al.* (1998), and Orhan *et al*. (1999). Numerous epidemiological surveys have shown an inverse relationship between the intake of fruit, vegetables and cereals and the incidence of coronary heart disease and certain cancers. Many constituents of these dietary components may contribute to their protective properties, including: vitamins C and E; selenium and other mineral micronutrients; carotenoids, phytoestrogens, allium compounds, glucosinolates and indoles; dithiolthiones, isothiocyanates, protease inhibitors, fiber and folic acid. These compounds may act independently or in combination as anti-cancer or cardioprotective agents by a variety of mechanisms. One such protective mechanism, attributed to vitamins C and E and the carotenoids, is antioxidant (radical-scavenging) activity. Recent work is also beginning to highlight the potential role of other phytochemical components, including the flavonoids, phenylpropanoids and phenolic acids, as important contributing factors to the antioxidant activity of the diet (Rice-Evans and Miller, 1996).

All plants produce an amazing diversity of secondary metabolites. One of the most important groups of these metabolites is phenolic compounds. Phenolics, especially flavonoids and phenylopropanoids, are oxidized by peroxidase, and act in H_2O_2 scavenging system. Their antioxidant action resides mainly in their chemical structure. There is some evidence of induction of phenolic metabolism in plants as a response to multiple stresses, including heavy metal stress (Michala, 2006). Phenolics are characterized by at least one aromatic ring bearing one or more hydroxyl (-OH) groups. Phenols are divided into several different groups, distinguished by the number of constitutive carbon atoms in conjunction with the structure of the basic phenolic skeleton (simple phenols, benzoic acids, phenylopropanoids and flavonoids). Phenolics have various functions in plants. An enhancement of phenylopropanoid metabolism and the amount of phenolic compounds can be observed under different environmental factors and stress conditions (Diaz *et al.* 2001; Grace and Logan, 2000; Sakihama and Yamasaki, 2002). The synthesis of isoflavones and some other flavonoids is induced when

plants are infected or injured (Takahama and Oniki, 2000; Ruiz *et al.* 2003), or under low temperatures and low nutrient conditions. Plants accumulate UV-absorbing flavonoids and other phenolic compounds mainly in vacuoles of epidermal cells, to prevent the penetration of UV-B into the deeper tissues of the plant (Kondo and Kawashima, 2000).

There have been many reports of induced accumulation of phenolic compounds and peroxidase activity in plants treated with high concentrations of metals. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. The induction of phenolic compound biosynthesis was observed in wheat in response to nickel toxicity (Diaz *et al.* 2001) and in maize in response to aluminium (Winkel-Shirley, 2002). *Phaseolus vulgaris* exposed to Cd^{2+} acccumulated soluble and insoluble phenolics and *Phyllantus tenellus* leaves contained more phenolics than control plants after being sprayed with copper sulphate (Diaz *et al*. 2001). Phenolics possess hydroxyl and carboxyl groups, able to bind particularly iron and copper (Jung *et al.* 2003). The roots of many plants exposed to heavy metals exude high levels of phenolics (Winkel-Shirley, 2002). They may inactivate iron ions by chelating and additionally suppressing the superoxide-driven Fenton reaction, which is believed to be the most important source of ROS (Rice-Evans and Miller, 1996; Arora *et al*. 1998). According to Morgan *et al.* (1997) this general chelating ability of phenolic compounds is probably related to the high nucleophilic character of the aromatic rings rather than to specific chelating group within the molecule. There is another basic mechanism of antioxidant ability of phenolics. Metal ions decompose lipid hydroperoxide (LOOH) by the hemolytic cleavage of the O-O bond and give lipid alkoxyl radicals ($LO^{\bullet}$), which initiate free radical chain oxidation. Phenolic antioxidants inhibit lipid peroxidation by trapping the lipid alkoxyl radical. This activity depends on the structure of the molecules, and the number and position of the hydroxyl group in the molecules (Milic *et al.* 1998). According to Arora *et al.* (2000), phenolics (especially flavonoids) are able to alter peroxidation kinetics by modifying the lipid packing order. They stabilize membranes by decreasing membrane fluidity (in a concentration-dependent manner) and hinder the diffusion of free radicals and restrict peroxidative reaction (Arora *et al*. 2000; Blokhina *et al*. 2003). Verstraeten *et al*. (2003) shows that in addition to

known protein-binding capacity of flavones and procyanidins, they can interact with membrane phospholipids through hydrogen bonding to the polar head groups of phospholipids. As a consequence, these compounds can be accumulated at the membranes surface, both outside and inside the cells. Through this kind of interaction, as they suggest, selected flavonoids help maintain membrane integrity by preventing the access of deleterious molecules to the hydrophobic region of the bilayer, including those that can affect membrane rheology and induce oxidative damage to the membrane components. *In vitro* studies have shown that flavonoids can directly scavenge molecular species of active oxygen: superoxide ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), singlet oxygen or peroxyl radical ($^1O_2$). Their antioxidant action resides mainly in their ability to donate electrons or hydrogen atoms (Khan *et al.* 2000; Inzé and Van-Montagu, 1995; Arora *et al*. 1998; Sakihama *et al*. 2000). Polyphenols possess ideal structural chemistry for this activity and have been shown to be more effective *in vitro* than vitamins E and C on molar basis (Rice-Evans *et al*. 1997).

Ancient and modern literature also emphasizes the importance of sprout vegetable seeds to possess potential anti-oxidant and other health related properties (Alvarez-Jubete *et al*. 2010). It has been reported that aqueous extracts from wheat sprouts inhibit the mutagenic effect induced by benzo[*a*]pyrene in strain TA98 of *Salmonella typhimurium* (Peryt *et al*. 1998). Benzo[*a*]pyrene induced sperm abnormalities in mice were found to diminish after oral administration of wheat sprout extract (Tudek *et al*. 1988). Wheat sprouts contain antioxidant compounds active in the protection of DNA against the oxidative stress induced by Fenton reaction (Fe^{2+}/H_2O_2) (Falcioni *et al.* 2002). Likewise, Brussels sprouts contain bioactive substances with a potential for reducing the physiological, as well as oxidative stress-induced, DNA damage in rats and humans (Verhagen *et al*. 1995, Deng *et al*. 1998).

The Human diet is enriched with young parts of plants (so called "green foods"), which can improve nutrient balance intake in natural way. Wheatgrass (*Triticum aestivum*) refers to young grass of the common wheat plant, which belongs to Poaceae family. This is the most commonly found herb in India. This plant is believed to have many nutritional values; it has been shown to have anti-

inflammatory, antioxidant, anti-carcinogenic, immunomodulatory, laxative, astringent, diuretic, antibacterial and anti-aging properties. Its use in acidity, colitis, kidney malfunctions, atherosclerosis and swelling has been shown to be beneficial. Wheatgrass juice helps in building red blood cells and stimulates healthy tissue cell growth. 100 g of wheatgrass powder is equal to 23 kg of fresh vegetables (Rana *et al*. 2011). Wheatgrass is embodied with many nutritional components including (per 3.5 grams) 860 mg proteins, 18.5 mg chlorophyll, 15 mg calcium, 38 mg lysine, 7.5 mg vitamin C and an abundance of micronutrients, such as B complex vitamins and amino acids. Wheat grass juice is high in vitamin K, which is a blood-clotting agent (Wheatgrass wonders, http://www.moscowfood.coop/archive/wheat-grass.html).

Phytochemical constituents of wheatgrass include alkaloids, carbohydrates, saponins, gum and mucilages. Its water soluble extractive value is found to be greater than its alcohol soluble extractive value. This is because of the chlorophyll content of wheatgrass, which is about 70% water soluble (Shirude, 2011). Wheatgrass has been shown to have potential anti-inflammatory and anti-aging properties (Smith, 2000). It has higher nutritive value than broccoli and spinach (Meyerowitz, 1999). Wheatgrass is promoted to treat a number of conditions including the common cold, cough, bronchitis, fever, infections, inflamed mouth and throat, and skin disorders like hemorrhoids, psoriasis, ivy, eczema, burns and thalassemia (Marwaha *et al*. 2004). A study by Ben *et al*. (2002) has found that patients who were given the extract of wheatgrass showed significant improvement in rectal bleeding and abdominal pain associated with ulcerative colitis, a type of inflammatory bowel disease. The vitamins A, C, E, which are present in wheatgrass, are examples of natural antioxidants. Wheatgrass contains all of the essential amino acids, mainly alanine, aspartic acid, glutamic acid, arginine, serine which are helpful in providing a sufficient amount of protein in the body (Duke, 1993). Other benefits of wheatgrass are: removal of toxins from the body, improvement of blood sugar balance, prevention of tooth decay, and maintenance of healthy hair, aiding digestion, and reducing high blood pressure levels (Duke, 1993).

Wheatgrass is rich in chlorophyll and enzymes. It contains more than 70% chlorophyll that is almost identical to the hemoglobin in human blood. The only difference is that the central element in chlorophyll is magnesium and in hemoglobin

it is iron. The molecular structure of chlorophyll in wheatgrass and hemoglobin in the human body is similar, and because of this wheatgrass is called 'Green Blood' (Ferruzzia and Blakesleeb, 2007). 70-83% increase in red blood cells and hemoglobin concentration was noted within 10-16 days of regular administration of chlorophyll derivatives (Kelentei *et al.* 1958). It was reported that chlorophyll enhanced the formation of blood cells in anemic animals (Borisenko and Sofonova, 1965). Chlorophyll is soluble in fat particles, which are absorbed directly into blood via the lymphatic system. Chlorophyll, abundant in wheatgrass, increases the function of heart. Wheatgrass has been claimed to reduce the blood pressure as it enhances the capillaries (Locniskar, 1988). Wheatgrass juice has a dilating effect on blood vessels; it makes the blood vessels larger so that blood flows through them more easily. Increased dilation means better nutrition to the cells, and more efficient removal of waste from them. Vitamin E, an antioxidant and fertility vitamin found in wheatgrass is a protector of the heart. This vitamin, present in wheatgrass, is ten times more easily assimilated by the body than synthetic vitamin E. Wheatgrass is a good source of calcium, which helps build strong bones and teeth, and regulates heartbeat, in addition to acting as a buffer that restores blood pH. Dried wheatgrass juice has as much calcium as milk (Wigmore, 1985). A study showed that an uncooked vegan diet, rich in lactobacilli, chlorophyll-rich drinks, and increased fiber intake, decreased subjective symptoms of rheumatoid arthritis (Shaikh, 2011). Another study showed that when 8.5g of fermented wheatgrass extract (Avemar) taken twice per day with water, in case of 15 Severe Rheumatoid Arthritis patients, showed decreased Ritchie index, and according to a health assessment questionnaire, morning stiffness showed significant improvement. Doses of steroids were reduced in half of patients. This may be due to presence of wheatgrass which contains vitamins A, B_1, B_2, B_3, B_5, B_6 and B_{12}, vitamin C, E and K, calcium, iodine, selenium, zinc, and many other minerals, along with superoxide dismutase, muco-polysaccarides, and chlorophyll. Its anti-inflammatory properties exert a positive effect on bone and joint problems, reducing pain and swelling (Nenonen, 1998).

Dietary compounds such as garlic, carotenoids, wheatgrass, etc are important due to their antioxidant properties. These dietary products protect against many diseases because food and degraded products come into direct contact with bowel

mucosa, and can influence its physiology and metabolism. Although many dietary compounds have been suggested to contribute to the prevention of cancer, there is a strong likelihood that wheatgrass extract, which contains chlorophyll, an antioxidant, may affect cancer prevention**.** Additionally, selenium and lactrile present in wheatgrass have anti-cancer properties. Selenium builds a strong immune system, and can decrease the risk of cancer (Scott Brain cancer & wheatgrass, http://www.ehow.com/about_5057457_brain-cancer-wheatgrass.html). Wheatgrass contains at least 13 vitamins (several of which are antioxidants), superoxide dismutase (SOD), cytochrome oxidase, muco-polysaccharide which can play effective role in enhancing apoptosis in cancer cells.

Bioaccessibility of some essential elements namely K, Mn, Zn, Fe and Na from wheatgrass, consumed as dietary supplement, have been demonstrated through *in vitro* gastric and gastro-intestinal digestion methods. Neutron activation analysis was used to determine bioaccessible concentration of these elements. Bioaccessibility of these elements in commercial wheatgrass tablets and wheat grains was also determined. From both the methods, it was found that bioaccessibility of the elements studied was the highest from fresh wheatgrass and the lowest for wheat seeds (Kulkarni *et al*. 2006). In addition, the effects of freeze drying and hot air drying on total phenolics, total flavonoids and antioxidant properties of flour from seven-day-old fresh wheatgrass have also been investigated by these authors. In the quantitative analysis of antioxidative components, fresh wheatgrass samples had the highest amount of ascorbic acid and chlorophyll, but the lowest amount of total flavonoids and phenolics. In the analysis of ferric-reducing antioxidant power assay (FRAP), ethanolic extract from freeze-dried wheatgrass gave the highest value, while the α-tocopherol gave the lowest value. In the analysis of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging ability, freeze-dried wheatgrass samples exhibited the highest activity (Das *et al*. 2011).

The sprouts of wheat grown with selenium under dark conditions and extracted with ethanol could be considered a potent and functional food ingredient or dietary food supplement for humans and animals because the selenium increased the total polyphenol content and antioxidative activity. The total polyphenol content in aqueous extract and ethanolic extract increased significantly in wheat (3 times). In

the selenite-treated sprouts of ryegrass and wheat, antioxidative activities in the ethanolic extracts increased significantly with the concentration of selenite (2 and 2.5 times, respectively). In the aqueous extracts, however, the effect of selenite on the antioxidative activities was less pronounced, with the exception of wheat (2.5 times) (Motomura *et al*. 2008). Sodium selenite treatment at doses of 5 and 10 ppm selenium increases the main antioxidant components of wheat seedlings, consecutive, as the content of total chlorophylls, chlorophylls a, chlorophylls b, carotenes and xanthophylls, vitamin C and antiradical activity. Sodium selenite had benefits on seedlings, manifested by a significant increase ($p<0.05$) of chlorophylls levels (until +173.03%), carotene and xanthophylls (until +318.71%), vitamin C (until +1728.33%) and improving of their antiradical capacity (until +600.77%) (Moldovan *et al*. 2011).

Keeping in view, the increasing emphasis on sprouted vegetables as nutritional ingredients and well known effect of selenium as an anti-oxidant, the present study examined the anti-oxidant profile of the wheat grass germinated from Se rich wheat grains. To the best of our knowledge, there is no report on the influence of bioavailable selenium on the anti-oxidant properties of the wheat grass cultivated under different environmental conditions, which is the objective focused in the present study.

3.0 Materials and methods

Sample collection

Harvested grains of wheat (*Triticum aestivum*, cv. PBW343) were collected from the two different sites of Selenium affected area, near the village Jainpur, geographically located at 31°13` N, 76°21` E between Nawanshahar and Hoshiarpur Districts of Punjab (India). Samples from Patiala district, which is located 75 miles away from the region, were collected to represent non-Seleniferous region. Collected grains were manually cleaned to remove broken and infected seeds, washed with water and air dried at room temperature for 48 h.

Seed sterilization

Approximately 50g of all three wheat samples were taken separately in wide mouth flask containing 100 ml water and 2-3 drops of Tween-80. The flasks were kept on shaker at 150 rpm for 30 min. After this flasks were kept under running tap water until frothing stop. 70% ethanol was added first for 5 min and decanted, rinsed 3 times with sterile distilled water before addition of 4% sodium hypochlorite for 10 min. Subsequently, the seeds were rinsed thoroughly with sterile water three times in bio safety cabinet for further use.

Seed germination

10 g of sterilized seeds were spread on plastic tray containing sterilized cotton bed soaked with 0.5 X Hoagland solutions. All three wheat samples were sown in a tray separately. Seeds were cultivated in triplicate. All three trays were incubated at 20° C for 2 days in dark. After 2 days, one tray was shifted under ultraviolet A (365 nm, 4-5 Watt m^{-2}) light, another one shifted to incandescent light and one tray was kept in dark. Trays were exposed to visible and ultraviolet light with photo period of 12h light/12h dark. 1X Hoagland solution was used for watering during germination and seeds were allowed to germinate for 6 days. Wheatgrass (8-10 cm long shoots of germinated seed) were harvested, washed and stored at -80° C for further use.

Selenium determination

1g wheatgrass samples were air dried at room temperature for 48 h, and selenium was determined in each sample by procedure given by Levesque and Vendette (1971). In brief 100 mg of each dried sample was acid digested with 3 ml HNO_3 and $HClO_4$ (3:1) and Se^{+6} reduced to Se^{+4} by using 5 ml of 2N HCl. Final volume of each digested solution was made up to 25 ml. 0.2 ml of digested solution was taken in a clean test tube containing 2 ml distilled water, 0.1 ml formic acid (1:1) and 0.1 ml stabilizing solution (40 mM EDTA and 10% Hydroxylamine hydrochloride). Final pH of the solution was adjusted to 1.8 using NH_4OH. Test tubes were incubated in water bath at 50° C for 10 min. 0.5 ml of 2, 3-diaminonaphthalene (DAN) solution was added to each tube and further kept at 50° C for 30 min. DAN solution was prepared by dissolving 0.1 g DAN in to 100 ml of 0.1 N HCl containing 0.5 g of Hydroxylamine hydrochloride in an amber bottle and kept at 50° C for 30 min in water bath. After cooling, solution was extracted twice with 20 ml cyclohexane to remove impurities. The aqueous layer was then separated and stored in an amber bottle. Se^{+4} make complex with DAN and forms a fluorescence compound piazselenol. 2 ml cyclohexane was added in each test tube for the extraction of piazselenol. The cyclohexane layer after being washed with 5ml 0.1 N HCl and fluorescence was measured at 360 nm of excitation wavelength to obtained emission spectrum of piazselenol at 520 nm using fluorescence spectrometer (Perkin Elmer LS-45). Se quantification in each sample was carried out by relative method using emission spectrum of NIST certified Selenium ICP standard solution (SRM-1349). Calibration curve was prepared by using 2-10 ng/ml

Preparation of different extracts

Approximately 0.5 g wheatgrass crushed in liquid nitrogen and wheatgrass extracts were prepared by maceration using pestle and mortar, with 5 ml of different solvents such as 20% methanol, 90% methanol and 5% (w/v) metaphosphoric acid. The homogenates were centrifuged at 7000 rpm for 20 minutes at 4°C and the resulting supernatants were analyzed for future experiment.

DPPH radical scavenging assay

The method described by Kitts *et al*. (2000) was used with slight modifications in order to assess the DPPH radical scavenging capacity of wheatgrass extracts. A 100 µM DPPH (1, 1-diphenyl-2-picrylhydrazyl) solution in methanol (1.0 ml) was mixed with 100 µl of various wheatgrass extracts (90% methanol) and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded after 30 min. The DPPH scavenging capacity of samples was expressed as µg Quercetin equivalents/g of fresh weight using a standard curve prepared with Quercetin (5-50 µg/ml). The absorbance of the remaining DPPH radicals was measured at 517 nm using spectrophotometer. The scavenging of DPPH was calculated according to the following equation.

$$\% \text{ scavenging} = \{(Abs_{control} - Abs_{sample})/Abs_{control}\} \times 100$$

Where $Abs_{control}$ = absorbance of DPPH radical + methanol; and Abs_{sample} = absorbance of DPPH radical + wheatgrass extract/standard.

Determination of total phenolic content

The phenolic compounds in the various extracts were determined using the Folin-Ciocalteu (FC) reagent in a manner proposed by Singleton and Rossi (1965), based on the reduction of mixture of phosphate tungsten and molybdenum oxides complex by phenolics to give blue coloured product. To 100 µl of wheatgrass extract (90% methanol)/standard (gallic acid) 1 ml water, followed by 50 µl FC (1 N Folin-Ciocalteu) reagent was added. The mixture was mixed and incubated for 10 minutes at room temperature. To the above mixture, 200 µl of 5% sodium carbonate solution was added and incubated for 1 hrs at room temperature. After incubation, the absorbance was taken at 760 nm. The total phenolic content (TPC) was expressed as µg gallic acid equivalents/gram of fresh weight.

Determination of lipid peroxidation (TBARS Assay)

Lipid peroxidation in the various extracts was determined by using the method proposed by Minotti and Aust (1987) with some modifications. Lipid peroxides were extracted by grinding in an ice-cold mortar 0.5 g of wheatgrass with

5 ml of 5% (w/v) metaphosphoric acid and 100 µl of 0.2% (w/v) butyl hydroxytoluene (in ethanol). Homogenates were centrifuged at 7000 rpm for 20 min. The chromogen was formed by mixing 0.5 ml of supernatant, 50 µl of 0.2% (w/v) butyl hydroxytoluene, 250 µl of 1% (w/v) TBA (in 50 mm NaOH) and 250 µl of 2N HCl and by incubating the reaction mixtures at 95°C for 30 min. A blank for all samples was prepared by replacing the sample with extraction medium, and controls for each sample were prepared by replacing TBA with 50 mm NaOH. Reaction was stopped by cooling the samples in an ice bath. The chromogen formed was extracted by adding 1.5 mL of 1-butanol, the tubes were vigorously shaken, the organic (upper) phase was separated by low speed centrifugation, and the emission spectra of TBARS were recorded by fluorescence spectrometer at excitation wavelength of 532 nm and emission at 550 nm. For MDA determination calibration curves were determined using malodialdehyde tetrabutyl ammonium salt as standard.

Determination of total protein content in wheatgrass (Lowry's method)

The protein content of the wheatgrass extracts (20% methanol) were measured by Lowry's Method with slight modifications (Lowry *et al*, 1951). 50 µl of wheatgrass extracts (20% methanol) were mixed with 1ml of analytical reagent. The mixture was incubated for 10 min at room temperature. 100 µl of Folin- ciocalteau reagent was added to the above mixture. Mixed well and incubated for 30 min at room temperature. Absorbance was measured at 660 nm. The absorbance was plotted against protein concentration to get a standard calibration curve. The concentration of the unknown sample was determined by using BSA as standard (0.5-4.0 mg/ml).

4.0 Results and Discussion

Selenium Concentration

Selenium concentrations in wheat flour and wheatgrass were determined by relative method using NIST certified Selenium ICP standard solution. Se concentration in three wheat samples **SeW1**, **SeW2** and **NSeW** were 56.45 ± 2.19 µg/g, 12.20 ± 0.12µg/g and 1.86 ± 0.20 µg/g respectively and on the basis of their Se concentrations these samples were represented as high, moderate and normal/non-seleniferous wheat respectively. The fresh weight (FW) to dry weight (DW) ratio of wheatgrass was 1:7. Se concentrations in wheatgrass grown under different conditions were shown in *Table-1*. Statistical analysis indicated that there is a significant difference in Se levels under all growth conditions.

Table-1. Selenium concentration in wheatgrass (µg/g DW) under different growth conditions. Data are represented as mean ± SD (*n=4*). Values followed by the same letter are not statistically different at $P \leq 0.05$ (t-test).

S. No	Sample	Se concentration in wheatgrass (µg/g DW)		
		Dark	Light	UV
1.	SeW1	59.38 ± 2.41^{a}	58.54 ± 2.22^{a}	54.0 ± 0.83^{a}
2.	SeW2	23.74 ± 3.09^{b}	23.96 ± 3.08^{b}	24.28 ± 0.56^{b}
3.	NSeW	2.85 ± 0.15^{c}	2.37 ± 0.37^{c}	2.64 ± 0.08^{c}

Wheat seeds are rich source of nutrients which provide nutrient reserves to sustain the growing seedling until it establishes itself as a self-sufficient autotrophic organism. It is well known that when Se enters to the plant, it get incorporated into protein moiety through Se-cystine (Se-Cys) and Se-methionine (Se-Met) (Terry *et al*. 2000). During the seed germination crude protein started decreasing earlier in grains than starch, indicating that faster protein breakdown and translocation to the embryonic tissue (Evelyn and Bienvenido, 1971). Results indicate that a major class of selenium rich storage proteins get metabolized and help the seedling growth. As mentioned above, SeMet is incorporated into general proteins and proteins having

the SeMet residue(s) are called Se-containing proteins. In contrast to Se-containing proteins, proteins having the SeCys residue(s) are named selenoproteins, e.g., glutathione peroxidase (GPx). Although there is mounting evidence that Se is required for the growth of algae (Price *et al*. 1987), the question of the essentiality of Se as a micronutrient in higher plants is unresolved and remains controversial. There are indications that Se may be required for Se-accumulating plants, which are endemic to seleniferous soils. Se existing predominantly as selenate is taken up by plants from soil. Sulfate competes with selenate for absorption by root, suggesting that selenate shares the sulfate transporter(s) expressed on roots (Ulrich and Shrift, 1968; Shrift and Ulrich, 1969). An independent study was carried out by Smrkolj *et al*. (2006) on pea plant wherein plants were treated foliarly once (OT) and twice (TT) with Se solution during their flowering period. Seeds obtained from these plants contained 383 and 743 ng Se g^{-1} respectively. After germination of these seeds higher Se contents were found in the leaves than in the stems of plants grown from both OT and TT seeds. The proportion of Se translocated from seeds to leaves and stems of progeny plants was calculated on the basis of Se contents of the individual parts and their lyophilized mass. In the OT and TT groups, 35%, on average, of the Se in the seed was translocated to the leaves and stems, irrespective of the initial Se content in the seeds. By contrast, in the UT group, 95% of the Se in the seed was transferred to leaves and stems. This suggests that Se has a specific role in the plant.

DPPH Radical Scavenging Assay

The DPPH method is considered as a simple, rapid and the most convenient method. It is independent of sample polarity for screening of many samples for radical scavenging activity. DPPH is a stable free radical in a methanolic solution. In its oxidized form the DPPH radical has deep violet color in solution and has an absorbance maximum centered at about 520 nm (Molyneux, 2004). When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of its violet color to a residual pale yellow color. Methanolic extract of wheatgrass rich in polyphenols, flavonoids, carotenoids etc. and these compounds easily donate H-atom for the reduction of DPPH radical. In present study, free radical scavenging capacity of 90% methanol

extracts obtained from wheatgrass grown under different condition were analyzed. µg Quercetin Equivalents/g of fresh weight and % Radical Scavenging capacity of different extracts were shown in *Table-2a & b*. Statistical analysis indicated that there is a significant difference in Quercetin equivalent antioxidant concentrations and % scavenging capacity with increase in Se concentration in wheatgrass.

Table-2a: µg Quercetin Equivalents/g of fresh weight of different extracts. Each column and row was tested separately. Data are mean±SD (*n=3*). Values followed by the same letter are not statistically different at $P \leq 0.05$ (t-test) and first shows significance difference in column and second in rows.

S. No	Sample	DPPH scavenging assay of wheatgrass (µg Quercetin Equivalents/g of fresh weight)		
		Dark	Light	UV
1	SeW1	602.80 ± 4.41^{aa}	597.47 ± 11.55^{aa}	622.67 ± 10.47^{ab}
2	SeW2	427.96 ± 1.98^{ba}	498.38 ± 14.35^{bb}	556.75 ± 7.16^{bc}
3	NSeW	354.04 ± 13.23^{ca}	462.33 ± 13.41^{cb}	528.96 ± 6.72^{cc}

Table-2b: Quercetin Equivalent % Radical Scavenging capacity of different extracts. Each column and row was tested separately. Data are mean±SD (*n=3*). Values followed by the same letter are not statistically different at $P \leq 0.05$ (t-test) and first shows significance difference in column and second in rows.

S. No	Sample	DPPH scavenging assay of wheatgrass (% radical scavenging)		
		Dark	Light	UV
1	SeW1	84.01 ± 0.62^{aa}	83.23 ± 1.63^{aa}	86.86 ± 1.48^{ab}
2	SeW2	59.27 ± 0.28^{ba}	69.24 ± 2.03^{bb}	77.50 ± 1.01^{bc}
3	NSeW	48.77 ± 1.87^{ca}	64.12 ± 1.90^{cb}	73.55 ± 0.95^{cc}

The results presented the decrease in absorbance of the DPPH radical due to the scavenging ability of soluble polyphenolics in extracts of different wheatgrass. Se enriched wheatgrass extracts were found to possess good DPPH scavenging activity. All samples showed a rapid decrease in absorbance. The estimation of DPPH scavenging activity of wheatgrass extracts was carried using straight line equation ($y = -10.358x+0.7355$; $r^2 = 0.9975$).

The radical scavenging capacity was expressed as µg Quercetin equivalents per gram of fresh weight of extract. Effect of growth conditions also showed significant difference on DPPH scavenging ability. The radical scavenging ability of wheatgrass extracts increased in the following order: Dark< Light< UV. Levels of Se in wheatgrass also seem to significantly influence the DPPH scavenging activity. High Se (SeW1) containing wheatgrass indicated higher scavenging activity than moderate (SeW2) and normal (NSeW) samples. Differences in DPPH scavenging capacity in between SeW1 and NSeW are 41%, 23% and 15% when grown under dark, light and UV respectively. Results obtained are clearly indicated that Se induces the antioxidant capacity in wheatgrass. Whereas effect of growth conditions shows less significant difference in scavenging capacity in the case of SeW1 rather than SeW2 and NSeW. Increase in the antioxidant ability of cereals grown under UV-B is due to increased flavonoids (saponarin and lutonarin) accumulation in both the lower epidermis and the mesophyll: about 40% of the saponarin and 20% of the lutonarin were in the lower epidermis of leaves (Lan *et al*. 1995), which seem to also be influenced by the presence of selenium. The growth of radish plants was promoted by the UV-A radiation, and the promotion by UV-A radiation was associated with an increase in chlorophyll content and photosynthetic activities. Furthermore, nitrate reductase activities, levels of soluble protein, levels of vitamin C (ascorbic acid + dehydroascorbic acid) and levels of nicotinamide coenzymes (NAD(H) and NADP(H)) in leaves and in the so-called globe "root", which includes stem tissue in radish plants, were also elevated by UV-A radiation (Tezuka *et al*. 1994).

Total Phenolic Content (TPC)

Plants are rich in natural antioxidants (Kamatha *et al*. 2004; Hollman *et al*. 1999). Among these, the phenolic compounds have ability to scavenge free radicals, super oxide and hydroxyl radicals through oxidation reactions. Total phenolic content of an extract can be evaluated with spectrophotometer method using Folin-Ciocalteu reagent. The principle of this method is reduction ability of phenol functional group. Oxidation and reduction reaction of phenolate ion takes place in basic environment. The reduction of phosphotungstate-phosphomolibdenum complex

(Folin-Ciocalteu reagent) by phenolate ion will change its color to be blue (Prior *et al*. 2005). The reduction of complex will increase when the extract contain more phenolic compounds. Thus, the color will be darker and the absorbance will be higher. The estimation of TPC of wheatgrass extracts was carried using straight line equation ($y = 0.006x - 0.0304$, $R^2 = 0.993$). The total phenolic content was expressed as µg gallic acid equivalents (GAE) per gram of fresh weight of extract. The results obtained in *Table.3* indicated that the selenium content also has prominent influence on TPC in wheatgrass with SeW1 extract showing higher TPC content in comparison to other wheatgrass extracts. TPC in SeW1 are 51%, 26% and 22% higher in comparison to NSeW, when grown under dark, light and UV respectively. Similarly effect of growth conditions showed significant difference in TPC and increased in following order Dark< Light< UV except SeW1. High selenium enriched wheatgrass indicated exceptionally high TPC when grown in dark and 15% more TPC in comparison to UV.

Table 3: Total Phenolic content of different extracts. Each column and row was tested separately. Data are mean±SD (*n=3*). Values followed by the same letter are not statistically different at $P \leq 0.05$ (t-test) and first shows significance difference in column and second in rows.

S. No	Sample	Total Phenolic content (µg Gallic Acid Equivalents/g of fresh weight)		
		Dark	Light	UV
1	SeW1	1133.82 ± 20^{aa}	944.36 ± 6.64^{ab}	972.26 ± 8.71^{ac}
2	SeW2	630.40 ± 10.74^{ba}	792.52 ± 15.35^{bb}	870.68 ± 11.27^{bc}
3	NSeW	550.44 ± 10.63^{ca}	695.64 ± 13.53^{cb}	749.21 ± 18.52^{cc}

Plant phenolics also have a beneficial role during oxidative burst, and improvement of vigour and germination under stress (Bradford, 1986; Bolwell *et al*. 1998). In the case of corn sprouts the general trend of developmental regulation of total phenolics was a steady increase with germination. This is justifiable because higher phenolic synthesis was required for lignification with growth and the total phenolic content of control (light) increased almost three-fold from day 1 (1.3 mg/g FW) to day 8 (4 mg/g FW) of dark germination (Randhir and Shetty, 2005). Phenolics, especially flavonoids and phenylopropanoids are oxidized by peroxidase

and act in H_2O_2 scavenging system. Their antioxidant action resides mainly in their chemical structure. There is some evidence of induction of phenolic metabolism in plants as a response to multiple stresses, including heavy metal stress (Michalak, 2006). Barley (*Hordeum vulgare*) and radish (*Raphanus sativus*) seedlings when continuously irradiated under UV-B for 5–10 d, in all plant species, the fresh weight, the leaf area, the amounts of chlorophylls, carotenoids and the galactolipids of the chloroplasts were reduced but the content of flavonoids increased in barley and radish seedlings by about 50%. UV radiation induces the accumulation of UV-absorbing flavonoids and other phenolic compounds mainly in vacuoles of epidermal cells, to prevent the penetration of UV-B into the deeper tissues of the plant (Kondo and Kawashima, 2000).

Total Protein Content

Wheatgrass is rich in protein and contains all 8 essential amino acids. It is also rich in metabolic enzymes like protease, amylase, lypase, cytochrome oxidase, transhydrogenase, superoxide dismutase (SOD) etc. total protein content in wheatgrass extracts were determined by Lowry's method. The principle behind the Lowry method of determining protein concentrations is under alkaline conditions cupric ions (Cu^{2+}) chelate with the peptide bonds resulting in reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{+}) and oxidation of tyrosine and tryptophan present in the protein. Cuprous ions (Cu^{+}) reduction of Folin Ciocalteu Reagent produces a blue color that can be read at 650-750nm. The estimation of total protein content in wheatgrass extracts was carried using straight line equation (0.2133x + 0.2191 R^2 = 0.9874). The total protein content was expressed as mg BSA equivalents per gram of fresh weight of extract. The results obtained in *Table-4* indicate that protein content in wheatgrass is Se dependent and significantly increases with increase in Se concentration. Effect of growth conditions also shows significant difference in total protein content and increased in following order Dark< Light< UV.

The major mechanism whereby high Se accumulation in plant tissues induces Se toxicity is almost certainly associated with the incorporation of SeCys and SeMet into proteins in place of Cys and Met, respectively (Brown and Shrift, 1981). The differences in size and ionization properties of S and Se may result in significant

alterations in protein structure. The bond between two Se atoms is approximately one seventh longer and one fifth weaker than the disulfide bond (Brown and Shrift, 1981).

Table 4: Total Protein content of different extracts. Each column and row was tested separately. Data are mean±SD (*n=3*). Values followed by the same letter are not statistically different at $P \leq 0.05$ (t-test) and first shows significance difference in column and second in rows.

S.No	Sample	Total Protein content (mg BSA Equivalents/g of fresh weight)		
		Dark	Light	UV
1	SeW1	28.79 ± 0.21^{aa}	29.17 ± 0.58^{aa}	42.98 ± 2.9^{ab}
2	SeW2	24.49 ± 0.11^{ba}	26.33 ± 0.50^{bb}	32.76 ± 0.26^{bc}
3	NSeW	22.24 ± 0.63^{ca}	24.24 ± 0.94^{bb}	30.52 ± 0.26^{cc}

Therefore, the incorporation of SeCys in place of Cys into protein could interfere with the formation of disulfide bridges, resulting in a slightly altered tertiary structure of S-proteins and a negative effect on their catalytic activity (Brown and Shrift, 1981). Furthermore, Se may diminish the rate of protein synthesis; this is because the substitution of SeMet for Met into proteins may be less effective as a substrate for peptide bond formation during translation (Eustice *et al*.1981). Most probably the first positive effect of Se on plant growth was reported by Singh *et al*. (1980), who showed that the application of 0.5 mg kg^{-1} Se as selenite stimulated growth and dry matter yield of Indian mustard (*Brassica juncea* L.). More recently, it was revealed that Se, applied at low concentrations, enhanced growth and antioxidative capacity of both mono- and dicotyledonous plants. The growth-promoting response to Se was demonstrated in lettuce and ryegrass (*Lolium perenne* L.) (Hartikainen *et al*. 1997; Hartikainen and Xue, 1999) and in soybean (*Glycine max* L.) (Djanaguiraman *et al*. 2005).

The first line of UV-B defense is to limit the penetration of UV-B within the tissue. Exposure results in the accumulation of flavonoids, synaptic esters and anthocyanins, pigments which selectively attenuate UV-B radiation (Caldwell *et al*. 1983). The increase in the level of these pigments is due to a co-ordinated increase in the expression and activity of the enzymes of the phenylpropanoid pathway

(Kubasek *et al*. 1992). Flavonoids are produced primarily in the epidermal layers of the leaves and are thought to absorb a large proportion of the incident UV-B radiation before it penetrates the tissues (Schmelzer *et al*. 1988). Specifically, as UV-B radiation leads to ROS generation and oxidative stress, exposure results in increases in the expression and activity of a number of antioxidant enzymes including ascorbate peroxidase, superoxide dismutase and glutathione reductase (A-H-Mackerness *et al*.1998).

TBARS Assay

The lipid perxidation of methanolic extract of wheatgrass was measured by TBARS assay. Thiobarbituric acid reactive species (TBARS) are formed as the byproduct of lipid peroxidation which can be detected by TBARS assay using Thiobarbituric (TBA) acid as a reagent. TBARS assay measures MDA (malondialdehyde) present in the sample. MDA is the byproduct formed via the decomposition of certain primary and secondary lipid peroxidation products. Malondialdehyde forms adduct with TBA. On boiling it produces pink colored fluorescent complex, which shows excitation at 532 nm and emission at 550 nm. In present study the lipid peroxidation of 5% metaphosphoric acid extract from wheatgrass grown under different conditions were analyzed. ng MDA Equivalents/g of fresh weight of different extracts was shown in *Table-5*. Statistical analysis indicated that there is significant difference in TBARS content and its concentration is significantly decreases with increase in Se levels in wheatgrass. TBARS content in SeW1 are 85%, 81% and 95% less in comparison to NSeW, when grown under dark, light and UV respectively.

Table 5: TBARS content of different extracts. Each column and row was tested separately. Data are mean±SD (*n=3*). Values followed by the same letter are not statistically different at $P \leq 0.05$ (t-test) and first shows significance difference in column and second in rows.

S.No	Sample	TBRS Assay (ng MDA Equivalents/g of fresh weight)		
		Dark	Light	UV
1	SeW1	35.53 ± 2.79^{aa}	24.79 ± 4.34^{ab}	18.73 ± 2.54^{ab}
2	SeW2	201.24 ± 11.40^{ba}	73.80 ± 1.47^{bb}	187.35 ± 13.39^{ba}
3	NSeW	228.04 ± 8.23^{ca}	131.68 ± 1.32^{cb}	371.37 ± 12.48^{cc}

Se has not yet been classified as an essential element for plants, although its role has been considered to be beneficial for plants that are is capable of accumulating large amounts of the element (Shanker, 2006).The role of Se in plant depends mainly on its concentration. According to Hamilton (2004), Se has three levels of biological activity: (1) trace concentrations are required for normal growth and development; (2) moderate concentrations can be stored to maintain homeostatic functions and (3) elevated concentrations can result in toxic effects. Studies on ryegrass (*Lolium perenne*) and lettuce (*Lactuca sativa*) showed that, although Se is harmful for the plants at high concentrations >10 and 1.0 mg kg^{-1}, respectively (reduction of biomass), it can exert beneficial effects at low concentrations, namely 0.1 mg kg^{-1} soil (Hartikainen *et al*. 2000; Xue *et al*. 2001). Rios *et al*. (2009) showed the effect of different application rates (5, 10, 20, 40, 60, 80 and 120 μM) of selenite or selenate on the production and detoxification of H_2O_2 in lettuce plant in non-stressed condition. The results indicate that the selenate form of Se is less toxic than selenite. On the contrary, the application of selenite triggered a higher foliar concentration of H_2O_2 and a higher induction of lipid peroxidation (MDA content and LOX activity) in comparison to that observed after the selenate application. Also, the plants treated with selenate induced higher increases in enzymes that detoxify H_2O_2, especially ascorbate peroxidase (APX) and glutathione peroxidase (GPX), as well as an increase in the foliar concentration of antioxidant compounds such as ascorbate (AsA) and glutathione (GSH).

Present data indicate that an application Se enriched wheatgrass can be used to promote the induction and enhancement of antioxidant system in plants. In addition, the Se induced anti-oxidant activity is significantly influence by the stress induced during growth conditions.

5.0 References

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