Selenium in Higher Plants: Physiological Role, Antioxidant Metabolism and Abiotic Stress Tolerance

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Abstract: It is now well established that virtually all abiotic and biotic stresses induce or involve oxidative stress to some degree and the ability of plants to control oxidant levels is highly correlated with stress tolerance. Recently, the role of Selenium (Se) as an antioxidant has generated a wide interest in it. In trace amounts, Se is an essential micronutrient and has important benefits for animal and human nutrition although it has not been confirmed to be an essential micronutrient in higher plants. Selenium has been shown to exert a positive effect on crop growth and stress tolerance at low concentrations. However, the specific physiological mechanisms that underlie the positive effects of Se in plants have not been clearly elucidated. There is a wealth of evidence that low concentrations of Se enhance the growth of plants and improve antioxidative capacity of plants either by acting as antioxidant directly or by increasing the activities of antioxidant enzymes and bring out the tolerance under stressful condition. In contrast at high concentrations, Se acts as a pro-oxidant and leads to drastic reduction in yield. In this study we reviewed available literature regarding the possible regulatory role of Se in the various physiological processes of plants as well as its protective role under abiotic stress condition.

Keywords: Selenium, reactive oxygen species, trace element, abiotic stress, antioxidant defense, plant metabolism

INTRODUCTION

Selenium (Se) is a non-metal, whose Greek name (Selene) means moon. Selenium was first discovered by J.J. Berzelius in 1817. He found a residual slime forming during the oxidation of sulfur (dioxide) from copper pyrites. Se, similar to sulfur (S) as regards its chemical properties, has been demonstrated as trace element in human and animal based on its presence in antioxidant defense systems which is needed for the maintenance of animal and human health (Schwartz and Foltz, 1957; Flohe et al., 1973; Rotruck et al., 1973) and in

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hormonal balance (Arthur et al., 1990; Pallud et al., 1997). During last two decades the physiological role of Se in plants has been explored by researchers. In plants Se can be found both in inorganic and organic Se forms, including selenocysteine acids and methylated compounds. Current interest in Se is focused on the health benefits of high-Se plants as a source of cancer preventative Se compounds (Finley et al., 2000, 2001) and the metabolism of Se in plant species that accumulate Se and are able to remEDIATE Se-polluted soils and prevent Se from entering into the food chain (Berkem et al., 2002). However, there is evidence that Se might be an essential micronutrient for accumulator plants species (Terry et al., 2000; Sors et al., 2005). Moreover, it has been shown that Se is required for an optimal growth of the unicellular green alga. In contrast, Se non-accumulator plants, including most species of crops, do not appear to require Se for their growth and in general, these plants have a low tolerance to this element (Terry et al., 2000). The essentiality of Se to higher plants, however, is still under debate. Although it is harmful for plants in high concentrations, it can exert beneficial effects at low concentrations (Germ et al., 2007a).

Although Se is not yet confirmed to be required by higher plants (Terry et al., 2000), several studies demonstrate that at low concentrations it may exert diverse beneficial effects, including growth-promoting activities (Hartikainen and Xue, 1999; Terry et al., 2000; Xue et al., 2001; Turakainen et al., 2004; Djanaguiraman et al., 2005). Moreover, some plant species grown in Se-enriched media have shown enhanced resistance to certain abiotic stresses, e.g. drought (Kuznetsov et al., 2003; Germ et al., 2007b; Yao et al., 2009), salinity (Kong et al., 2005; Djanaguiraman et al., 2005; Hawrylak-Nowak, 2009), chilling (Cmi et al., 2010; Hawrylak-Nowak et al., 2010), heavy metals (Fargashova et al., 2006; Hawrylak et al., 2007, Pedreno et al., 2008, Filek et al., 2008, Srivastava et al., 2009; Cartes et al., 2010) and UV-imradiation (Valkama et al., 2003; Hartikainen and Xue, 1999; Yao et al., 2010a, b) stresses. Se exerts beneficial effects on growth and stress tolerance of plants by enhancing their antioxidative capacity (Hartikainen and Xue, 1999, Xue and Hartikainen, 2000, Xue et al., 2001, Djanaguiraman et al., 2005; Kong et al., 2005; Rios et al., 2009). Se increases plant resistance against oxidative stress caused by free oxygen radicals. However, agricultural crop plants are sensitive to high Se concentrations which vary among plant species (Hartikainen et al., 2000, 2001; Rani et al., 2005; Lyons et al., 2005).

Nevertheless, the specific physiological and molecular mechanisms that underlie the beneficial effects of Se in plants have not been fully explained yet. In this review, we discuss the progress in understanding the possible role of Se in plant growth and its involvement in the antioxidant metabolism towards abiotic stress tolerance in plants.

**SELENIUM IN SOIL ENVIRONMENT**

In the soil, Se may be present in four different oxidation states: selenate (+6), selenite (+4), elemental Se (0) and as inorganic and organic selenide (-2). The chemical form, the soil redox potential, pH and clay content determine the bioavailability of Se in the soil (Gissel-Nielsen, 1971; Mikkelsen et al., 1989; McNeal and Balistreri, 1989). The predominant Se inorganic forms in cultivated soils are selenate and selenite. Selenate is more soluble and available for plants under oxidized and alkaline soil conditions (Masscheleyn et al., 1990; Mayland, 1994). Selenite is less available to plants than selenate because it is adsorbed more strongly by iron oxide surfaces and soil clays (Ylaranta, 1983a, 1985; Mikkelsen et al., 1989).

Selenium content of most soils varies between 0.1 and 2.0 mg kg⁻¹ depending on geographical area (Mayland, 1994; Dhillon and Dhillon, 2003). The Se concentration of soil
depends on the composition of bedrock from which the soil component derives and the geochemical processes that produce the soil. In clay soils, Se content was generally higher than one coarse mineral soils. The mean Se content in clay soils was 0.29 mg kg⁻¹ and in coarse mineral soils 0.17 mg kg⁻¹. The highest Se concentration (0.46 mg kg⁻¹) was measured from organic soils (Koljonen, 1975; Sippola, 1979; Ylaranta, 1983b).

The plant-availability of Se in soil is largely depend on the predominant Se species and the soil factors controlling their behavior, such as the quantity of the sorption components (Al and Fe oxides), pH and redox status. Furthermore, the presence of anions (sulfate, phosphate, organic anions, etc.) competing for the same sorption surfaces contributes to the retention of Se (Elrashidi et al., 1987; Neal, 1995; Fordyce, 2005; Hartikainen, 2005).

SELENIUM UPTAKE AND METABOLISM IN PLANTS

Plants play a unique role in recycling and delivering Se from the soil to the food chain. The concentration of Se in agricultural products and fodder depends on the content of Se in the soil and its bioavailability (Sippola, 1979; Koivistoinen, 1980; Ylaranta, 1983a, 1985). Availability of Se is restricted in soils and its content is relatively low as a result of reduced weathering status and acidity (Koljonen, 1975; Sippola, 1979; Ylaranta, 1983b). Plant roots take up Se from soil water in either the selenate or the selenite ionic forms. In higher plants metabolism of Se is closely related to that of sulfur due to their chemical similarity. Quantities in the soil solution are governed by the solubility of adsorbed forms and by the biological transformation of organic forms (Fig. 1).

Fig. 1: Selenium in the Soil-Plant-Water consumer system. Plant roots take up selenate or selenite forms of Se from the soil water. The Se concentration in the soil solution depends on the solubility of the forms of Se present and the biological transformation of organic forms (Burau, 1985)
Higher plants have different capacities to accumulate and tolerate Se. They are classified into non-accumulators, indicators and accumulators (Rosenfeld and Beath, 1964; Terry et al., 2000; Dhillon and Dhillon, 2003; White et al., 2004). Approximately 25 genera of plants are classed as Se accumulators (Bura, 1985) which includes garlic (Allium sativum L.), onion (Allium cepa L.), broccoli (Brassica oleracea L.) and wild leek (Allium tripaecum L.) (Neuhriel et al., 1999; Whanger, 2002). Some particular plant species are termed Se hyperaccumulators. The largest group of hyperaccumulators belongs to the genus Astragalus and Stanleya (Rosenfeld and Beath, 1964; Neuhriel et al., 1999; Terry et al., 2000). The Se hyperaccumulators are placed into two groups: the primary Se accumulators are able to accumulate thousands of milligrams of Se kg⁻¹ (>4000 mg kg⁻¹) and the secondary accumulators hundreds of milligrams Se kg⁻¹. Brassicaceae species including Indian mustard (Brassica juncea L.), broccoli (Brassica oleracea botrytis L.) and canola (Brassica napus sp. oleifera L.) have been classified as primary accumulators. Plant species with a high capacity to accumulate and tolerate Se could be used in the phytoremediation of Se-contaminated sites (Terry et al., 2000; Berken et al., 2002). However, most cultivated crop plants have a low tolerance to high Se levels. Generally, they contain less than 25 μg Se g⁻¹ DW and are considered to be non-accumulators. Potato is classified as a Se non-accumulator (White et al., 2004). Although non-accumulators are sensitive to high Se concentration, they can tolerate as well as accumulate even high concentrations of Se without growth reduction when grown in Se-enriched soils (Rani et al., 2005). The critical Se concentration in plant tissues, which decreased the yield in Indian mustard was 105 μg g⁻¹ DW, in maize (Zea mays L.) 77 μg g⁻¹ DW, in rice (Oryza sativa L.) 42 μg g⁻¹ DW and in wheat 19 μg g⁻¹ DW, a levels attained by Se addition as selenite of 5 μg g⁻¹ soil for Indian mustard and maize, 4 μg g⁻¹ soil for wheat and 10 μg g⁻¹ soil for rice (Rani et al., 2005).

Se uptake and metabolism also differ due to the plant species, growth stage and the plant organs. Broccoli (Brassica oleracea var. italica) is known for its ability to accumulate high levels of Se, with the majority of the selenocysteine acids in the form of Se-Met (SeMeSeCys) (Lyi et al., 2005). Increased Se fertilization results in Se concentration in potato (Munshi and Mondy, 1992; Poggi et al., 2000; Turakainen et al., 2004). The majority of plants accumulate more Se in shoot and leaf than in root tissues, but there are exceptions (Zayed et al., 1998). Turakainen (2007) observed that the Se concentrations in the upper leaves, roots, stolons and tubers of potato increased with increasing Se supplementation. The highest Se concentration was reached in young upper leaves, roots and stolons, indicated that added selenite was efficiently utilized and taken up at an early stage. During the growing period the Se concentration declined in the aerial parts, roots and stolons of potato plants whereas an intensive accumulation took place in immature and mature tubers (Turakainen et al., 2006; Turakainen, 2007). Se accumulation was also affected by the methods of application. In tea plants, foliar application with selenite significantly increased Se content in the leaves (Hu et al., 2003). The results of Smrkolj et al. (2006) showed that the Se content of pea seeds obtained from untreated and once and twice foliarly-treated plants was directly proportional to the number of sprayings.

From several studies it is clear that Se is taken up from the soil by plants primarily as selenate (SeO₄²⁻) or selenite (SeO₃⁻) (Ellis and Salt, 2003). Lyons et al. (2005) suggested that for higher toxicity of selenite compared to selenate is due to the faster incorporation of selenite than selenate. In addition, the uptake of selenate into roots and its distribution in plants is much faster than that of selenite (Arvy, 1993; De Souza et al., 1998; Pilon-Smits et al., 1998; Cartes et al., 2005). De Souza et al. (1998) reported that total Se accumulation in a plant was about 10-fold higher from selenate compared to selenite.
Kabakachhi et al. (2004) stated that actively growing tissues usually contain the largest amounts of Se. The metabolism of Se in plants postulated by Dumont et al. (2006) is shown in Fig. 2.

**ROLE OF SELENIUM IN PLANT GROWTH AND PHYSIOLOGY**

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 capable of accumulating large amounts of the element (Shanker, 2006). The role of Se in plants 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).

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). Se can also delay senescence and promote the growth of aging seedlings (Hartikainen and Xue, 1999; Xue et al., 2001).

Se has also demonstrated its effect on germination. Carvalho et al. (2003) reported that at higher supplementation level than 29 mg kg⁻¹ soil, Se inhibited the growth and germination of tomato, lettuce and radish (*Raphanus sativus* L.) seeds. In contrast, priming of seeds with
selenite promoted germination of bitter gourd (*Momordica charantia* L.) seeds at suboptimal temperatures (Chen and Sung, 2001). The positive effect on germination was linked to antioxidative activity.

Foliar application, performed manually only on plants, is the most appropriate way to add Se, since contamination of the soil is minimal. Foliar application to barley at 10 and 20 g Se ha⁻¹, as sodium selenate, increased the Se contents of barley grain and straw and red clover forage (MacLeod et al., 1998). A stimulatory effect of foliar application of Se on growth has been reported for ryegrass (Hartikainen et al., 2000), lettuce (Xue et al., 2001), potatoes (Turakainen et al., 2004) and green tea leaves (Hu et al., 2003). Se affected plant growth promotion might be the result of increased starch accumulation in chloroplasts (Penna et al., 2002) and that protected cell content (Xue et al., 2001).

Simojoki (2003) reported that small Se addition that increased Se contents in lettuce shoots up to 1-5 mg kg⁻¹ dry matter (DM) to enhance plant growth. But the yields drop drastically at Se contents above 20 mg kg⁻¹ DM. Se fertilization changes root morphology and the effects are diverse in different parts of the root system. Moderate Se additions decrease the specific length and specific surface area of basal and lateral roots, whereas large additions increase the specific volume of roots (Simojoki, 2003). In potato plant, Se increased carbohydrate accumulation in the young upper leaves and in stolons, roots and tubers at maturity. However, it could not be explained by increased production of photoassimilates as net photosynthesis did not differ among Se treatments. The Se treated plants produced higher tuber yields than control plants and at the highest Se concentration (0.3 mg kg⁻¹) lower numbers of larger tubers were harvested. Increased yield of Se treated plants suggested that Se may enhance the allocation of photoassimilates for tuber growth, acting as a strong sink for both Se and for carbohydrates. It was also observed that Se improves the processing and storage quality of potato tubers (Turakainen et al., 2004; Turakainen, 2007). The positive impact of Se on the yield of potato plants could be related to its antioxidative effect in delaying senescence. Se at a concentration of 1.5 mg L⁻¹ increased yield in pumpkins (*Cucurbita pepo*) (Germ et al., 2005). Some possible roles of Se studied by different researchers are presented in Table 1.

<table>
<thead>
<tr>
<th>Se mediated effects</th>
<th>Observed plant species</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Stimulate growth and dry matter accumulation</td>
<td>Indian mustard</td>
<td>Singh et al. (1980)</td>
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<td>Beneficial effect on the growth</td>
<td>Alfalfa, subterranean clover</td>
<td>Breyer et al. (1966)</td>
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<td></td>
<td>Lettuce</td>
<td>Pennanen et al. (2002)</td>
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<td></td>
<td>Green tea</td>
<td>Hu et al. (2003)</td>
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<tr>
<td>Enhanced growth and antioxidative capacity</td>
<td>Lettuce, ryegrass</td>
<td>Hartikainen et al. (1997), Hartikainen and Xue (1999)</td>
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<td></td>
<td>Soybean</td>
<td>Djumaguliryan et al. (2005)</td>
</tr>
<tr>
<td>Increase growth and quality of tuber</td>
<td>Potato</td>
<td>Turakainen (2007)</td>
</tr>
<tr>
<td>Promote shoot and root growth</td>
<td>Ryegrass</td>
<td>Cartes et al. (2010)</td>
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<tr>
<td>Nitrogen assimilation</td>
<td>Barley</td>
<td>Azam et al. (1996)</td>
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<tr>
<td>Increase respiratory potential</td>
<td><em>Ptoxo sativa</em></td>
<td>Germ and Oswald (2005)</td>
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<td>Induced starch accumulation in chloroplasts</td>
<td>Lettuce</td>
<td>Pennanen et al. (2002)</td>
</tr>
<tr>
<td>Increased seed dry matter production</td>
<td>Soybean</td>
<td>Djumaguliryan et al. (2005)</td>
</tr>
<tr>
<td>Promote germination</td>
<td>Bitter gourd</td>
<td>Chen and Sung (2001)</td>
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<tr>
<td>Increase the antioxidative capacity of senescing plants</td>
<td>Lettuce, ryegrass</td>
<td>Xue et al. (2001)</td>
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<td>and delay senescence</td>
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<tr>
<td>Regulate the water status of plants</td>
<td>Wheat</td>
<td>Kurnetsov et al. (2003)</td>
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<tr>
<td>Delay the death of plants subjected to severe UV-stress</td>
<td>Lettuce</td>
<td>Pennanen et al. (2002)</td>
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<tr>
<td>Promote growth of plant subjected to short UV-B episodes</td>
<td>Lettuce</td>
<td>Hartikainen and Xue (1999)</td>
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ANTIOXIDATIVE ROLE OF SELENIUM

Oxidative stress describes a condition when the generation of reactive oxygen species (ROS) in a system exceeds the system’s ability to neutralize and eliminate them (Sies, 1985, 1986; Sies and Cadenas, 1985). The imbalance can result from a lack of antioxidant capacity caused by disturbance in production, distribution, or by an overabundance of ROS from endogenous sources or environmental stressors. If not regulated properly, excess ROS can damage cellular lipids, proteins or DNA, thus inhibiting signal transduction pathways, and, in general, normal cellular function.

Recent researches have demonstrated that Se not only able to promote growth and development of plants but also increase resistance and antioxidant capacity of plants subjected to various stresses (Hartikainen and Xue, 1999, Djanaguiraman et al., 2005; Peng et al., 2002). The beneficial effect of Se in plants subjected to stress conditions has in most cases been attributed to increased antioxidant activity. Research works conducted by Xue et al. (2001) and afterwards by Djanaguiraman et al. (2005) showed the effect of Se application in the form of selenate on senescence in lettuce and soy, confirming that the decline in antioxidant enzyme activity was milder in plants treated with this element, which offsets oxidative damage by boosting growth in plants treated with Se.

However, 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 H₂O₂ 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 H₂O₂ 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 H₂O₂, 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). These data indicate that an application of selenate at low rates can be used to promote the induction in plants of the antioxidant system, thereby improving stress resistance.

Nowak et al. (2004) showed a significant change in the activities of oxidoreductase enzymes in response to added Se in wheat plants. The nature of the changes can not be clearly determined, however, they were found to depend on both the concentration of Se and
the enzyme. However, the greenhouse experiment revealed an increase in activity of both catalase (CAT) and peroxidase (POD) in plants, treated with Se at 0.05 mM kg\(^{-1}\), whereas these activities were respectively, reduced and increased at 0.15 mM kg\(^{-1}\). The highest concentration (0.45 mM kg\(^{-1}\)) caused a reduction in both enzyme activities. Comparing these results to those of other authors, it can be generalized that the lowest Se concentration positively affected the antioxidant defense in wheat plants, but higher concentrations provoked stress responses. Hence, higher concentrations can be regarded as a prooxidant. Nowak et al. (2004) also reported that, a significant increase in nitrate reductase activity was observed in the later development stages of wheat plants grown on Se treated soil, probably due to incorporation of selenocystein to one of the active sites, where NAD(P)H is bound. Enzymes with selenocystein in the active site acquire a greater, more powerful redox potential towards their substrates, because selenocysteine has a greater nucleophilic power and a lower pK than cysteine (Eshkhat et al., 1997; Campbell, 2001).

Se acted as an antioxidant, inhibiting lipid peroxidation in ryegrass (Lolium perenne) in the concentrations 0.1 and 1.0 mg Se kg\(^{-1}\) (Hartikainen et al., 2000). 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). Several studies have shown that a protective role of Se against the oxidative stress in higher plants coincided with enhanced GPX activity and decreased lipid peroxidation (Xue and Hartikainen, 2000; Djanaguiraman et al., 2005; Cartes et al., 2005). Senescence processes are partly delayed due to enhanced antioxidation, which is associated with an increase of GPX activity (Hartikainen et al., 2000). The positive relationship between the Se concentration and GPX activity suggests the presence of Se-dependent GPX (Hartikainen et al., 2000). Cartes et al. (2005) demonstrated that selenite was more efficient than selenate as an inducer of GPX activity in ryegrass plants. Takeda et al. (1997) demonstrated that in algae, \(\text{H}_2\text{O}_2\) was eliminated primarily by the enzyme APX in the absence of Se; however, when this trace element was applied, the \(\text{H}_2\text{O}_2\) was detoxified mainly by GPX. However, apart from GPX, other enzymes such as SOD form an integral part in oxidative metabolism and function to transform the radical superoxide into \(\text{H}_2\text{O}_2\) (Grato et al., 2005). In this respect, Kong et al. (2005), in sorrel plants under saline stress found stronger SOD activity. In addition, Se affected the activity of CAT and glutathione S-transferase (GST) (Xue and Hartikainen, 2000). In potato, moreover, Xue et al. (2001) and Pemmanen et al. (2002) showed that Se retarded the decline of tocophersols, especially the more biologically active form, \(\alpha\)-tocopherol. Sreekala et al. (1999) observed that mitochondrial SOD activity exhibited a progressive enhancement with Se supplementation, 3-fold at 0.5 ppm, 4-fold at 0.75 ppm and 7-fold at 1.0 ppm. The increase was seen with respect to both total and specific activities. Soluble SOD decreased by 60% in the Se at 0.5 ppm, but an increase of 70% was elicited at 0.75 ppm with no further enhancement at 1.0 ppm. Sreekala et al. (1999) also observed that levels of mitochondrial CAT activity remained unaffected in the Se group (0.5 ppm) with respect to both total and specific activities. In the Se groups (0.75 and 1.0 ppm), both total and specific activities registered 60% and 70% decreases, respectively. The response of mitochondrial APX to Se exposure was similar to that of CAT activity (Sreekala et al., 1999) with a decrease in both total and specific activities up to 50-70% with Se supplementation at 0.5 ppm and no further changes in the Se groups (0.75 and 1.0 ppm).

**ROLE OF SELENIUM IN ABIOTIC STRESS TOLERANCE**

In short, the effect of Se in promoting antioxidant activity has been reported basically in plants subjected to any type of stress, while the possible action of this trace element
Salinity

The production of ROS is the important cause of damage to plants when subjected to salt stress, thus leading to the growth suppression (Dionisio-Sese and Tobioa, 1998; Zhu, 2001; Hasanuzzaman et al., 2009; Nahar and Hasanuzzaman, 2009; Hassain and Fujita, 2010). Research results obtained by different researchers showed the ability of Se to protect plants from salt stress-induced damages when applied at low concentration. Kong et al. (2005) reported that at low concentrations (1-5 μM), Se tended to stimulate the growth, the activities of SOD and POD enzymes, as well as the accumulation of water-soluble sugar in leaves of sorrel seedlings. However, at higher concentrations (10-30 μM), Se exerted diminished beneficial effects on growth and enzyme activities. Results revealed that SOD and POD activities of salt-stressed seedlings increased when exposed to concentrations ranging 1-5 μM Se. At concentrations between 10 and 30 μM, there were adverse effects on both enzymes compared with that at 5 μM Se. In cucumber leaves, Se treatments at 5 and 10 μM significantly improved the growth rate and increased the photosynthetic pigments and proline contents when subjected to salt stress (Hawrylak-Nowak, 2009). Additionally, Se enhanced the salt tolerance of seedlings by protecting the cell membrane against lipid peroxidation Se subdued MDA formation also in experiments performed by Djanaguiraman et al. (2005). Recently, in our laboratory we observed that Se-treated salt-stressed seedlings showed higher tolerance compared to the seedling treated with salt alone which was supported by the lower level of MDA and H2O2 content and higher enzymatic activities as well as non-enzymatic antioxidant viz. AsA and GSH. Khedr et al. (2003) reported that proline induces the expression of salt-stress-responsive proteins and improves the salt-tolerance in the desert plant Pancratium maritimum. Increase of proline content in Se-treated soybean plants has also been reported by Djanaguiraman et al. (2005). However, the mechanisms and the reasons for proline accumulation in Se-supplied plants have not been fully investigated.

The interaction of Se with soil salinity has also been studied by Terry et al. (2000). It is hardly surprising that sulfate salinity drastically inhibits plant uptake of selenate (Mikkelsen et al., 1988; Wu and Huang, 1991; Zayed et al., 1998). Not all plant species are affected to the same extent of sulfate salinity. In Se-accumulators, selenate is taken up preferentially over sulfate. Chloride salinity had much less effect on selenate uptake than sulfate salinity (Wu and Huang, 1991). Generally, there is a small decrease in shoot accumulation of Se with increasing salt levels (Bamuels et al., 1996).

Drought

Drought is a multi-dimensional stress, which causes various physiological and biochemical changes on plants (Dhandh et al., 2000; Wang et al., 2003; Verhagen et al., 2004; Hossain et al., 2009). One of the earliest responses of plants to drought is the accumulation of active oxygen species such as O2−, •OH, H2O2 and O3 (Mittler, 2002; Shigeoka et al., 2002; Apel and Hart, 2004). Plants protect cell systems from the cytotoxic effects of these active radicals using enzymes such as SOD, APX, glutathione reductase (GR), CAT and non-enzymatic antioxidants: GSH, AsA and carotenoid (Car) (Foyer et al., 1997; Foyer and Noctor, 2000). However, there are few reports on the protective role of exogenous Se on drought stress in plant.
In common buckwheat, Tadina et al. (2007) observed that in water deficit plants the stomatal conductance (gs) was significantly lower, while Se-supplemented water-deficit plants had significantly higher gs. A significantly higher actual photochemical efficiency of PSII was obtained in Se- and water-deficit plants, which was possibly due to improvement of plant water management during treatment. A significant interaction was also observed between the effects of water deficit and Se on respiratory potential.

Recently, Yao et al. (2009) suggested that optimal Se supply is favorable for growth of wheat seedlings during drought condition. The growth and physiological responses of seedlings were different, depending on the Se concentration. Use of higher (3.0 mg Se kg\(^{-1}\)) and lower amount used (0.5 mg Se kg\(^{-1}\)) did not significantly affect on biomass accumulation. Treatments with 1.0 and 2.0 mg Se kg\(^{-1}\) promoted biomass accumulation of wheat seedlings. Treatments at 1.0, 2.0 and 3.0 mg Se kg\(^{-1}\) significantly increased root activity, proline content, POD and CAT activities, Car content, chlorophyll content and reduced MDA content of wheat seedlings. Lower Se treatment did not significantly effect on chlorophyll content and MDA content, although it also increased some antioxidant index (proline and Car content, POD and CAT activities) in wheat seedlings. However, Xiaojin et al. (2009) observed that extra Se supply did not effect on the activities of CAT and POD, while the combination of drought and Se significantly increased activities of POD and CAT, but the increase did not counteract the lipid peroxidation.

**Chilling**

Many crops often encounter cold stress in growing periods, which brings greater loss to agricultural production. It has already been established that Se increases resistance and antioxidant capacity of plants, whereas there has been little effort to understand the role of Se in plants under cold stress. In a recent study on wheat plant, Chu et al. (2010) reported the protective role of Se under cold stress. They observed that Se treatments with 1.0 mg kg\(^{-1}\) significantly reduced MDA content and the rate of O\(_2^{-}\) production in wheat seedlings grown under cold stress, which indicated that suitable Se treatment reduced membrane lipid peroxidation and oxidative stress in seedlings subjected to stressful condition. Additionally, compared with the control, Se treatments significantly increased contents of anthocyanins, flavonoids and phenolic compounds of seedlings subjected to cold stress (Chu et al., 2010) which have the ability to scavenge free radicals and inhibit membrane lipid peroxidation of seedlings. The effects of different Se treatments on POD and CAT activities in seedlings exposed to cold stress were also reported by Chu et al. (2010). They showed a significant increase in activities of POD and CAT in Se treated wheat seedlings under cold stress. The increases in antioxidant enzymes’ activities and contents of antioxidant compounds probably decreased the toxicity of ROS (Chu et al., 2010). The results of the study indicated that Se could provide an ecological adaptation for young seedlings subjected to stress conditions by the increase in antioxidant levels and enzymes’ activities.

In another recent study, Hawrylak-Nowak et al. (2010) reported that under short-term chilling, the contents of chlorophylls and carotenoids showed no significant change after Se supplementation. Hawrylak-Nowak et al. (2010) also observed that, compared with the control, the Se-treated plants showed an increase of proline content in leaves, once after chilling and again after 7 days of re-warming. After stress, the MDA content in the root of plants decreased directly when it was treated with 2.5-10 µM Se. This was in comparable with the plants grown without Se, whereas MDA level increased in roots and leaves of plants exposed to 20 µM Se. Seven days later, the MDA level in the root of plants grown in the
presence of Se was still lower than those of plants not treated with Se and generally witnessed no significant change in leaves. Nevertheless, Se at concentrations of 2.5-10 μM modified the physiological response of cucumber to short-term chilling stress, causing an increase in proline content in leaves and diminishing lipid peroxidation in roots, the resistance of plants to low temperature was not clearly enhanced (Hawrylak-Nowak et al., 2010).

Heavy Metals and Al

Although many heavy metals in trace amounts are essential for various metabolic processes in organisms, they create physiological stress leading to generation of free radicals when in high concentration (Hossain et al., 2010). Last few years several researches have been conducted on the role of Se on heavy metal stress tolerance in plants. Results obtained by Vorobets (2006) suggest that the size of GSH pool shows a marked alteration in response to a combined lead and Se treatment. Vorobets (2006) concluded that during heavy metal stress Se might prevent its toxic effect in plants. It has been suggested that the protective effects of Se are due to the formation of non toxic Se-metal complexes (Whanger, 1992; Vorobets and Mykytyievich, 2000). Pedero et al. (2008) observed that the proportion of α-tocopherol was similar in the control plants and in those supplied with Se separately or in combination with cadmium (Cd). However, the percentage of α-tocopherol concentration increased to the level found in control and Se-enriched plants when Se was added simultaneously with Cd. It has been reported that an increase of α-tocopherol favors the stress tolerance of plants as it favors the scavenging of singlet oxygen species in chloroplasts (Munne-Bosch and Alegre, 2002; Munne-Bosch, 2005). Therefore, the increase of α-tocopherol in plants exposed to Se and Cd simultaneously, in comparison to those grown only in Cd, shows evidence that Se assists the plants in the adaptation.

Srivastava et al. (2009) observed that Se acted as an antioxidant, inhibiting lipid peroxidation (reduced by 26-42%) via increased levels of thiols and GSH (increased by 24%). The results suggest that Se is either an antioxidant or it activates plant protective mechanisms, thereby alleviating oxidative stress and improving arsenic uptake in Pteris vittata. The added Se significantly inhibited lipid peroxidation in the fronds. Compared to the controls, addition of 5 and 10 μM Se reduced the TBARS (thiobarbituric acid reactive substances) concentrations in the fronds by 26-42 and 27-35%, respectively. There were no significant differences in TBARS between two application rates of Se (5 and 10 μM) or two exposure times (5 and 10 day). Recently, a beneficial effect of Se on heavy metal toxicity has been reported. Pedero et al. (2008) studied the response of broccoli submitted to Cd toxicity together with the application of Se. This study reported that Se application diminished the MDA content and decreased the translocation of Cd towards the shoot, thereby reducing oxidative stress provoked by this heavy metal. Pedero et al. (2008) showed that, when plants were exposed simultaneously to Se and Cd, the MDA level noticeably decreased to the level found in the control. In the plants supplied only with Se, the level of MDA was the lowest. These findings can be attributed to the antioxidative effect of Se reported in previous studies (Hartikainen et al., 2000; Djanaguiraman et al., 2005).

Under heavy metal stress, there are some possible mechanisms by which Se confers tolerance to stress. Fikek et al. (2008) observed a protective role of Se ions on the Cd stress in the changes in growth and fresh weight of rape seedlings followed by considering two possible mechanisms: (1) removal of Cd from metabolically active cellular sites and (2) reduction of oxygen radicals. However, their results suggest that the Se effect is linked mainly to a reduction of oxygen radicals that are produced in the presence of Cd.
Non-enzymatic deactivation of oxygen radicals by Se can also be enhanced at the low (2 μM) Se concentration. In that study, Se-induced production of antioxidants was high enough to ameliorate the toxicity of Cd at concentration levels of 400-600 μM. In contrast, compared with Cd concentration, Se concentration seemed to be too low to remove Cd from proteins, especially at higher concentration levels. Very recently, Sun et al. (2010) observed the detoxification action of Se on garlic growth under Cd stress. They also considered the mechanisms for the protective role of Se ions on the Cd stress which were: (1) removal of Cd from metabolically active cellular sites, (2) induction of Se to harm the Cd induced free oxygen radical and (3) the regulation of Se to phytochelatin activity. The three actions mitigated the effects of Cd on garlic. In our laboratory, we observed the pre-treatment of mustard seedling with exogenous Se showed better performance in response to enzymatic and non-enzymatic antioxidant when subjected to short-term Cd stress.

Cartes et al. (2010) first suggested that at low concentration Se alleviated the Al-induced oxidative stress by means of two mechanisms: (1) the improvement of the spontaneous dismutation of superoxide free radicals to H$_2$O$_2$, as previously hypothesised by (Hartikainen et al., 2000) and (2) through the activation of POD enzyme, an H$_2$O$_2$ scavenger, in response to the increased disproportion of O$_2^-$ which possibly occurred by effect of the applied Se. Cartes et al. (2010) observed that Al enhanced lipid peroxidation and activated the POD, APX and SOD enzymes in the roots of ryegrass. Interestingly, Se application up to 2 μM improved root growth and steadily decreased TBARS accumulation in plants treated with 0 and 0.2 mM Al. However, above 2 μM, Se induced stress in plants grown with or without Al. Significant changes in antioxidant enzymes activities were also found as a result of the added Se. At low Se addition levels POD was activated, whereas APX activity decreased irrespective of added Al. Furthermore, Se supplied up to 2 μM greatly decreased root SOD activity in Al-stressed plants. Qi et al. (2004) and Peng et al. (2002) reported that Se enhanced resistance to oxidizing ability and reduced membrane lipid peroxidation of rice roots under ferrous stress.

**UV-Radiation**

Depletion of the stratospheric ozone layer in the atmosphere is known to result in an increase in ultraviolet-B (UV-B) radiation at the earth’s surface (Madronich et al., 1998). Enhanced UV-B radiation alter transpiration and photosynthesis (Gaberskik et al., 2002a), respiration potential (Gaberskik et al., 2002b) and growth, development and morphology of plants (Gao et al., 2003, Flint et al., 2003). Several reports indicated the protective role of Se against UV-B radiation.

In their recent report, Yao et al. (2010a) reported that appropriate amount of Se could significantly enhance the antioxidant ability and reduce membrane lipid peroxidation in aboveground parts of wheat seedlings exposed to enhanced UV-B radiation. In addition, Yao et al. (2010b) demonstrated that Se treatments significantly reduced the rate of O$_2^-$ production and MDA content in roots of wheat seedlings grown under enhanced UV-B radiation. The results were similar with the previous study on aboveground parts of wheat seedlings (Yao et al., 2010a). This indicated that Se treatments could reduce active oxygen content and oxidative stress in aboveground and belowground parts of wheat seedlings subjected to stressful condition. Se supply alleviated the damage of enhanced UV-B on wheat seedlings to some extent. Previously, Xue and Hartikainen (2000) observed that Se reduced the lipid peroxidation irrespective of light conditions, even though in the second assay the antioxidative effect was relatively more pronounced in the plants subjected to the short wave length light.
Although UV irradiation generally diminished the activities of enzymes other than GPX, in combination with Se it increased CAT in lettuce and rye grass, SOD in the lettuce and GST in ryegrass. This suggests that Se may increase the antioxidative capacity of plants by multiple systems that act alone or synergistically and are likely different at different growing stages. For instance, the antioxidative capacity of ryegrass against UV was strengthened by simultaneous increase in CAT, GST and GPX. Se treatments also induced an increase in activities of POD and SOD of wheat roots (Yao et al., 2010b). Similarly, an increase in antioxidant enzymes' activities of aboveground parts in seedlings subjected to stress condition has also been reported earlier (Xue et al., 2001; Kong et al., 2005; Yao et al., 2010a), indicating that optimal Se could provide an ecological adaptation for aboveground and belowground parts of seedlings by enhanced contents of antioxidant compounds and activities of antioxidant enzymes under stress conditions. It is noteworthy that APX did not increase in order to resist UV-induced oxidative stress and always responded negatively to exogenous Se, although it shares a common substrate H$_2$O$_2$ with GPX and CAT (Xue and Hartikainen, 2000). Analysis of SOD and APX expression carried out by Willekens et al. (1994) in ozone sensitive tobacco revealed that the induction of cytosolic copper/zinc, SOD and cAPX under ozone stress occurs only with the onset of visible damage. The results show that the synergistic effect of UV and Se added at non-toxic levels on plant growth is at least partly associated with the antioxidative role of Se through increased activity of GPX. The contribution of CAT and GST to the synergistic growth-promoting effect can be concluded from their analogous behavior, under normal light they responded indifferently or even negatively to Se but increased under UV-light (Xue and Hartikainen, 2000). In addition, the slight toxicity-alleviating effect of UV at the high Se addition level seemed to be associated with antioxidative enzymes: the high-energy light increased CAT in ryegrass and SOD in lettuce.

**NEGATIVE EFFECT SELENIUM IN PLANTS AND ENVIRONMENT**

Despite the widespread occurrence of Se deficiency globally, Se toxicity (selenosis) is a problem in some areas. Some soils and mineral deposits are naturally Se rich and exploitation of these seleniferous soils or fossil fuels can lead to toxic accumulation of Se in the environment. Se contamination of sediments, soils and drainage water particularly occurs in arid seleniferous areas with intensive crop irrigation (Oholendt et al., 1986).

Spallholz and Hoffman (2002) suggested three major Se toxicity mechanisms: (1) generation of superoxide radicals, (2) substitution of Se for sulfur (S) in proteins and (3) inhibition of methylation. Uptake of high concentration of Se by plant's root may exhibit symptoms of injury including stunting of growth, chlorosis, withering and drying of leaves, decreased protein synthesis and premature death of the plant (Trelease and Beath, 1949; Mengel and Kirkby, 1987). There are striking differences between the Se-accumulating plants and the non-accumulators in the maximum amount of Se they can absorb without showing symptoms of toxicity. In nonaccumulators, the threshold Se concentration in shoot tissue resulting in a 10% reduction in yield varied from 2 mg Se kg$^{-1}$ in rice to 330 mg Se kg$^{-1}$ in white clover (Mikkelsen et al., 1989). Se accumulators, on the other hand, may contain Se concentrations in excess of 4000 mg Se kg$^{-1}$ without exhibiting any negative effects on growth (Shrift, 1969). The threshold Se concentration may also vary with plant age and with sulfate supply. For example, Rosenfeld and Beath (1964) observed that in the nonaccumulators, wheat and corn, younger plants were more susceptible and growth inhibition was greater than in mature plants. Tolerance to Se toxicity may increase with
increasing sulfate supply so that threshold Se concentrations may not be the same at different sulfate concentrations in the root environment (Mikkelsen et al., 1989). In non-accumulators, the threshold toxicity concentration is also dependent on the form of Se accumulated. Selenate and selenite are the major forms that are toxic to plants because both are readily absorbed by the plant and assimilated to organic Se compounds. Some studies indicate that selenite is more toxic than selenate.

Kápolna et al. (2009) observed that when carrot plants are exposed to high concentrations of Se in their root medium or exposed to excessive Se concentrations in foliar sprays, they may show symptoms of damage like stunting of growth, chlorosis, withering and necrosis of leaves (Trelease and Beath, 1949). Such phenomena, like chlorotic and necrotic leaves were observed in this study when Se(+4) was applied on the leaves at 100 μg Se mL⁻¹, whereas no symptoms developed when the leaves were sprayed with the 10 μg Se mL⁻¹ concentration. On the other hand, Se did not cause any visible damage to the leaves at 10 and at the 100 μg Se mL⁻¹ concentrations. This indicated that the upper practical Se concentration for Se (+6) had been reached at 100 μg Se mL⁻¹. Oxidative stress in ryegrass (*Lolium perenne*) was found with the addition of Se at 10 mg kg⁻¹ or more, which resulted in drastic loss of yield. 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⁻) 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 (O₂⁻). A higher Se dosage (1.0 mg kg⁻¹ soil) was toxic to lettuce and reduced the yield of young plants. Breznik et al. (2004) indicated a slightly negative effect of Se on primary branching and on seed production in buckwheat.

Most recently, Aggarwal et al. (2010) reported that at 4 and 6 ppm Se levels, the growth of bean (*Phaseolus vulgaris* L.) seedlings was inhibited appreciably, which was associated with increase in stress injury measured as damage to membranes and decrease in cellular respiration, chlorophyll and leaf water content. The oxidative injury as elevation of lipid peroxidation was larger compared to H₂O₂ accompanied by reduced levels of enzymatic (SOD, CAT, APX and GR) and non-enzymatic (AsA and GSH) antioxidants. Proline content was significantly higher at 1 and 2 ppm Se but diminished considerably at 4 and 6 ppm levels concomitant with the reduced growth. However, exogenous application of proline (50 μM) resulted in substantiation of its endogenous levels that antagonised the toxic effects of Se by improving the growth of seedlings.

**CONCLUSION**

The moon goddess ‘Selene’ appears to have conferred not only her name, but also her nature on this micronutrient. The facts of Se is intriguing, enigmatic and challenging (even capricious) for researchers. Although, a number of report indicated the protective role of Se under abiotic stress condition, to date the research works conducted on the physiological role of Se under stressful condition is scarce. The studies reported in this issue increase our knowledge of Se in soil and plant and also raise further questions for researchers to try to answer. Controversy exists over the question of whether Se is an essential plant micronutrient. On a cautionary note, the appropriate concentration of exogenous Se is still a matter of intensive research. Complete elucidation of the role of Se as well as detailed protective mechanisms would be helpful for developing stress tolerance in plants.
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