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INFLUENCE OF SELENIUM ON GROWTH AND METABOLISM IN RICE (*ORYZA SATIVA* L.) AND ITS POSSIBLE INTERACTION WITH SULPHATE

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SUMMARY

The effect of selenate (Na_2SeO_4) with or without sulphate (Na_2SO_4) was studied on the growth and metabolism in rice seedlings cv. Khitish and cv. Satabdi. In the test cultivars, selenate at 2µM concentration showed growth promoting effects in comparison to 20µM selenate and above, where mostly inhibitory effects were observed. The levels of total chlorophyll, chlorophyll a, chlorophyll b, carotene, xanthophyll and intensity of chlorophyll fluorescence were also similarly affected. Selenium has been attributed with a protective role against formation of reactive oxygen species (ROS) in plants subjected to stress. High concentration of selenate induced differential effects in the activities of antioxidant scavenging enzymes, viz., SOD, CPX, AOX and CAT in rice seedlings. Activities of SOD and AOX were decreased, whereas CAT and CPX activities were increased. In these seedlings receiving high concentrations of selenate , the levels of oxidative stress markers, viz., proline, H_2O_2 and MDA were enhanced. Application of sulphate jointly with selenate reversed the inhibitory effects of high concentration of selenate treatment alone on all parameters tested, which ultimately led to enhanced growth and metabolism in rice seedlings. Thus selenium has a dose dependent effect on the physiological and biochemical responses of rice seedlings, with low concentration of selenate inducing a stimulatory effect on growth and metabolism as against high concentration of selenate which proved to be toxic to the rice seedlings.

***Key words*: Biochemical changes, growth, rice, selenate, sulphate**

INTRODUCTION

Selenium is a trace element and a Group IV A, Period 4 metalloid belonging to oxygen sulphur family with some important functions in living organisms particularly in plants and animals. Selenium plays an important role in various agricultural aspects. Selenium affects oxidative stress, DNA methylation, DNA repair, apoptosis, cell proliferation, carcinogen metabolism, hormone production and immune function in different animal systems (Navarro-Alarcon and Cabrera-Vique 2008). Current interest in selenium is focused on health benefits using plants with high selenium contents as a source of cancer preventive selenium compounds (Finley *et al.* 2001).

Although selenium is not classified as a micronutrient for higher plants, numerous studies have shown that at low concentration selenium exerts beneficial effect on growth and stress tolerance (Hartikainen *et al.* 2000, Xue *et al.* 2001). Selenium increases plant resistance against oxidative stress caused by production of free radicals (Djanaguiraman *et al.* 2005). Thus, selenium

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increases the tolerance of plants to UV-induced oxidative stress, delays senescence, and promotes the growth of seedlings (Kong *et al.* 2005). Furthermore, selenium has the ability to regulate the water status of plants under conditions of drought (Kuznetsov *et al.* 2003). Selenium accumulating plants limit the integration of seleno-amino acids into proteins by converting selenium into soluble non protein seleno-amino acids like seleno-methylselenocysteine and selenocystathionine (Terry *et al.* 2000, Whanger 2002). Seleno- methylselenocysteine in particular has been shown to have chemoprotective effects against cancer (Finley *et al.* 2001).

The selenium concentration in plants depends on the chemical form of selenium, its concentration and bioavailability in soil and the accumulation capacity of the plant. Selenate is more soluble and available for plants under oxidizing and alkaline soil conditions (Mayland 1991). Selenite is less available to plants than selenate because it is absorbed more strongly by iron oxide surfaces and soil clays (Yläranta 1983a, Mikkelsen *et al.* 1989). Plants take up selenate and transform it into organic selenium compounds, mainly selenomethionine. This affects human nutrition by increasing the selenium content of foods of both animal and vegetable origin (Aro *et al.* 1995). In higher plants, metabolism of selenium is closely related to that of sulfur because of their chemical similarity. It has been proposed that selenate, chemically analogous to the sulfate ion, is actively transported into roots via sulfate transporters and subsequently is rapidly translocated into shoots (Arvy 1993, Terry *et al.* 2000). Many plants including broccoli and cabbage have been shown to accumulate selenium very efficiently (Terry *et al.* 1992, Zayed *et al.* 1998). Selenium stimulates the growth of *Astragalus* and *Stanleya* and the presence of sulfate and phosphate ions can affect uptake of selenium in these species (Sors *et al.* 2005).

In the present study, we investigated how selenium and sulphate could induce morphological and biochemical changes occurring in rice seedlings. Rice was selected as the study material owing to its significance as one of the major food crops of India particularly in West Bengal. The study includes how selenium in the form of selenate affects the growth and metabolism in rice seedlings. With this view, we measured the level of chloroplastic pigments, assayed various antioxidant enzymes for the understanding of the metabolism of redox-active compounds, measured the level of different oxidative stress markers, viz., proline, total peroxide content (H_2O_2) and malondialdehyde (MDA) from selenium and sulphate treated rice seedlings. The experiments have been primarily focused to study the beneficial as well as the toxic effects of selenium in rice and its possible reversal by sulphate. Such studies may help in the future to develop selenium enriched nutritious rice grains which may provide a better alternative to expensive drugs in countering lethal diseases in humans.

MATERIALS AND METHODS

Plant material

Rice (*Oryza sativa* L.) seeds cv. Khitish and cv. Satabdi obtained from the State Rice Research Station, Chinsura, Hooghly, West Bengal, India and used as experimental material.

Growth conditions and salt treatments

The rice (*Oryza sativa* L.) seeds were surface sterilized with sodium hypochlorite solution (5 %, w/v) for about 20 min and washed thoroughly with distilled water. About 50 seeds for each batch were spread over in Petri dishes (ϕ 10cm) lined with filter papers containing various concentrations of sodium selenate (Na_2SeO_4) and sodium sulphate (Na_2SO_4), purchased from Loba-chemie. The seeds were kept in dark and humid conditions for 48 hours in a germinator at 30 ± 2°C, and then exposed to 16 h photoperiod (260 µmol m^{-2} s^{-1}PFD) for eight days. The seedlings were harvested after ten days and used for various physiological and biochemical analysis.

Morphological and anatomical studies

Data for growth measurement were collected from ten days old rice seedlings. The root and shoot lengths of rice seedlings treated with sodium selenate with or without sulphate were measured from ten seedlings at a time that were randomly selected from petriplates of the same set of the experiment and averaged.

Chlorophyll and carotenoid

Total chlorophyll, chlorophyll-a, chlorophyll-b contents were measured from the rice leaves according to Arnon (1949). For this, 1 g of fresh leaves were extracted with 80% acetone (v/v) and chlorophyll contents were estimated spectrophotometrically at 645 nm and 663 nm using a Hitachi U-2000 spectrophotometer. The chlorophyll contents were expressed in terms of mg chlorophyll present g^{-1} fresh weight. The fluorescence of chlorophyll was measured at an excitation wavelength of 640 nm and emission wavelength of 680 nm with the help of a Hitachi-650-40 spectrofluorometer.

Carotene and xanthophyll contents were estimated according to the method described by Mukherji and Biswas (1979). Carotene and xanthophyll contents were measured by utilizing the values of absorbance at 425 nm and 450 nm respectively using Hitachi U-2000 spectrophotometer and data were expressed in terms of optical density g^{-1} fresh weight.

Proline

1 g tissues of rice seedlings were extracted with 0.1 M sulphosalicylic acid and centrifuged at 5000 g for 30 min (Bates *et al.* 1973). The proline content of the supernatant was measured at 520 nm, calculated from standard curve and expressed as µg g^{-1} fresh weight.

H_2O_2

The H_2O_2 content from the seedlings was measured at 390 nm and calculated using an extinction coefficient (e) of 0.28 μM^{-1} cm^{-1} and expressed as µg g^{-1} fresh weight (Velikova *et al.* 2000).

Malondialdehyde (Lipid peroxidation)

For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test was used to measure MDA level as an end product of lipid peroxidation (Hodges *et al.* 1999). The absorbance value of the supernatant was measured at 532 nm. The value for non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex present was calculated using an extinction coefficient (ε) of 155 $mM^{-1}cm^{-1}$and expressed as µM g^{-1} fresh weight.

Enzyme extraction and assays

Enzyme extraction procedure was carried out at 4°C. 1 g plant sample was homogenized in pre-chilled 0.1 M sodium phosphate buffer (pH 7.0), centrifuged at 12,000 g for 20 min and supernatant was used to assay the activities of the enzymes.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by using the nitroblue tetrazolium method (Giannopolitis and Ries, 1977). One unit of SOD was defined as the amount of enzyme that produced 50 % inhibition of NBT reduction under assay conditions. Catalase (CAT, EC 1.11.1.6) activity was determined as the amount of $KMnO_4$ consumed in terms of H_2O_2 (Gasper and Lacoppe, 1968). The enzyme activity was expressed in terms of mg H_2O_2 decomposed h^{-1} g^{-1} fresh weight. Ascorbic acid oxidase (AOX, EC 1.10.3.3) activity was measured according to Olliver (1967). The difference between blank and sample reading gave the AOX activity and expressed as mg ascorbic acid decomposed h^{-1} g^{-1} fresh weight. Catechol peroxidase (CPX, EC 1.11.1.7) activity was assayed spectrophotometrically (Chance and Maehly, 1955) using a Hitachi U-2000 spectrophotometer. The absorbance value of reaction mixture was recorded at 420 nm at 0 time and after incubation for 1 min. The enzyme activity was expressed in terms of ΔO.D at 420nm min^{-1} g^{-1} fresh weight.

Statistical analysis

The experiments were carried out in a completely randomized design (CRD) with five replicates, each replication comprised a single Petri dish containing an average of 50 seeds. The data and significant differences among mean values were compared by descriptive statistics (± SE).

RESULTS

Effect of selenate and/or sulphate on growth of rice seedlings

Exposure of rice seedlings (cv. Khitish and cv. Satabdi) to different concentrations of selenate showed both stimulatory and inhibitory effects on elongation of

root and shoot lengths (Fig. 1-4). Maximum growth was recorded in 2µM selenate treated rice seedlings where the shoot lengths were increased by about 14% in cv. Khitish and 17% in cv. Satabdi and the root length was increased by about 20% and 24% in cv. Khitish and cv. Satabdi respectively. On the contrary, application of 20µM and 50µM concentrations of selenate significantly reduced shoot and root lengths in both the rice cultivars. The effect was more prominent on root than shoot lengths. In 50µM concentration of selenate treated test seedlings, the root lengths were reduced by about 65%

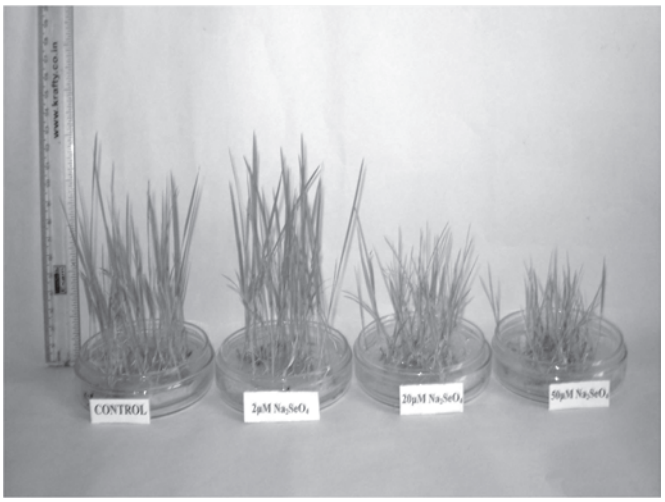

Fig. 1A. Effect of different concentrations of sodium selenate (Na_2SeO_4) on the growth of ten days old rice (cv. Khitish) seedlings

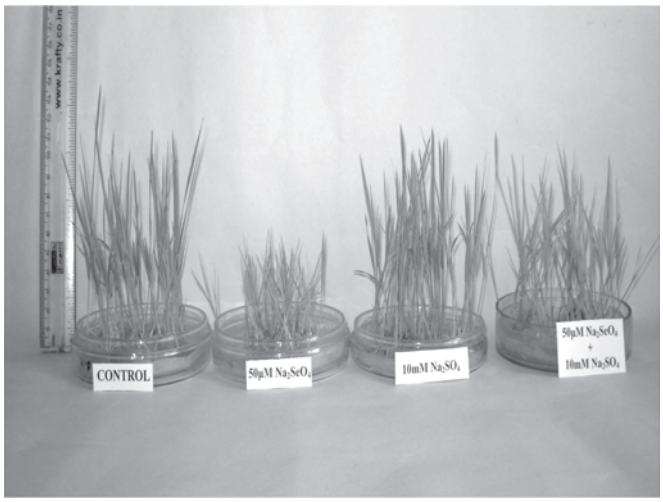

Fig. 1B. Effect of sodium selenate (Na_2SeO_4) and sodium sulphate (Na_2SO_4) applied singly or in combination on the growth of ten days old rice (cv. Khitish) seedlings

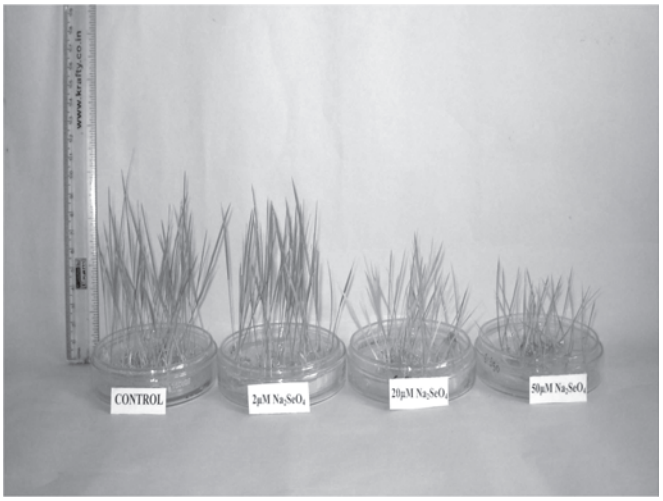

Fig. 2A. Effect of different concentrations of sodium selenate (Na_2SeO_4) on the growth of ten days old rice (cv. Satabdi) seedlings

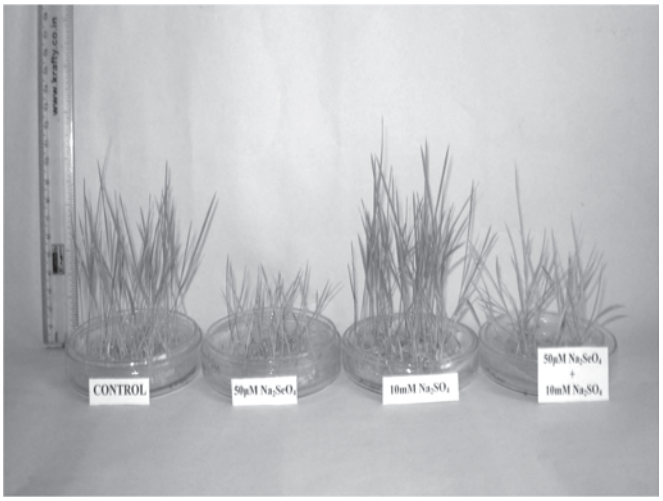

Fig. 2B. Effect of sodium selenate (Na_2SeO_4) and sodium sulphate (Na_2SO_4) applied singly or in combination on the growth of ten days old rice (cv. Satabdi) seedlings

in cv. Khitish and 35% in cv. Satabdi while the shoot lengths were reduced by 53% and 26% in cv. Khitish and cv. Satabdi respectively from water control. Application of sulphate (10mM) with selenate (50µM) showed an increase in root lengths of about 16% and 12% (cv. Khitish) and 18% and 33% (cv. Satabdi) as compared to water control by application of 20µM and 50µM concentrations of selenate respectively. The inhibition of shoot growth caused by 20µM and 50µM concentrations of selenate was totally reversed by sulfate. Although the effect was little in cv. Khitish but

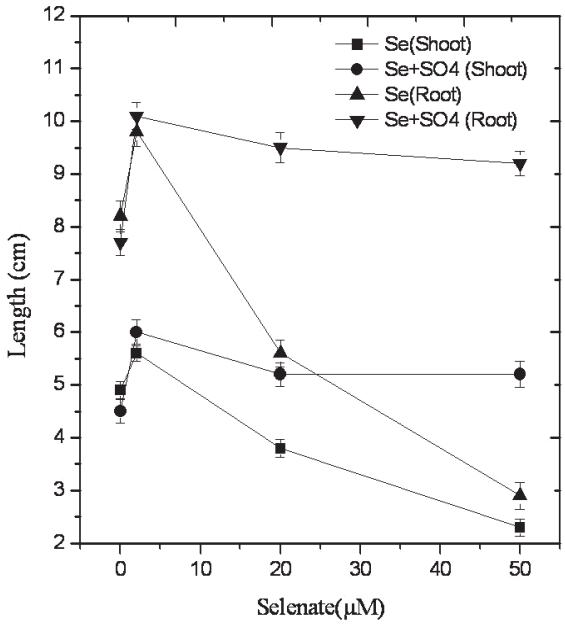

Fig. 3. Effect of sodium selenate (Na_2SeO_4) and sodium sulphate (Na_2SO_4) applied singly or in their combination on shoot and root length of ten days old rice (cv. Khitish) seedlings. Data are expressed as mean values ± SE (n=5)

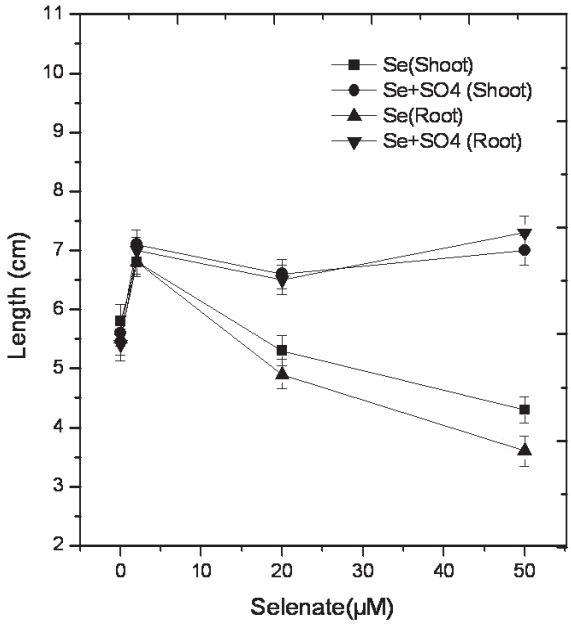

Fig. 4. Effect of sodium selenate (Na_2SeO_4) and sodium sulphate (Na_2SO_4) applied singly or in their combination on shoot and root length of ten days old rice (cv. Satabdi) seedlings. Data are expressed as mean values ± SE (n=5)

the rate of amelioration was about 14% and 21% in cv.Satabdi under 20µM and 50µM selenate treatment respectively.

Effect of selenate and/or sulphate on pigment contents of rice seedlings

Under 2µM concentration of selenate treatment, the total chlorophyll, chlorophyll a and chlorophyll b contents were increased in rice seedlings (Table 1) that were about 14% and 25% for total chlorophyll, 22% and 26% for chlorophyll a and 36% and 29% for chlorophyll b in cv. Khitish and cv. Satabdi respectively. Rice seedlings treated with 50µM selenate showed a decrease in total chlorophyll contents by about 19% in cv. Khitish and 13% in cv. Satabdi. Simultaneous application of sulphate (10mM) along with selenate (50µM) showed a recovery of inhibition in total chlorophyll contents by about 9% and 19%, chlorophyll a by about 16% and 18% and chlorophyll b by about 18% and 14% in cv. Khitish and in cv. Satabdi respectively.

Table 1a. Effect of selenate applied either alone or in combination with sulphate on chlorophyll contents in rice (cv. Khitish) seedlings. The data were recorded from ten days old seedlings.

Treatment	Total Chlorophyll (mg g^{-1} f w)	Chl – a (mg g^{-1} f w)	Chl – b (mg g^{-1} f w)	Fluorescence intensity
Control	0.43 ± 0.03	0.32 ± 0.05	0.11 ± 0.04	655.0 ± 2.81
Selenate (µM)				
2	0.49 ± 0.02	0.39 ± 0.03	0.15 ± 0.04	700.0 ± 2.38
20	0.38 ± 0.02	0.28 ± 0.06	0.09 ± 0.02	587.5 ± 2.27
50	0.35 ± 0.03	0.25 ± 0.04	0.07 ± 0.02	537.5 ± 2.34
Sulfate (10mM)	0.43 ± 0.04	0.33 ± 0.04	0.10 ± 0.03	645.0 ± 2.28
+Selenate (50 ìM)	0.47 ± 0.04	0.37 ± 0.05	0.13 ± 0.04	677.5 ± 2.74

The values are mean of 5 replicates ±SE

Table 1b. Effect of selenate applied either alone or in combination with sulphate on chlorophyll contents in rice (cv. Satabdi) seedlings. The data were recorded from ten days old seedlings.

Treatment	Total Chlorophyll (mg g^{-1} f w)	Chl – a (mg g^{-1} f w)	Chl – b (mg g^{-1} f w)	Fluorescence intensity
Control	0.48 ± 0.04	0.34 ± 0.06	0.14 ± 0.05	427.5 ± 2.90
Selenate (µM)				
2	0.60 ± 0.06	0.43 ± 0.04	0.18 ± 0.06	485.0 ± 2.44
20	0.44 ± 0.04	0.31 ± 0.06	0.12 ± 0.04	367.5 ± 2.58
50	0.42 ± 0.04	0.29 ± 0.03	0.09 ± 0.02	332.5 ± 2.36
Sulfate (10mM)	0.47 ± 0.05	0.33 ± 0.04	0.14 ± 0.06	405.0 ±2.23
+Selenate (50 ìM)	0.57 ± 0.07	0.40 ± 0.05	0.16 ± 0.03	455.0 ± 2.19

The values are mean of 5 replicates ± SE

Table 1c. Effect of selenate applied either alone or in combination with sulphate on carotene and xanthophylls content in rice seedlings. The data were recorded from ten days old seedlings

	cv. Khitish		cv.Satabdi	
Treatment	carotene (A_{425} g^{-1}fw)	xanthophyll (A_{450} g^{-1} fw)	carotene (A_{425} g^{-1}fw)	xanthophyll (A_{450} g^{-1} fw)
Control	0.30 ± 0.04	0.53 ± 0.05	0.47 ± 0.04	0.36 ± 0.09
Selenate (µM)				
2	0.36 ± 0.09	0.60 ± 0.09	0.49 ± 0.05	0.41 ± 0.06
20	0.22 ± 0.04	0.50 ± 0.04	0.39 ± 0.09	0.30 ± 0.06
50	0.20 ± 0.05	0.48 ± 0.09	0.34 ± 0.06	0.27 ± 0.09
Sulfate (10mM)	0.30 ± 0.07	0.52 ± 0.06	0.45 ± 0.08	0.39 ± 0.07
+Selenate (50 µM)	0.37 ± 0.09	0.56 ± 0.03	0.48 ± 0.02	0.40 ± 0.08

The values are mean of 5 replicates ± SE

An increase in fluorescence activity of chlorophyll of about 7% and 13% were observed in rice seedlings, cv. Khitish and cv. Satabdi respectively under 2µM concentration of selenate treatment. The fluorescence activity was linearly decreased with increasing concentrations of selenate. Maximum inhibition in fluorescence activity was found to occur in 50 µM selenate treated rice seedlings. Application of 10mM sulphate along with 50µM selenate increased very little the fluorescence activity of chlorophyll.

The carotene contents were increased by about 20% in cv. Khitish and little in cv. Satabdi when treated with 2 µM selenate in these seedlings. This increment over control was reversed with resultant inhibition when treated with high concentrations of selenate. Maximum inhibition was recorded in 50µM selenate treated rice seedlings where the carotene contents were decreased by about 33% and 28% in cv.Khitish and cv. Satabdi respectively. Application of sulphate (10mM) along with selenate (50 µM) increased the carotene contents by about 23% in cv. Khitish and very little in cv. Satabdi over water control.

The xanthophyll levels were also increased in rice seedlings by the application of 2 µM selenate. The increment of xanthophyll content was about 13%. Application of 20 µM and above concentrations of

selenate to the seedlings of the said cultivars caused a decrease in xanthophyll levels by an average of about 8% in cv. Khitish and 18% in cv. Satabdi. Application of sulphate (10mM) along with selenate (50 µM) caused an ameliorative effect on xanthophyll levels in the seedlings and the detrimental effect on xanthophyll production was overcome by about 6% in cv. Khitish and 11% in cv. Satabdi.

Effect of selenate and/or sulphate on antioxidant enzyme activities of rice seedlings

Exogenous application of selenate exhibited variable response in the activity of SOD (superoxide dismutase) in root and shoot of rice seedlings over control (Table 2). In 2µM selenate treated rice seedlings the increase in SOD activity was very little in cv. Khitish and 11% (shoot) and 9% (root) in cv. Satabdi. Higher concentrations of selenate particularly over 20 µM were detrimental to the activity of SOD enzyme. Maximum inhibition in SOD activity occurred in rice seedlings treated with 50µM selenate solution. The decrease in SOD activity was about 14% (shoot) and 20% (root) in cv. Khitish and 25% (shoot) and 29% (root) in cv. Satabdi. Further, during joint application of sulphate along with 50 µM selenate, the activity of SOD was increased in an average by 12% in cv. Satabdi over control, whereas the effect was negligible in cv. Khitish.

The AOX activity was increased in shoot and root by about 5% and 9% respectively in cv. Khitish and 8% and 10% respectively in cv. Satabdi. Treatment with 20 µM selenate solution decreased AOX activity in an average of 8% in shoot and 16% in root of both cultivars. Maximum inhibition occurred in AOX activity in 50 µM selenate treated rice seedlings by about 15% in shoot and 33% in root in cv. Khitish and 14% in shoot and 27% in root in cv. Satabdi. Sulphate application along with selenate (50µM) showed little increase in AOX activity over control in the seedlings of both cultivars (Table 4).

The CPX activity in 2 µM selenate treated rice seedlings were about 9% and 8% in shoot and root respectively of cv. Khitish and that effect was negligible in cv. Satabdi. The activity of the enzyme was increased with increasing concentrations of selenate (Table-3). Rice seedlings treated with 20 µM selenate recorded an increase of about 13% in shoot and 15% in root (cv. Khitish) and 10% in shoot and 13% in root (cv. Satabdi) over control. The highest activity of peroxidase was observed in 50µM selenate treated rice seedlings where the increase in enzyme activity was observed in both shoot (61%) and root (49%) samples in cv. Khitish and 49% and 26% in shoot and root respectively in cv. Satabdi. This is quite opposite to the behaviour of SOD, the activity of which was promoted at low and inhibited

Table 2. Effect of selenate applied either alone or in combination with sulphate on SOD activity of rice seedlings. The data were recorded from ten days old seedlings

Treatment	SOD activity (E.U SOD g^{-1} min^{-1})			
	cv. Khitish		cv. Satabdi	
	shoot	root	shoot	root
Control	1.52 ± 0.04	1.32 ± 0.03	1.16 ± 0.04	1.29 ± 0.06
Selenate (µM)				
2	1.57 ± 0.08	1.40 ± 0.09	1.29 ± 0.04	1.41 ± 0.05
20	1.38 ± 0.06	1.17 ± 0.05	1.10 ± 0.08	1.00 ± 0.05
50	1.30 ± 0.08	1.05 ± 0.04	0.87 ± 0.06	0.92 ± 0.07
Sulfate (10mM)	1.49 ± 0.04	1.30 ± 0.05	1.12 ± 0.09	1.21 ± 0.07
+Selenate (50 µM)	1.58 ± 0.09	1.38 ± 0.08	1.13 ± 0.04	1.44 ± 0.05

The values are mean of 5 replicates ± SE

Table 3. Effect of selenate applied either alone or in combination with sulphate on CPX activity of rice seedlings. The data were recorded from ten days old seedlings

Treatment	CPX activity (ΔOD_{420} min^{-1} g^{-1})			
	cv. Khitish		cv. Satabdi	
	shoot	root	shoot	root
Control	0.87 ± 0.09	0.53 ± 0.08	0.74 ± 0.05	0.47 ± 0.06
Selenate (µM)				
2	0.79 ± 0.07	0.49 ± 0.05	0.69 ± 0.04	0.44 ± 0.07
20	0.98 ± 0.05	0.61 ± 0.08	0.81 ± 0.03	0.53 ± 0.06
50	1.40 ± 0.04	0.79 ± 0.06	1.10 ± 0.08	0.59 ± 0.05
Sulfate (10mM)	0.84 ± 0.06	0.52 ± 0.08	0.72 ± 0.05	0.46 ± 0.06
+Selenate (50 µM)	0.77 ± 0.07	0.47 ± 0.04	0.70 ± 0.06	0.45 ± 0.04

The values are mean of 5 replicates ± SE

Table 4. Effect of selenate applied either alone or in combination with sulphate on AOX activity of rice seedlings. The data were recorded from ten days old seedlings

Treatment	AOX activity (mg AOX g^{-1} h^{-1})			
	cv. Khitish		cv. Satabdi	
	shoot	root	shoot	root
Control	3.9 ± 0.07	5.4 ± 0.06	3.6 ± 0.03	4.80 ± 0.07
Selenate (µM)				
2	4.1 ± 0.07	5.9 ± 0.04	3.9 ± 0.04	5.28 ± 0.04
20	3.6 ± 0.08	4.6 ± 0.05	3.3 ± 0.08	3.96 ± 0.06
50	3.3 ± 0.07	3.6 ± 0.07	3.1 ± 0.06	3.50 ± 0.05
Sulfate (10mM)	3.8 ± 0.06	5.2 ± 0.09	3.6 ± 0.07	4.60 ± 0.07
+Selenate (50 ìM)	4.1 ± 0.08	5.7 ± 0.09	3.8 ± 0.07	5.10 ± 0.08

The values are mean of 5 replicates ± SE

at high concentrations of selenate. The effect of 50µM selenate on the enzyme activity was reversed by the joint application of 50µM selenate with sulphate, whereby the decline in CPX activity was observed on an average to about 11% in shoot and marginal in root of cv. Khitish and cv. Satabdi, respectively.

Since catalase is a major protectant against accumulation and toxicity of H_2O_2, alterations in catalase activity by selenium was studied in rice seedlings. In 2 µM selenate treated rice seedlings, catalase activity was found to decrease by about 8% in shoot of cv. Khitish whereas it increased by about 23% in cv. satabdi. Thereafter, catalase activity increased with increasing concentrations of selenate in shoot of both cultivars (Table 5). Maximum activity of the enzyme was recorded in 50 µM selenate treated rice seedlings which were about 85% in cv. Khitish and in a significant amount in cv. Satabdi. In both the cultivars an almost opposite effect was observed on catalase activity of the roots treated with increasing concentrations of selenate. Inhibition in catalase activity was recorded by application

Table 5. Effect of selenate applied either alone or in combination with sulphate on catalase (CAT) activity of rice seedlings. The data were recorded from ten days old seedlings

Treatment	CAT activity (mg H_2O_2 g^{-1} f.w. min^{-1})			
	cv. Khitish		cv. Satabdi	
	shoot	root	shoot	root
Control	0.88 ±0.04	6.21 ± 0.04	0.83 ± 0.08	8.68 ± 0.07
Selenate (µM)				
2	0.81 ±0.07	6.40 ±0.06	1.02 ± 0.07	7.76 ± 0.04
20	1.02±0.06	6.16 ± 0.08	3.48± 0.06	6.93 ± 0.09
50	1.63 ± 0.05	4.94 ± 0.05	3.66± 0.04	9.30 ± 0.07
Sulfate (10mM)	1.33 ± 0.08	7.53 ± 0.08	1.22 ± 0.07	7.49 ± 0.07
+Selenate (50 ìM)	0.68 ± 0.07	4.48 ± 0.06	1.43 ± 0.05	8.13 ± 0.06

The values are mean of 5 replicates ± SE

of 50 µM selenate where decrease in enzyme activity was about 20% in cv. Khitish as opposed to slight increase in root of cv. Satabdi. Application of sulphate (10 mM) along with selenate (50 µM) showed a decrease in enzyme activity by about 23% in shoot and 28% in root of cv. Khitish while the catalase activity increased by about 72% in shoot and very little in root in cv. Satabdi.

Effect of selenate and/or sulphate on proline contents of rice seedlings

A variable effect in proline contents with increasing concentrations of selenium salt was recorded in both cultivars (Table 7). Under 2 µM selenate treatment, the level of proline contents was less by about 25% in shoot and 16% in root of cv.Khitish and by about 18% in shoot and 13% in root of cv. Satabdi. Proline level was significantly increased in the 50µM selenate treated seedlings over control which was about 38% in shoot and 67% in root (cv. Khitish) and 25% in shoot and 40% in root (cv. Satabdi). Furthermore, joint application of sulphate along with selenate (50µM) decreased proline contents to an extent of about 25% and 11% in shoot and root respectively in cv.Khitish and about 29% and 21% in shoot and root in cv. Satabdi.

Effect of selenate and/or sulphate on total peroxide (H_2O_2) contents of rice seedlings

There was a gradual decrease in total peroxide contents of rice seedlings treated with increasing selenate concentrations over water control except in 2 µM selenate application (Table 6). An increased level of peroxide content was observed in 2 µM selenate, where the increments were about 44% in shoot and 32% in root of cv. Khitish while 11% in shoot and 16% in root of cv. Satabdi. The peroxide contents suffered a decline in 50 µM selenate treated rice seedlings which were at about 37% (shoot) and 28% (root) in cv.Khitish while 33% (shoot) and 24% (root) in cv. Satabdi. Application of sulphate (10 mM) along with selenate (50 µM) exhibited increased peroxide contents in both the cultivars that were about 25% and 18% in shoot and root respectively of cv. Khitish and 28% and 11% in shoot and root respectively of cv. Satabdi over water control.

Effect of selenate and/or sulphate on malondialdehyde (MDA) contents of rice seedlings

Selenate (2 µM) treatment diminished lipid peroxidation in the rice seedlings as measured by malondialdehyde contents. The decrease in MDA

Table 6. Effect of selenate applied either alone or in combination with sulphate on total peroxide (H_2O_2) contents of rice seedlings. The data were recorded from ten days old seedlings

Treatment	H_2O_2 (µM g^{-1} f.w.)			
	cv. Khitish		cv. Satabdi	
	shoot	root	shoot	root
Control	0.57 ± 0.04	0.79 ± 0.08	0.64 ± 0.07	0.71 ± 0.04
Selenate (µM)				
2	0.82 ± 0.04	1.04 ± 0.06	0.71 ± 0.05	0.82 ± 0.07
20	0.43 ± 0.03	0.68 ± 0.07	0.54 ± 0.05	0.61 ± 0.07
50	0.36 ± 0.03	0.57 ± 0.06	0.43 ± 0.03	0.54 ± 0.05
Sulfate (10 mM)	0.54 ± 0.06	0.75 ± 0.06	0.68 ± 0.07	0.68 ± 0.04
+Selenate (50 µM)	0.71 ± 0.05	0.93 ± 0.05	0.82 ± 0.04	0.79 ± 0.06

The values are mean of 5 replicates ± SE

Table 7. Effect of selenate applied either alone or in combination with sulphate on proline contents of rice seedlings. The data were recorded from ten days old seedlings

Treatment	Proline content (µM g^{-1} f w)			
	cv. Khitish		cv. Satabdi	
	shoot	root	shoot	root
Control	402 ± 0.97	451 ± 0.94	280 ± 1.18	383 ± 1.67
Selenate (µM)				
2	301 ± 1.14	381 ± 1.28	232 ± 1.13	333 ± 0.82
20	481 ± 1.17	603 ± 0.91	330 ± 1.41	481 ± 1.75
50	550 ± 1.20	751 ± 1.21	351 ± 1.78	531 ± 1.58
Sulfate (10 mM)	362 ± 1.26	440 ±1.17	253 ± 1.37	350 ± 0.96
+Selenate (50 µM)	300 ± 1.18	403 ± 0.92	200 ± 1.19	302 ± 0.87

The values are mean of 5 replicates ± SE

contents was not pronounced in shoot, whereas an appreciable decrease of about 16% was recorded in root of both cultivars. The MDA contents on the other hand, increased significantly in rice seedlings treated with 20 µM and above concentrations of selenate (Table-8). The maximum increment in MDA production was observed in 50 µM selenate treated rice seedlings which were about 47% (shoot) and 11% (root) in cv. Khitish and about 36% (shoot) and 23% (root) in cv. Satabdi. The increment in MDA levels in the seedlings treated with selenate (50 µM) and sulphate (10 mM) solution were reduced to about 27% in shoot and 5% in root in cv. Khitish and about 35% in shoot and 24% in root in cv. Satabdi over water control.

DISCUSSION

In order to determine the effect of selenium on germination and growth of rice seedlings, solutions of

Table 8. Effect of selenate applied either alone or in combination with sulphate on malondialdehyde (MDA) contents in rice seedlings. The data were recorded from ten days old seedlings

Treatment	MDA content (µM g^{-1} f w)			
	cv. Khitish		cv. Satabdi	
	shoot	root	shoot	root
Control	1.50 ± 0.04	1.85 ± 0.06	1.67 ± 0.05	0.71 ± 0.02
Selenate (µM)				
2	1.49 ± 0.03	1.56 ± 0.04	1.62 ± 0.08	0.59 ± 0.06
20	1.92 ± 0.03	1.90 ± 0.06	1.78 ± 0.07	0.80 ± 0.06
50	2.21 ± 0.05	2.05 ± 0.03	2.27 ± 0.08	0.87 ± 0.04
Sulfate (10 mM)	2.02 ± 0.02	1.82 ± 0.06	2.47 ± 0.07	0.70 ± 0.03
+Selenate (50 µM)	1.90 ± 0.08	1.95 ± 0.05	2.26 ± 0.06	0.88 ± 0.07

The values are mean of 5 replicates ± SE

selenium (as selenate) were supplied in factorial combinations. Treatment of rice seedlings with 2µM concentration of selenate has a promotive effect, while 20 µM and above concentrations of selenate exhibited inhibitory effect on root and shoot growth. The inhibition of rice seedling growth was alleviated under combined application of selenate and 10mM concentration of sulfate (Fig. 1-4). Growth inhibition as seen to occur at high selenium concentrations may result from impaired photosynthesis. Increasing supplies of selenate are associated with a reduction in chlorophyll content (Table 1a,1b). The observations suggest that chloroplasts are important targets in the mechanism of selenium toxicity. Furthermore, the inhibition of photosynthetic electron transport evaluated by chlorophyll fluorescence induction confirms this hypothesis and demonstrates that selenate disrupts the photosynthetic electron chain. Our observations are in agreement with those of Pennanen *et al.* (2002) who reported that selenium showed an inhibitory effect at high concentration and promotive effect at low concentration on lettuce plants in response to UV radiation. Similar patterns in the development of root and shoot lengths were demonstrated in *Spirulina platensis* by Zhi Yong *et al.* (2003) and in *Eruca sativa* by Khattab (2004). The synergistic effect of selenate and sulfate was partly attributable to the antioxidative function of selenium, evidenced by inhibition of lipid peroxidation and promotion of other important antioxidative enzymes.

Exposure of rice seedlings to 2µM concentration of selenate induced an increase in chlorophyll (Table 1a,1b) and carotenoid contents (Table 1c). The increase in chlorophyll contents is possibly due to protective effect of selenium towards chloroplast enzymes which in turn increase the biosynthesis of photosynthetic pigments (Pennanen *et al.* 2002). The decrease in chlorophyll contents in rice seedlings by application of high concentrations of selenate is consistent with the results obtained in mungbean by Padmaja *et al.* (1989) and in rocket plant by Khattab (2004). Reactive oxygen species (ROS, e.g. O_2^- and H_2O_2) are produced as by-products of plant cellular metabolism and cause rapid cell damage by triggering off a chain of reactions (Noctor and Foyer 2000, Imlay 2003). Plants are equipped with oxygen radical detoxifying enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT) and antioxidant redox-active molecules like ascorbic acid and reduced glutathione in order to survive under stress conditions. The antioxidant enzymes play an important role in scavenging ROS and therefore, their involvement could increase the ability of stress tolerance and delay the senescence programme (Alscher *et al.* 2002). Antioxidant molecules can interact with ROS directly,

thereby removing ROS from cells. Carotenoids are synthesized by plants and serve to act as powerful anti-aging antioxidants, protecting the cells from damage caused by free radicals.

The SOD catalyses the conversion of superoxide to hydrogen peroxide. Exogenous application of 20 µM and above concentrations of selenate showed a dose dependent decrease in SOD activity in root and shoot of rice seedlings from control (Table 2). According to Kong *et al.* (2005), 1–5 µM concentrations of selenate caused stimulation of growth, activities of superoxide dismutase and peroxidase enzymes and also accumulation of water-soluble sugar in leaves of *Rumex acetosa* seedlings whereas under 10µM to 30µM concentrations of selenate, the beneficial effects on growth and enzyme activities were diminished and said effects were reversed by the application of sulfate along with selenate.

Hydrogen peroxide is toxic to cells and has to be further detoxified by CAT and/or peroxidases to water and oxygen. Low concentration of selenate treatment in rice seedlings showed an opposite effect on catechol peroxidase (CPX) activity compared to superoxide dismutase (SOD) activity (Table 3). The highest level of CPX activity was observed in 50 µM selenate treatment. The enhanced CPX activity may either increase the scavenging of superoxide produced as a result of stress or indirectly by modifying redox and cell signaling process (Sandalio *et al.* 2001).The stimulatory effects by low concentration of selenate treatment on SOD and CPX activities may challenge the free radical mediated lipid peroxidation of the membrane.

Catalase (CAT) is a major protectant against accumulation and toxicity of H_2O_2. Catalase activity is generally measured by determining the rate of decomposition of hydrogen peroxide (Maehly and Chance 1954). Selenium induced increases in CAT activity was in a dose dependent pattern (Table 5). Further, catalase activity was reduced in rice seedlings exposed to simultaneous application of selenate and sulfate. Reduction in catalase activity, therefore, does not necessarily imply accumulation of H_2O_2 to damaging levels. The absence of a clear inverse relationship between catalase activity and H_2O_2 concentration suggests the continued activity of other reactions that remove H_2O_2 which may be important in the tolerance of plants to oxidative attack. Loss of catalase activity may result from the inability of damaged peroxisomal membranes to transport catalase precursors into the peroxisome. Higher selenate concentrations lead to higher selenium accumulation and increase the activities of SOD and CAT.

The results showed that there was differential effects in ascorbic acid oxidase (AOX) activity in shoot and root of rice seedlings treated with increased concentrations of selenate from water control (Table 4). The ascorbate oxidase (AOX) has a role in controlling the cell wall redox state which in turn affect growth and ozone resistance (Pignocchi and Foyer 2003). Selenate at high concentrations induced considerable reduction in the activities of AOX in the rice seedlings. The decline in AOX activity was more in case of root than shoot in both rice cultivars. The inhibitory action of selenate at higher concentrations was reversed by the joint application of selenate with sulfate in this system.

The reactive oxygen species function as signaling molecules that mediate responses to various stimuli (Desikan *et al.* 2004).Hydrogen peroxide occurs naturally in animals and plants, and can help to protect plants from diseases or signal the plant subject to stress. There is a dramatic increase in H_2O_2 level observed in rice seedlings treated with 20 µM and above concentrations of selenate (Table 6). Proline is reported to be an universal osmolyte accumulated in response to several stresses (Mansour 2000). In the present work, proline level is significantly increased in rice seedlings treated with high concentrations of selenate (Table 7). Joint application of 10mM sulfate and 50 µM selenate in rice seedlings caused a decrease in proline contents in comparison to rice seedlings treated with 20 µM selenate only.

Under adverse conditions, some of the most reliable and widely used indicators of stress are plant biomass, lipid peroxidation and the oxidation state of the proteins determined by the content in carbonyl groups (Fu and Huang 2001, Sairam *et al.* 2002). With respect to the stress indicators used in this experiment, we found that the external application of 2 µM concentration of

selenate diminishes malondialdehyde (MDA) production whereas 20 μM and above concentrations of selenate augmented concentration of MDA (Table-8). The linear increase in malondialdehyde (MDA) contents by increasing selenate application in roots and leaves of rice seedlings thus obtained indicates the influence of enhanced oxidative stress. Selenate at low concentration acted as an antioxidant, inhibiting lipid peroxidation, whereas at higher concentrations, it was a pro-oxidant, enhancing the accumulation of lipid peroxidation products.

At high concentrations (20–50 μM), selenium has been shown to exert inhibitory effects on growth and enzyme activities. The beneficial effects in presence of low concentration of selenate have received little attention compared to toxic effects that typically occur at higher concentrations. In plants, selenium has been shown to play a role analogous to that of sulfur. Selenium and sulfur are chalcogen elements that share many chemical properties. Because of its chemical similarities, sulfur can interfere with the absorption of selenium by plants. Sulfur competes with selenium compounds for uptake by plants, causing a decrease in available selenium in crop plants (Sors *et al*. 2005). But no conclusive evidence of selenium deficiency due to competition by sulfur has been recorded. Understanding the beneficial role of elements in plants is important to improve crop productivity and enhance plant nutritional value for a growing world population. As research continues to advance our understanding in the area of nutritional value of selenium in plants, additional role of selenium may be discovered to further understand its metabolism and benefits in human welfare.

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REFERENCES

Alscher, R.G., Erturk, N. and Heath, L.S. (2002). Role of superoxide dismutases (SOD) in controlling oxidative stress in plants. *J Exp Bot.* **53:** 1331-1341.

Arnon, D.I. (1949). Copper enzyme in isolated chloroplast. *Plant Physiol*. **24:** 1-15.

Aro, A. and Alfthan, G. (1995). Effects of Supplementation of Fertilizers on Human Selenium Status in Finland. *Analyst*. **120:** 841-843.

Arvy, M.P. (1993). Selenate and selenite uptake and translocation in bean plants (*Phaseolus vulgaris*). *J. Exp. Bot*. **44:** 1083-1087.

Bates, L.S., Waldren, R. P.and Treare, I.D. (1973). Rapid estimation of free proline for water stress determination. *Plant and Soil*. **39:** 205-207.

Chance, B. and Maehly, A.C. (1955). Assay of catalases and peroxidases. *Methods in Enzymol*. **2:** 764-817.

Desikan, R., Cheung, M.K., Bright, J., Henson, D., Hancock, J.T. and Neill, S.J. (2004). ABA, hydrogen peroxide and nitric oxide signaling in stomatal guard cells. *J Exp Bot.* **55:** 205-212.

Djanaguiraman, M., Durga Devi D., Shanker, A.K., Sheeba, J.A. and Bangarusamy, U. (2005). Selenium—an antioxidative protectant in soybean during senescence. *Plant Soil*. **272:** 77-86.

Finley, J.W., Ip, C., Lisk, D.J., Davis, C.D., Hintze, K.J. and Whanger, P.D. (2001). Cancer-protective properties of high-selenium broccoli. *J. Agric. Food Chem.* **49:** 2679-2683.

Fu, J. and Huang, B. (2001). Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environmental and Experimental Botany*. **45:** 105-114.

Gasper, T. and Lacoppe, J. (1968). The effect of CCC and AMO-1618 on growth, catalase, peroxidase, IAA oxidase activity of young barley seedlings. *Physiol. Plant.* **2:** 1104-1109.

Giannopolitis, C.N. and Ries, S.K. (1977). Superoxide dismutases I. Occurrence in higher plants. *Plant Physiol*. **59:** 309-314.

Hartikainen, H., Xue, T. and Piironen, V. (2000). Selenium as an antioxidant and prooxidant in ryegrass. *Plant and Soil*. **225:** 193-200.

Hodges, D.M., DeLong, J.M., Forney, C.F. and Prange, R.K. (1999). Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta.* **207:** 604-611.

Imlay, J.A. (2003). Pathways of oxidative damage. *Annu Rev Microbiol.* **57:** 395-418.

Khattab, H. (2004). Metabolic and oxidative responses associated with exposure of *Eruca sativa* (Rocket) plants to different levels of selenium. *Int. J. Agric. Biol.* **6:** 1101-1106.

Kong, L., Wang, M. and Bi, D. (2005). Selenium modulates the activities of antioxidant enzymes, osmotic homeostasis and promotes the growth of sorrel seedlings under salt stress. *Plant Growth Regulation.* **45:** 155-163.

Kuznetsov, V.V., Kholodova, V.P., Kuznetsov, V.I.V. and Yagodin B.A. (2003). Selenium regulates the water status of plants exposed to drought. *Dokl. Biol. Sci.* **390:** 266-268.

Maehly, A.C. and Chance, B. (1954). The assay of catalases and peroxidases, in *Methods of Biochemical Analysis* (Glick P.), **Vol. 1**, pp. 357–424. Interscience Publ. lnc., New York .

Mansour, M.M.F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol. Plant.* **43:** 491-500.

Mayland, H.F., Gough, L.P. and Stewart, K.C. (1991). Selenium mobility in soils and its absorption, translocation, and metabolism in plants, p. 55-64. In: R.C. Severson, S.E.Fisher, Jr., and L.P. Gough (eds.),Selenium in arid and semiarid environments, Western United States. U.S. Geol. Sur. Cir. 1064.

Mikkelson, R.L., Page, A.l. and Bingham, A.T. (1989). Factors affecting selenium accumulation by agricultural crops. *Soil Sci. soc. Am. Special Publications*, **23**: 65-94.

Mukherji, S. and Biswas, A.K. (1979). Modulation of chlorophyll, carotene and xanthophylls formation by penicillin, benzyladenine and embryonic axis in mungbean (*Phaseolus aureus* L.) cotyledons. *Ann. Bot.* **43:** 225-229.

Navarro-Alarcon, M. and Cabrera-Vique, C. (2008). Selenium in food and the human body: A review. *Science of the Total Environment*, **400:** 115-141.

Noctor, G. and Foyer, C.H. (2000). Homeostasis of adenylate status during photosynthesis in a fluctuating environment. *J. Exp. Bot.* **51:** 347-356.

Olliver, M. (1967). Ascorbic acid estimation. In: Sebrell, W.H, Harris, R.S., editors, The Vitamins. Academic Press, New York, p.338.

Padmaja, K., Prasad, D.D.K., and Prasad, A.R.K. (1989).Effect of selenium on chlorophyll biosynthesis in mung bean seedlings. *Phytochemistry*, **28:** 3321-3324.

Pennanen, A., Xue, T. and Hartikainen, H. (2002). Protective role of selenium in plant subjected to severe UV irridiation stress. *J. Appl. Bot*., **76:** 66-76.

Pignocchi, C., and Foyer C.H. (2003). Apoplastic ascorbate metabolism and its role in the regulation of cell signalling. *Current Opinion in Plant Biology*, **6:** 379-389.

Sandalio, L.M., Dalurzo, H.C., Gomez, M., Romero–Puertas, M.C. and del Rio, L. A. (2001). Cadmium–induced changes in the growth and oxidative metabolism of pea plants. *J. Exp. Bot.* **52:** 2115–26.

Sairam, R.K.,Veerabhadra,K., and Srivastava, G.C. (2002). Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. *Plant Science*, **163:** 1037-1046.

Sors, T.G., Ellis, D.R. and Salt, D.E. (2005). Selenium uptake, translocation, assimilation and metabolic fate in plants. *Photosynthesis Research*. **86:** 373-389.

Terry, N., Carlson, C., Raab, T. K. and Zayed, A.M. (1992). Rates of selenium volatilization among crop species. *J. Environ. Qual*. **21:** 341-344.

Terry, N., Zayed, A., De Souza,M.P. and Tarun A.S. (2000). Selenium in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **51:** 401-432

Velikova, V., Yordanov, I. and Edreva, A. (2000). Oxidative stress and some antioxidant systems in acid rain-treated bean plants. *Plant Science*. **151:** 59-66.

Whanger, P.D. (2002). Selenocompounds in plants and animals and their biological significance. *J. Am. Col.l Nutr.* **21:** 223-232.

Xue, T., Hartikainen, H. and Piironen, V. (2001). Antioxidative and growth promoting effect of selenium in senescing lettuce. *Plant Soil*. **237:** 55-61.

Yläranta, T. (1983a). Selenium in Finnish agricultural soils. *Annales Agriculturae Fenniae.* **22:** 122-136.

Zayed, A., Lytle, C.M. andTerry, N. (1998). Accumulation and volatilization of different chemical species of selenium by plants. *Planta*. **206:** 284-292.

Zhi-Yong, L., Si-Yuan, G., and Lin L. (2003). Bioeffects of selenite on the growth of *Spirulina platensis* and its biotransformation. *Biores. Techn*. **89:** 171-176.