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Influence of Selenium on Growth, Antioxidants Production and Physiological Parameters of Rice (*Oryza sativa* L.) Seedlings and Its Possible Reversal by Coapplication of Sulphate

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Abstract

The effect of selenate (Na_2SeO_4) and sulphate (Na_2SO_4) was studied on growth and metabolism in two rice cultivars cv. satabdi and cv. khitish. Selenate at low concentration (2 μM) expressed growth promoting effect on rice seedlings as opposed to its high concentration (≥20 μM) where the test seedlings showed stunted growth with browning at the apices of both roots and shoots. The chlorophyll contents showed a dose dependent effect. Both chlorophyll a and chlorophyll b contents were inhibited with increase in selenate concentrations. The effect was more pronounced in cv. satabdi compared to cv. khitish.The level of accessory pigments was deferentially affected by selenium treatment. Simultaneously, the fluorescence intensity and Hill activity decreased with increase in selenate concentrations in the test seedlings. It is assumed that selenium plays a protective role in plants subjected to stress and prevents the formation of reactive oxygen species (ROS) in the cells. Higher selenate concentrations (≥20 μM) exerted variable effect on the activities of enzymatic antioxidants viz.; superoxide dismutase (SOD), catechol peroxidase (CPX) and catalase (CAT) in the test seedlings. The activity of SOD increased with increase in selenate concentrations, whereas activities of CAT and CPX decreased. Under high selenate concentrations, the levels of oxidative stress markers, viz.; proline, H_2O_2 and MDA were also enhanced. Selenium induced accumulation of total soluble sugar and increased the level of both reducing and non reducing sugars in both the test cultivars. The starch contents concomitantly decreased with rise in selenate concentrations. Moreover, the nutrient contents of test seedlings were significantly influenced by selenium. The Na and K levels gradually increased whereas Ca, Mg and Fe

levels decreased on application of selenate. Joint application of 10 mM sulphate and selenate showed significant alterations on all parameters tested with respect to selenate treatment alone. Partial to complete amelioration occurred in the test seedlings treated with high concentrations of selenate and sulphate. Our study shows that selenium at low concentration had a stimulatory effect on growth and metabolism as against high concentrations which proved to be toxic to the rice seedlings obtained from both the cultivars. Effects were more pronounced in cv. satabdi than in cv. khitish which is considered to be comparatively tolerant to selenium. The dose dependent influence of selenium on the physiological and biochemical responses of test seedlings may be reversed by co-application with sulphate.

Keywords

Rice, Selenate, Sulphate, Growth, Biochemical Changes

1. Introduction

Selenium (Se), belonging to oxygen sulphur family, acts as an essential micronutrient as well as an antioxidant in microbes and animals but its role in plants is debatable. The narrow margin between the beneficial and harmful levels of Se has important implications for human health. Selenium evidently possesses antidiabetic, immunostimulating, cardio-protective, antiviral, anti-carcinogenic and detoxification properties [1]. Plant sources of selenium are more bioavailable than animal sources but the translocation of selenium from root to shoot is dependent on the form of selenium supplied [2]. In plants, Se is primarily absorbed as selenite or selenate from soil. It is then translocated to the chloroplast, where it follows the sulfur assimilation pathway [3]. Selenium and sulphur being chalcogens compete readily for their uptake in plants as both anions are taken up via sulphate transporter(s) in the root plasma membrane [4] [5].

Although Se is not regarded as a micronutrient for higher plants [6], studies in different plant species have shown that at low concentration selenium exerts beneficial effect on growth and stress tolerance [7] [8] [9]. Selenium at low concentration stimulates the growth of *Astragalus*, *Arabidopsis*, *Brassica* and *Stanleya* but the presence of sulphate ions influences the uptake of Se in these species [10] [11] [12] [13]. Selenium not only regulates water status of plants under drought conditions [14], it also confers resistance against oxidative stress in them [15] by enhancing the activities of both enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), catechol peroxidase (CPX) and non enzymatic antioxidants like GSH, ascorbate and carotenoids [16]. However, at high concentrations, Se is phytotoxic and accelerates accumulation of reactive oxygen species (ROS) [17]. Uptake of high concentration of Se by plant roots may lead to symptoms of injury including stunted growth, chlorosis, withering and drying of leaves, decreased protein synthesis and also premature death [18].

Selenosis due to high Se accumulation leads to malformed selenoproteins that alter redox potential, induce oxidative stress and affect enzyme kinetics [19]. Selenate also compromises membrane integrity accompanied by increased activity of ROS-scavenging enzymes, including superoxide dismutase, catalase and peroxidase [20] [21].

Fe-S cluster proteins of chloroplast and mitochondria are susceptible to Se-induced damages and interfere with chlorophyll synthesis as well as respiratory processes [22]. Se induced interference in pigment synthesis possibly affects the photosynthetic machinery which reduces synthesis of starch and alters carbohydrate metabolism. Detoxification of ROS is however accomplished by an up regulation in enzymatic and non-enzymatic scavenging systems inside the plant [23].

Malnutrition is predominant in developing countries where almost half the population suffers from diseases caused by deficiency in essential mineral uptake. Nutritional disorders due to the mineral elements may manifest as deficiency or excesses of particular elements. Minerals are important chemical elements which act as essential nutrients for plants and aid in various metabolic activities. They are absorbed from the soil by plants in the form of organic and inorganic ions. Plant tissues are able to bind metals as the various functional groups present in them can attract and sequester the metallic ions [24]. In plants the sequestered ions act as structural components in carbohydrates and proteins; organic molecules in metabolism (viz., magnesium in chlorophyll and phosphorus in ATP), enzyme activators and channel facilitators like potassium and sodium, and also as osmoticum for maintaining osmotic balance. Ions like Cu, Fe, Se, Mn and Zn form an integral part of enzymatic antioxidants and sufficient amount of trace elements like Fe, Cu and Zn are required for energy production as well as to provide protection from the highly toxic reactive oxygen species. Trace metals or minerals have significant roles to play in the catalytic activities of major antioxidant enzymes during maturation, activation and defence mechanisms of the cell [25].

Surplus of Se inhibits the absorption of metals, mainly Mn, Zn, Cu, and Cd and causes deficiency of nutrients in plants [26]. Deficiency in essential mineral uptake not only affects physiological processes but also generates nutritional disorders in plants and subsequently in humans. The mineral constituents are either directly or indirectly influenced by various factors which affect their availability to humans and animals from different dietary sources. Knowledge of the importance of the mineral elements in plants is essential as the global trend in nutrition and medicine is shifting towards the use of plant as fruits and vegetables and phyto-medicines [27]. Part of our work was to evaluate the mineral composition of test seedlings under the influence of Se salt on nutrients accumulation in the two rice cultivars cv. khitish and cv. satabdi. The potentially deleterious effects of Se contamination in agricultural areas with large selenate content in the soil may be controlled by S application as a close relationship exist

between Se and S metabolism in plants due to their physical and chemical similarities. Selenate competes with the high-affinity sulfate transporter sultr1; 1 for influx in the roots and its toxicity is progressively reduced in presence of increasing levels of sulphate in the rhizosphere because high sulphate concentrations in the rhizosphere reduce selenate uptake [28] [29].

Biofortification or enriching crop tissues which act as potential dietary source by exogenous supplementation of bioavailable elements is a promising way to counteract the menace posed by nutrient deficiencies [30]. With this point in view the importance of obtaining an adequate crop yield with suitable nutrient concentration of macronutrients especially Na, K, Ca and micronutrient such as Fe have been studied. The knowledge obtained on the nutritional status of a plant in presence of Se and under the influence of co-application of Se and S will subsequently shed light on the functional state of the test cultivars cv. satabdi and cv. khitish.

Rice is an important crop plant which forms the staple food for about half of the world population. Rice (*Oryza sativa* L.) also forms one of the most predominant cereal crops in Eastern India including West Bengal. Presently farmers prefer to cultivate hybrid rice varieties as they are cost productive and can withstand or counteract stresses induced by various biotic and abiotic factors. Hybrid rice varieties such as cv. Satabdi (drought sensitive) and cv. Khitish (drought resistant) are much in demand in West Bengal due to the finer quality of the grains as well as higher market value. So, we based our study on these two cultivars of rice. Se biofortified rice may act as important dietary source to human and provide the necessary nutrients required to alleviate health issues related to its deficiency. Standardization of ideal strategy to increase Se level in rice plants has to be developed which is both economically and agronomically suitable for human consumption [1]. With this perspective, our study was focused on the influence of selenate, the readily bioavailable form of inorganic Se in the environment on the above mentioned cultivars of rice.

2. Plant Material and Selenium Treatment

Rice (*Oryza sativa* L.) seeds cv. Satabdi (IET 4786) and cv. khitish (IET 4094) were obtained from the State Rice Research Station, Chinsurah, Hoogly, West Bengal. The seeds were surface sterilized with sodium hypochlorite (5% w/v) and thoroughly washed with distilled water. The petridishes of diameter-10 cm lined with filter papers contained about 100 seeds for each treatment. The petridishes were kept in dark and humid condition for 72 hours at 30°C ± 2°C in a germinator. After germination, the seedlings were grown hydroponically in modified Hoagland solution with various concentrations (2 μM, 10 μM, 20 μM, 50 μM) of sodium selenate ($Na_2SeO_4 \cdot 7H_2O$; Loba-Chemie, India)with or without 10 mM sodium sulphate (Na_2SO_4; Merck, India). The test seedlings were then exposed to 16 h photoperiod (260 μmol·m^{-2}·s^{-1} PFD) and harvested after a total of 21 days for the following studies.

2.1. Growth and Water Content Measurements

From 21 days rice seedlings the root and shoot lengths of selenate treated and untreated seedlings were measured. Then, 10 seedlings from each set were harvested and weighed. The selected seedlings were allowed to dry at 70°C for 4 days and then at 100°C for 3 days after which the dry weights of the samples were taken. To determine water contents, the differences between fresh weight and dry weight of the seedlings were recorded [31].

2.2. Extraction and Estimation of Selenium and Mineral Content

Total selenium contents were measured from 21 days old test seedlings treated with selenate with or without sulphate by acid digestion of oven dried samples. Dried samples (2 g each) of test seedlings were digested in a microwave digestor using 7 ml HNO_3 (65%), 5 ml HCl and 2 ml H_2O_2 for 60 minutes [32]. Selenium contents of the rice samples were determined by Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) Model No 6300 DUO (Thermofischer) with flow injection hydride generation system, using a standard curve prepared from known concentrations of selenium respectively. The selenium contents were expressed in terms of mg·kg^{-1} DW. Element concentrations of K, Ca, Na, Mg and Fe were analyzed with a Perkin-Elmer 2001 Model Inductively Coupled Plasma-Mass Emission Spectrometry (ICP-MS) and Atomic absorption Spectrometry (AAS).The ion contents were also expressed in terms of mg·kg^{-1} DW.

2.3. Chlorophyll Contents and Fluorescence Activity

Total chlorophyll, chlorophyll-a and chlorophyll-b contents were measured from the rice leaves according to Arnon [33]. 1 g of fresh leaves was extracted with 25 ml of 80% alkaline acetone (0.1 M Na_2CO_3) (v/v). Chlorophyll contents were estimated spectrophotometrically by recording the OD at 645 nm and 663 nm using a Hitachi U-2000 spectrophotometer and calculations were done according to Arnon's (1949) formula as follows:

$$\text{Total chlorophyll} = \left[20.2 \left(A_{645} \right) + 8.02 \left(A_{663} \right) \right] \times V/1000 \times 1/W$$

$$\text{Chlorophyll-a} = \left[12.7 \left(A_{663} \right) - 2.69 \left(A_{645} \right) \right] \times V/1000 \times 1/W$$

$$\text{Chlorophyll-b} = \left[22.9 \left(A_{645} \right) - 4.68 \left(A_{663} \right) \right] \times V/1000 \times 1/W$$

(V = Final volume of chlorophyll extract in 80% acetone, W = fresh weight of leaf in grams, A = OD value).

The chlorophyll contents were expressed in terms of mg chlorophyll present g^{-1}. FW. The fluorescence of the chlorophyll was monitored at an excitation wavelength 640 nm and emission wavelength 680 nm with the help of a Hitachi650-40 spectro fluorometer and results were expressed as fluorescence intensity of the chlorophyll obtained from g^{-1} FW.

2.4. Carotenoid Contents

Carotene and xanthophyll contents were estimated according to the protocol

described by Mukherji and Biswas [34]. 20 ml of cyclohexane was mixed with the pigmented alkaline acetone solution in a separating funnel. The hexane layer was washed with 20 ml of water. Xanthophylls were removed from the upper hexane layer containing carotene by repeated extraction with 20 ml of 90% methanol (v/v). Carotene and xanthophyll contents were measured by utilizing the values of absorbance at 425 nm and 450 nm respectively using a Hitachi U-2000 spectrophotometer and data were expressed in terms of OD g^{-1} FW.

2.5. Hill Activity

Hill activity was assayed according to Vishniac [35]. 1 g fresh green leaves were extracted in 5 ml sucrose-phosphate buffer (0.4 M sucrose in 0.05 M sodium phosphate buffer) at pH 6.2 and centrifuged at 2000 g for 10 minutes. The pellet was discarded and the supernatant was recentrifuged at 8000 g for 20 minutes. Now the pellet containing chloroplasts were resuspended in 5 ml sucrose-phosphate buffer. The reaction mixture consisted of 1 ml chloroplast suspension, 4 ml sucrose phosphate buffer, 0.5 ml 0.03% DCPIP and initial absorbance was recorded at 610 nm using Hitachi U-2000 spectrophotometer. The reaction mixtures were kept in bright sunlight for 5 minutes and discolouration of DCPIP was recorded at 610 nm. The difference in OD was converted in terms of μmole DCPIP photoreduced from a standard curve using known concentrations of DCPIP. Also the chlorophyll contents of the isolated chloroplast were measured according to the method of Arnon [33]. Hill activity was expressed as μmole DCPIP photoreduced mg^{-1} chlorophyll min^{-1}.

2.6. Extraction and Assays of Antioxidant Enzymes

Enzyme extraction procedures were carried out at 4˚C. 1 g of plant sample from each treatment was homogenized in 5 ml of pre-chilled 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 12,000 g for 20 minutes and supernatant was used to assay the enzymes activities.

2.6.1. Assay of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (EC 1.15.1.1) activity was assayed by using the nitroblue tetrazolium method [36]. The reaction mixture contained 2.5 ml of 80 mM Tris-HCl buffer (pH 7.5) containing 0.12 mM EDTA and 10.8 mM TEMED, 0.1 ml 0.0033% BSA, 0.1 ml 6 mM NBT, 0.1 ml of 0.6 mM riboflavin and 0.1 ml enzyme extract. The reaction was started by the addition of riboflavin, and the glass tube was shaken and placed under a fluorescent lamp (60 μmol·m^{-2}·s^{-1}). The reaction was allowed to proceed for 10 minutes and was stopped by switching off the light. The absorbance was measured at 560 nm. Blank and control were run in the same manner but without illumination and enzyme respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay condition. Enzyme activity was expressed as EU SOD mg^{-1} protein min^{-1}.

2.6.2. Assay of Catalase (CAT) Activity

Catalase (EC 1.11.1.6) activity was determined as the amount of $KMnO_4$ consumed in terms of H_2O_2 [37]. The reaction mixture contained 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.0), 1 ml of 3% H_2O_2 (v/v) and 1 ml of enzyme extract. After incubation, the reaction was stopped by adding 3 ml of 10% H_2SO_4 (v/v). The residual H_2O_2 was titrated against 0.02 N $KMnO_4$. The enzyme activity was expressed in terms of mg H_2O_2 decomposed mg^{-1} protein min^{-1}.

2.6.3. Assay of Catechol Peroxidase (CPX) Activity

Catechol peroxidase (EC 1.11.1.7) activity was assayed spectrophotometrically [38] using a Hitachi U-2000 spectrophotometer. The reaction mixtures contained 5 ml of 0.1 M sodium phosphate buffer (pH 7.0), 1 ml of 10% H_2O_2 (v/v), 1 ml of 0.5% catechol (w/v) and 1 ml enzyme extract. The absorbance values of reaction mixtures were recorded at 480 nm at 0 time and after incubation for 1 minute. The enzymatic activity was expressed in terms of change in absorbance, A_{480} mg^{-1} protein min^{-1}.

2.7. Proline Content

1 g of sample from 21 days old rice seedlings was extracted with 5 ml of 0.1 M sulpho salicylic acid and centrifuged at 5000 g for 30 minutes [39]. To 2 ml of supernatant, 5 ml GAA and 5 ml of 140 mM acid ninhydrin were added and shaken vigorously. The mixture was heated in a boiling water bath and after cooling, the mixture was extracted in 10 ml of toluene in a separating funnel and aqueous layer was discarded. The absorbance of the mixture was measured at 520 nm. The proline content was calculated from standard curve and expressed as $\mu g \cdot g^{-1}$ FW.

2.8. Hydrogen Peroxide (H_2O_2) Content

The H_2O_2 content from root and shoot samples from 21 days old rice seedlings were measured as described by Velikova *et al.* [40]. 1 g tissue was extracted with 5 ml of trichloro acetic acid (0.1%, w/v) at 4°C and homogenate was centrifuged at 12,000 g for 15 minutes. To 0.5 ml of supernatant, 0.5 ml of 0.05 M sodium phosphate buffer (pH 7.0) and 1 ml of 1 M potassium iodide (KI) solution were added. The absorption of the mixture was measured at 390 nm. The H_2O_2 content was determined using an extinction coefficient (ε) of 0.28 $\mu M^{-1} \cdot cm^{-1}$ and expressed as $\mu mol \cdot g^{-1}$ FW.

2.9. Malondialdehyde Content (Lipid Peroxidation)

Lipid peroxidation in terms of malondialdehyde (MDA) contents was determined to assess the membrane damage in rice seedlings. For the measurement of lipid peroxidation, the thiobarbituric acid (TBA) test was used to measure MDA level as an end product of lipid peroxidation [41]. 1 g tissue was homogenized in 4 ml of 1% trichloroacetic acid (TCA) solution (w/v) and centrifuged at 10,000 g for 10 minutes. The supernatant was mixed with 1 ml of 0.5% TBA in 20% TCA

(w/v). The mixture was incubated in boiling water for 30 minutes and the reaction was stopped by placing the tubes in an ice bath. The samples were re-centrifuged at 10,000 g for 5 minutes and the absorbance values of the supernatants were measured at 532 nm. The values for nonspecific absorption at 600 nm were subtracted. The amount of MDA-TBA complex present was calculated using an extinction coefficient (ε) of 155 mM^{-1}·cm^{-1} and expressed as µmol·g^{-1} FW.

2.10. Total Soluble Sugar Content

The amount of total soluble sugar was estimated by phenol sulphuric acid reagent method [42]. 1 g of fresh shoot and root samples from each treatment set were homogenized with 5 ml 80% ethanol (v/v). The alcoholic extracts were centrifuged at 2000 g for 20 minutes. To 1 ml of supernatant, 0.05 ml of 5% phenol solution (v/v) and 5 ml 98% H_2SO_4 were added. The mixtures were incubated in water bath at 30°C for 20 minutes. The OD values of the characteristic yellow orange colour were measured at 490 nm in a Hitachi-2000 spectrophotometer. By using standard curve of glucose, the quantity of the total soluble sugar was calculated and expressed as mg·g^{-1} FW.

2.10.1. Reducing and Non-Reducing Sugar Contents

Estimation of reducing sugar contents was done by the method of Miller [43]. 1 g of plant material was crushed with 5 ml of 80% ethanol (v/v) and centrifuged at 2000 g for 20 minutes. To 1 ml of alcoholic extract, 0.5 ml of 1% (w/v) DNSA reagent was added and boiled in water bath for 5 minutes. After cooling, the absorbance was measured at 515 nm using a Hitachi-2000 spectrophotometer. From a standard curve of glucose, the quantity of reducing sugar was calculated and expressed as mg·g^{-1} FW. The amount of non-reducing sugar was measured by subtracting the value of reducing sugar from the value of total soluble sugar.

2.10.2. Starch Content

Estimation of starch was done by the method of Mc Cready *et al.* [44]. The residual mass, obtained after centrifugation for the extraction of total soluble sugar was suspended in 2.5 ml of distilled water followed by the addition of 3.25 ml of 52% (v/v) $HClO_4$. After stirring, the mixture was centrifuged at 2000 g for 20 minutes. The supernatant was collected and poured in conical flasks and the total volume was made up to 50 ml by adding distilled water for each set. After filtration through Whatman (No. 42) filter paper, 1 ml of filtrate from each set was taken and starch contents were measured following the same procedure as the total soluble sugar. Quantity of starch was calculated in terms of glucose and factor 0.9 was used to convert the values of glucose to starch. The quantity of starch was expressed as mg·g^{-1} FW.

2.11. Estimation of Protein Content

In all the enzyme preparations, protein contents were estimated by the method

of Lowry *et al.* [45] using bovine serum albumin (BSA, Sigma) as standard.

2.12. Statistical Analysis

The experiments were carried out in a completely randomized design (CRD) with means of three repeats with two replicates in each time; each replication comprised a single petridish containing 100 seeds. The data and significant differences among mean values were compared by descriptive statistics (±SE).

3. Results

3.1. Effect on Growth

A marked inhibition in growth of rice seedlings with increase in concentrations of selenium were observed in both roots and shoots of cv. satabdi and cv. khitish (Figure 1(a) and Figure 1(b)). The growths of roots were more affected than shoots along with browning of the apices. At 2 µM selenate, the root length increased on an average, by about 5% whereas for shoot the increase was by about 3% over water control in cv. satabdi. On the contrary, in cv. khitish there was almost negligible increase in growth both in root and shoot over control. However, under 10 µM, 20 µM and 50 µM selenate treatments, the root lengths were
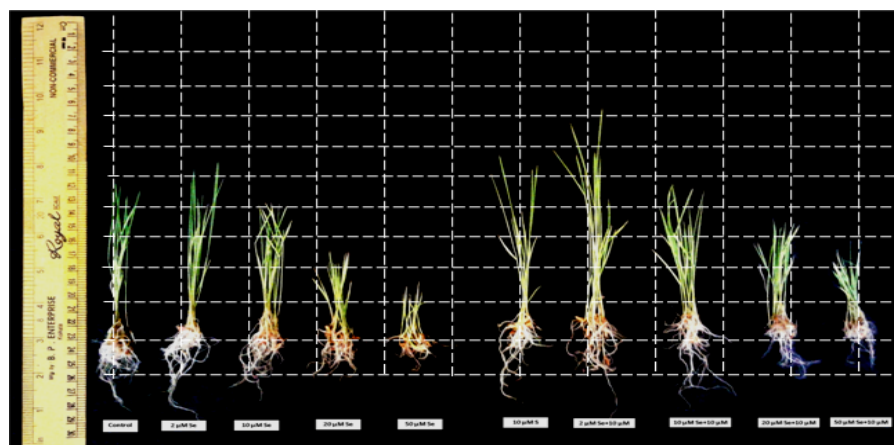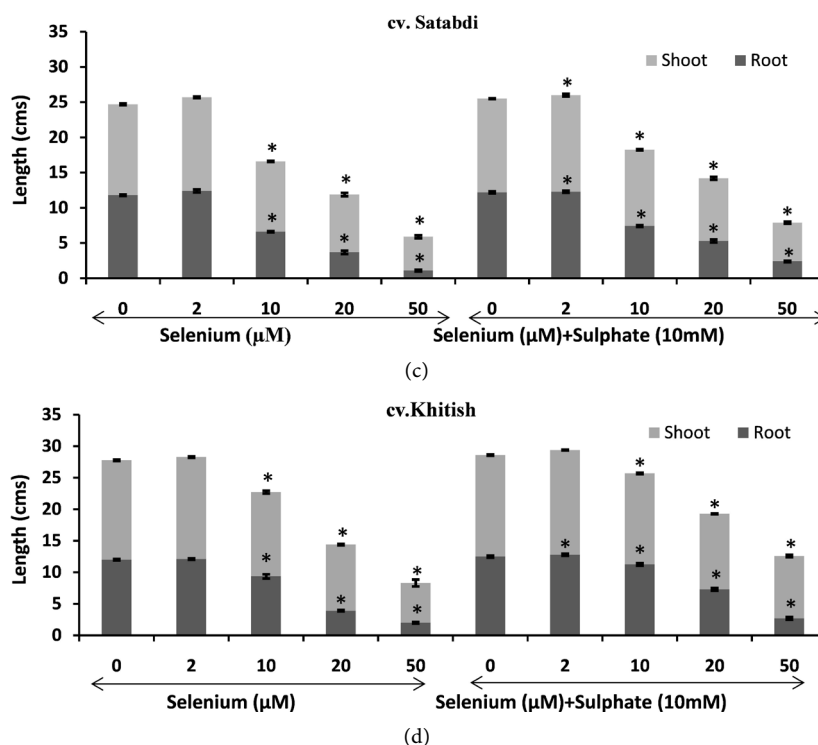

(a)

(b)

Figure 1. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the growth of rice (cv. Satabdi) seedlings. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the growth of rice (cv. Khitish) seedlings. (c) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the growth of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at $p \leq 0.05$ respectively compared to control. (d) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the growth of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at $p \leq 0.05$ respectively compared to control.

reduced significantly on an average, by about 44%, 69%, 91% in cv. satabdi and by about 22%, 68%, 83% in cv. khitish respectively over water control. Decrease in shoot lengths by about 23%, 36%, 63% in cv. satabdi and about 15%, 34%, 60% in cv. khitish also occurred under similar concentrations respectively over control.

Application of sulphate (10 mM) with 2 μM selenate ameliorated the effect caused by selenate alone and increased root lengths of both cv. satabdi and cv. khitish on an average, by about 4% and 7% respectively over control. However, the margin of inhibitory effect was narrowed down in root on an average, by about 37%, 55%, 80% in cv. satabdi and by about 6%, 39%, 78% in cv. khitish under 10 μM, 20 μM and 50 μM selenate and (10 mM) sulphate treatments over control. Similarly, under 2 μM selenate and sulphate treatment, the shoot length increased by about 6% in cv. satabdi and 5% in cv. khitish with respect to control. However, the shoot length decreased by about 16%, 31%, and 57% in cv.

satabdi and by about 9%, 24%, and 37% in cv. khitish under 10 μM, 20 μM and 50 μM selenate and (10 mM) sulphate treatments respectively over water control. Therefore, the inhibitory effects observed in shoots of test seedlings treated with combined solutions of selenate and sulphate is less compared to those treated with selenate alone in both cultivars (**Figure 1(c)** and **Figure 1(d)**).

3.2. Effect on Water Content

The water content showed a dose dependent effect of selenate on the test seedlings. At 2 μM selenium, water content increased on an average, by about 2% in the test seedlings of both cultivars. Application of 10 μM, 20 μM and 50 μM selenate reduced the water content by about 3%, 9%, 38% in cv. satabdi and by about 12%, 13%, 19% in cv. khitish respectively over control.

The water content increased on an average by about 5% in 2 μM selenate and sulphate treated test seedlings of both cultivars. Addition of sulphate together with higher concentrations of selenate narrowed down the inhibitory effect on water content of the rice seedlings. Co-application of 10 mM sulphate and 10 μM, 20 μM and 50 μM selenate reduced the water content by about 1%, 9%, 27% in cv. satabdi and by about 4%, 9%, 16% in cv. khitish respectively over water control (**Figure 2**).

3.3. Effect on Pigments

Treatment with 2 μM selenate enhanced chl a and chl b contents by about 8% and 13%, while total chlorophyll contents increased by about 9% in cv. satabdi. On the other hand, in 2 μM selenate treated seedlings of cv. khitish, an increment of about 3% and 12% in chl a and chl b contents respectively were noted along with 5% increase in total chlorophyll contents over control. At higher selenate doses both chl a and chl b levels linearly decreased with increase in selenate concentrations in both the test cultivars. Chl a contents decreased by about 8%, 28% and 45% in cv. satabdi and by about 18%, 23% and 26% in cv. khitish under 10 μM, 20 μM and 50 μM selenate treatments respectively. The chl b contents also
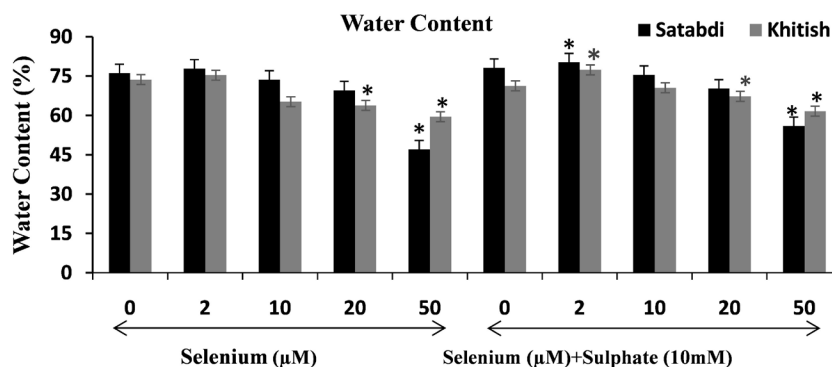
Figure 2. Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the water content of rice (cv. satabdi & cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

declined under similar treatments by about 6%, 25% and 38% in cv. satabdi and by about 6%, 18% and 29% in cv. khitish respectively over control. As a result, under 10 μM, 20 μM and 50 μM selenate treatment, the inhibitory effect on total chlorophyll gradually increased in cv. satabdi seedlings by about 7%, 23% and 43% and in cv. khitish by about 8%, 13% and 23% respectively over water control (Table 1 and Table 2).

Co-application of selenate along with sulphate significantly ameliorated the

Table 1. Effect of selenate and/or sulphate on pigment contents in twenty one days old rice (cv. Satabdi) seedlings.

Treatment	Chlorophyll a (mg·g⁻¹f w)	Chlorophyll b (mg·g⁻¹ f w)	Total Chlorophyll (mg·g⁻¹ f w)	Fluorescence Intensity	Carotene (A₄₂₅ g⁻¹ f w)	Xanthophyll (A₄₅₀ g⁻¹ f w)
Control	0.40 ± 0.05	0.16 ± 0.06	0.56 ± 0.05	555.0 ± 2.73	0.38 ± 0.06	0.37 ± 0.04
Selenate						
2 μM	0.43 ± 0.04	0.18 ± 0.04	0.61 ± 0.04	600.0 ± 2.81	0.40 ± 0.04	0.39 ± 0.05
10 μM	0.37 ± 0.04	0.15 ± 0.05	0.52 ± 0.05	$480.3^* \pm 2.80$	0.36 ± 0.06	0.33 ± 0.06
20 μM	$0.29^* \pm 0.05$	$0.12^* \pm 0.06$	$0.43^* \pm 0.06$	$440.0^* \pm 2.74$	0.36 ± 0.04	0.31 ± 0.04
50 μM	$0.22^* \pm 0.04$	$0.10^* \pm 0.06$	$0.32^* \pm 0.05$	$410.0^* \pm 2.77$	$0.31^* \pm 0.04$	$0.30^* \pm 0.06$
Sulphate (10 μM)	0.41 ± 0.06	0.18 ± 0.05	0.59 ± 0.06	545.0 ± 2.73	0.43 ± 0.05	0.45 ± 0.06
+ Selenate						
2μM	0.45 ± 0.05	0.19 ± 0.06	0.64 ± 0.06	570.5 ± 2.62	0.42 ± 0.04	0.44 ± 0.06
10μM	0.38 ± 0.07	0.16 ± 0.07	0.54 ± 0.05	550.0 ± 2.68	0.41 ± 0.06	0.36 ± 0.06
20μM	0.32 ± 0.04	0.14 ± 0.05	$0.47^* \pm 0.05$	$474.0^* \pm 2.67$	0.39 ± 0.05	0.35 ± 0.05
50μM	$0.28^* \pm 0.06$	$0.13^* \pm 0.06$	$0.41^* \pm 0.06$	$428.0^* \pm 2.70$	0.34 ± 0.05	0.34 ± 0.05

Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

Table 2. Effect of selenate and/or sulphate on pigment contents in twenty one days old rice (cv. Khitish) seedlings.

Treatment	Chlorophyll a (mg·g⁻¹f w)	Chlorophyll b (mg·g⁻¹ f w)	Total Chlorophyll (mg·g⁻¹ f w)	Fluorescence Intensity	Carotene (A₄₂₅ g⁻¹ f w)	Xanthophyll (A₄₅₀ g⁻¹ f w)
Control	0.39 ± 0.04	0.17 ± 0.10	0.56 ± 0.04	525.8 ± 2.82	0.51 ± 0.03	0.45 ± 0.04
Selenate						
2 μM	0.40 ± 0.06	0.19 ± 0.12	0.59 ± 0.04	530.0 ± 2.79	0.48 ± 0.06	0.43 ± 0.05
10 μM	0.32 ± 0.05	0.16 ± 0.12	0.48 ± 0.05	490.0 ± 2.73	0.44 ± 0.05	0.38 ± 0.04
20 μM	0.30 ± 0.06	0.14 ± 0.10	$0.44^* \pm 0.04$	457.5 ± 2.80	0.38 ± 0.07	0.34 ± 0.06
50 μM	$0.29^* \pm 0.05$	0.12 ± 0.12	$0.41^* \pm 0.06$	$412.5^* \pm 2.76$	0.36 ± 0.06	0.32 ± 0.06
Sulphate (10 μM)	0.38 ± 0.06	0.22 ± 0.11	0.60 ± 0.05	515.0 ± 2.82	0.49 ± 0.06	0.48 ± 0.04
+Selenate						
2 μM	0.41 ± 0.06	0.20 ± 0.13	0.61 ± 0.06	540.0 ± 2.71	0.50 ± 0.05	0.46 ± 0.04
10 μM	0.36 ± 0.04	0.18 ± 0.12	0.54 ± 0.04	510.0 ± 2.77	0.43 ± 0.06	0.42 ± 0.05
20 μM	0.33 ± 0.04	0.15 ± 0.14	0.48 ± 0.06	480.0 ± 2.69	0.38 ± 0.05	0.37 ± 0.05
50 μM	0.32 ± 0.04	0.13 ± 0.13	$0.45^* \pm 0.05$	$440.0^* \pm 2.78$	0.37 ± 0.05	0.36 ± 0.05

Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

effect of selenate alone on the test seedlings. Joint treatment with 2 μM selenate and sulphate enhanced chl a by about 13% and chl b by about 19% in cv. satabdi while in cv. khitish, similar treatment increased the level of chl a and chl b by about 5% and 18% respectively over water control. Simultaneous application of sulphate along with 10 μM, 20 μM and 50 μM concentrations of selenate showed a recovery of inhibition in total chlorophyll contents by about 4%, 16% and 27% in cv. satabdi and by about 3%, 5% and 7% in cv. khitish seedlings with respect to control.

The fluorescence intensity (F.I) initially increased on an average by about 4% in seedlings of both cultivars under 2 μM selenate treatments. Subsequently a significant drop in F.I were recorded at 10 μM, 20 μM and 50 μM selenate treatment which were about 14%, 21% and 26% respectively in cv. satabdi and about 7%, 13% and 22% respectively in cv. khitish over control. Combined application of selenate and sulphate mitigated the inhibitory effect on F.I to some degree in the test seedlings. In cv. satabdi, application of 10 mM sulphate along with 10 μM, 20 μM and 50 μM selenate narrowed down the inhibitory effect on F.I by about 1%, 15% and 23% respectively whereas in cv. khitish, the F.I decreased by about 3%, 9% and 16% respectively over water control.

The Hill Reaction Activity (HRA) was negatively affected with increase in selenate concentration in both test cultivars. The HRA was reduced on an average by about 5% in the 2 μM treated rice seedlings of cv. satabdi and cv. khitish. A linear decline by about 11%, 34% and 42% in HRA activity were observed in 10 μM, 20 μM and 50 μM selenate treated rice seedlings of cv. satabdi with respect to water control. Similar pattern was observed in cv. khitish where the HRA also decreased by about 17%, 34% and 36% in the said concentrations of selenate over control. However joint application of sulphate and selenate ameliorated the negative effect of selenate alone on Hill Reaction Activity considerably which coincided with its promotive influence on the chlorophyll contents of the test tissue.

Selenium exerted an inhibitory effect on the level of accessory pigments, carotene and xanthophyll in seedlings of cv. satabdi and cv. khitish. At 2 μM selenate concentration, both carotene and xanthophyll levels increased by about 5% in cv. satabdi while in cv. khitish, the level of both the accessory pigments declined on an average by about 5% with respect to water control. A gradual decline in both carotene and xanthophyll contents at higher concentrations of selenate in both the test cultivars were noted. Carotene contents decreasedby about 5% under both 10 μM and 20 μM selenate treatments and by about 13% under 50 μM selenate treatment in cv. satabdi compared to about 14%, 26% and 29% in cv. khitish seedlings. Xanthophyll contents similarly decreased by about 11%, 16% and 19% in cv. satabdi and by about 16%, 24% and 29% in cv. khitish under 10 μM, 20 μM and 50 μM selenate treatment respectively. Therefore, selenate treatment affected the chlorophyll contents as well as accessory pigments of cv. satabdi to a greater extent compared to that of cv. khitish.

On application of sulphate along with 2 μM selenate, carotene contents increased by about 11% while xanthophyll contents increased by about 19% in cv.

satabdi seedlings over control. However, there was very little change in carotene and xanthophyll levels on seedlings of cv. khitish treated with 2 μM selenate and sulphate. Under combined treatments, carotene contents in cv. satabdi increased by 8% and 3% under 10 μM and 20 μM selenate and sulphate treatments respectively over control. The margin of decline in carotene contents was however, narrowed down by about 11% in 50 μM selenate and sulphate treated rice seedlings of cv. satabdi in comparison to the test seedlings treated individually with 50 μM selenate. Similarly in cv. khitish, the carotene level declined by about 17%, 26% and 28% under the said concentrations of selenate and sulphate. Following an identical pattern the xanthophyll contents also declined by about 3%, 5% and 8% and by about 7%, 18% and 20% in cv. satabdi and cv. khitish respectively under combined treatment with sulphate and said concentrations of selenate over control.

3.4. Effect on Antioxidant Enzymes Activity

3.4.1. Superoxide Dismutase (SOD)

Exogenous application of selenate showed a dose dependent increase in SOD activity in roots and shoots of both cultivars of rice over control. In case of 2 μM selenate treatment very little increase in SOD activity were observed in test seedlings of cv. satabdi and cv. khitish over control. Thereafter, the SOD activity in roots increased remarkably by about 69%, 144%, 147% and in shoots by about 78%, 109%, 160% in 10 μM, 20 μM and 50 μM selenate treated rice seedlings of cv. satabdi respectively. The enzyme activity also increased significantly by about 75%, 145%, 150% in roots and by about 42%, 82%, 83% in shoots of cv. khitish respectively over water control (**Figure 3(a)** and **Figure 3(b)**).

Administration of sulphate and 2 μM selenate together inhibited SOD activity in root and shoot by about 9% and 4% in cv. Satabdi and by about 5% and 33% in cv. khitish respectively over control. The inhibitory effect was further reduced under 10 μM selenate, sulphate treatment by about about 9% in root and very little in shoots of cv. satabdi. Although in cv. khitish the SOD activity increased at 10 μM selenate, sulphate treatment by about 42% in root and by about 9% in shoot, the increment was much less in comparison to seedlings treated with selenate only. Similar trend was observed in cv. satabdi where the rise in SOD activity by about 60% and 123% in roots and by about 79% and 128% in shoots respectively under the influence of combined 20 μM and 50 μM selenate and sulphate solutions was less than that observed in seedlings treated with same concentration of selenate alone. The same effect was reflected in cv. khitish where the enhanced enzyme activity of about 42%, 89% and 138% in roots and about 8%, 35% and 37% in shoots under 10 μM, 20 μM and 50 μM selenate and sulphate treatment were less compared to rice seedlings treated with selenate only.

3.4.2. Catechol Peroxidase (CPX)

An opposite effect was observed in case of catechol peroxidase (CPX) activity in the test seedlings under the influence of selenate. The enzyme activity decreased
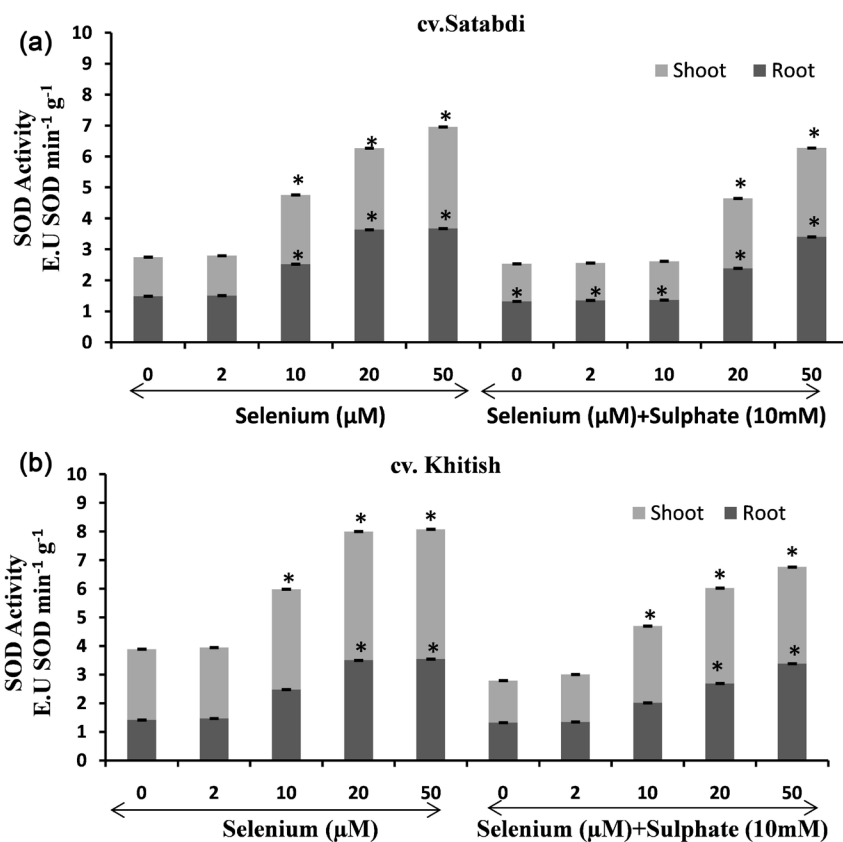
Figure 3. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the SOD activity of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the SOD activity of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

with increase in concentrations of selenate in roots and shoots of both test cultivars. The activity of CPX under 2 µM selenate treatment decreased in root and shoot by about 36% and 8% in cv. satabdi and by about 7% in root and very little in shoot of cv. khitish over control. Exposure to 10 µM, 20 µM and 50 µM selenate inhibited enzyme activity by about 18%, 44% and 60% in roots and 22%, 24% and 25% in shoots of cv. satabdi and by about 10%, 47% and 55% in roots and very little in shoots of cv. khitish respectively over control (Figure 4(a) and Figure 4(b)).

Co-application of 10 mM sulphate and selenate inhibited CPX activity in test seedlings but the inhibitory effect was less pronounced than observed in seedlings treated with selenate alone. Factorial combination of 2 µM selenate and sulphate reduced enzyme activity by about 35% and 22% in root and shoot respectively in cv. Satabdi. However, under same treatment, the enzyme activity decreased by about 3% in roots and increased very little in shoots of cv. khitish over water control. Further in cv. satabdi the CPX activity declined on an average
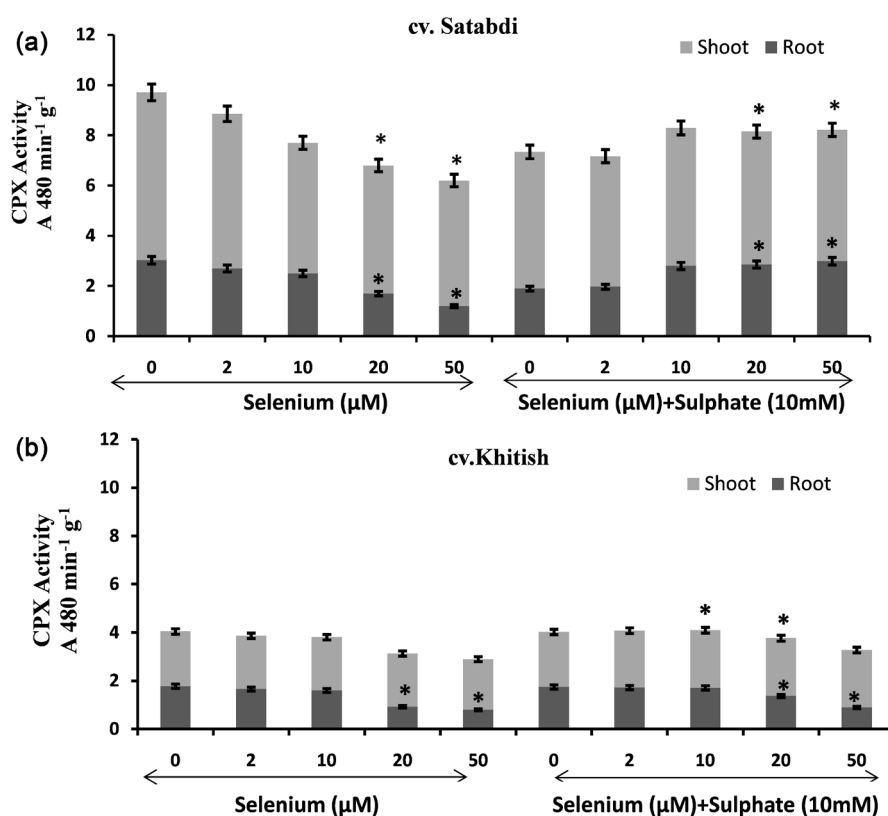
Figure 4. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the CPX activity of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the CPX activity of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

by about 5% in roots and 20% in shoots of 10 μM, 20 μM and 50 μM selenate and sulphate treated test seedlings. On the contrary the enzyme activity decreased by about 4%, 23% and 49% in roots but increased on an average by about 5% in shoots of cv. khitish with respect to control.

3.4.3. Catalase (CAT)

Selenate exerted a variable effect on the catalase activity in roots and shoots of the test seedlings. In both the cultivars, the catalase activity in roots decreased with increasing concentrations of selenate. However, at 2 μM selenate treatment the catalase activity increased by about 14% and 54% in roots and shoots of cv. satabdi respectively over control. Under same concentration of selenate, very little increment in the enzyme activity occurred in roots while it increased by about 28% in shoots of cv. khitish with respect to control. Thereafter application of 10 μM, 20 μM and 50 μM selenate inhibited catalase activity by about 6%, 28% and 54% in roots and initially increased by about 15% and then decreased by about 4% and 14% in shoots of cv.satabdi respectively. Similar trend was ob-

served in cv. khitish where the enzyme activity gradually decreased by about 6%, 17% and 35% in roots under 10 μM, 20 μM and 50 μM selenate treatment. However, in shoots of cv. khitish, the catalase activity increased by about 28% and 8% under 10 μM and 20 μM selenate treatment respectively followed by significant decrease of about 25% under 50 μM selenate treatment with respect to control (Figure 5(a) and Figure 5(b)).

Co-application of sulphate and selenate increased catalase activity in both test cultivars. The rice seedlings treated with 2 μM selenate and sulphate registered an increase in catalase activity by about 18% in roots and by about 70% in shoots of cv. satabdi. However, under same concentration the catalase activity decreased by about 7% in roots but increased by about 28% in shoots of cv. khitish over control. Exposure to combined 10 μM selenate and sulphate increased catalase activity in roots and shoots by about 22% and 70% in cv. satabdi and by about 10% and 42% in cv. khitish respectively. This positive trend was altered under joint application of 20 μM and 50 μM selenate and sulphate where the enzyme activity decreased slightly on an average in roots and increased on an
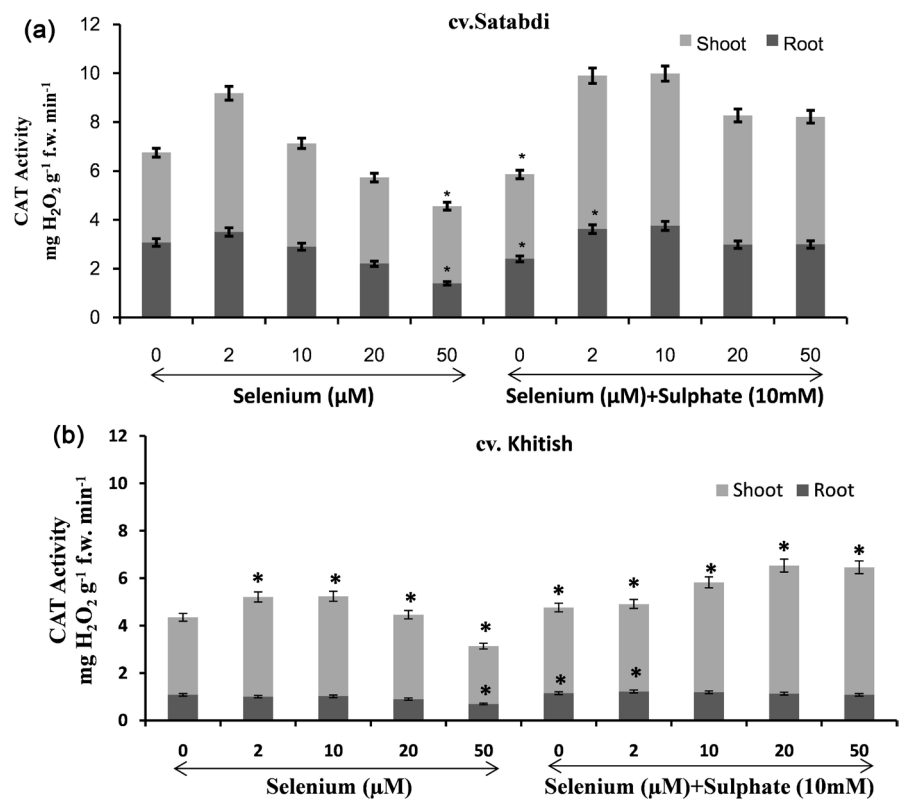

Figure 5. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the CAT activity of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the CAT activity of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

average by about 43% in shoots of cv. satabdi. In cv. khitish under same condition the negligible enhancement in enzyme activity in roots were countered by significant increase on an average by about 65% in shoots over water control.

3.5. Effect on Oxidative Stress Markers

3.5.1. Effect on Proline Content

Selenium treatment caused a significant increase in proline contents in root and shoot of test seedlings over water control. The proline contents decreased in 2 µM selenate treated seedlings by about 17% and 9% in root and shoot of cv. satabdi and by about 3% and 20% in root and shoot of cv.khitish respectively. Thereafter, the proline contents increased considerably with increase in concentrations of selenate. Maximum increase was observed in 50 µM selenate treatment where it increased by about 58% and 86% in root and shoot of cv. satabdi and by about 45% and 48% in root and shoot of cv.khitish respectively over water control (**Figure 6(a)** and **Figure 6(b)**).
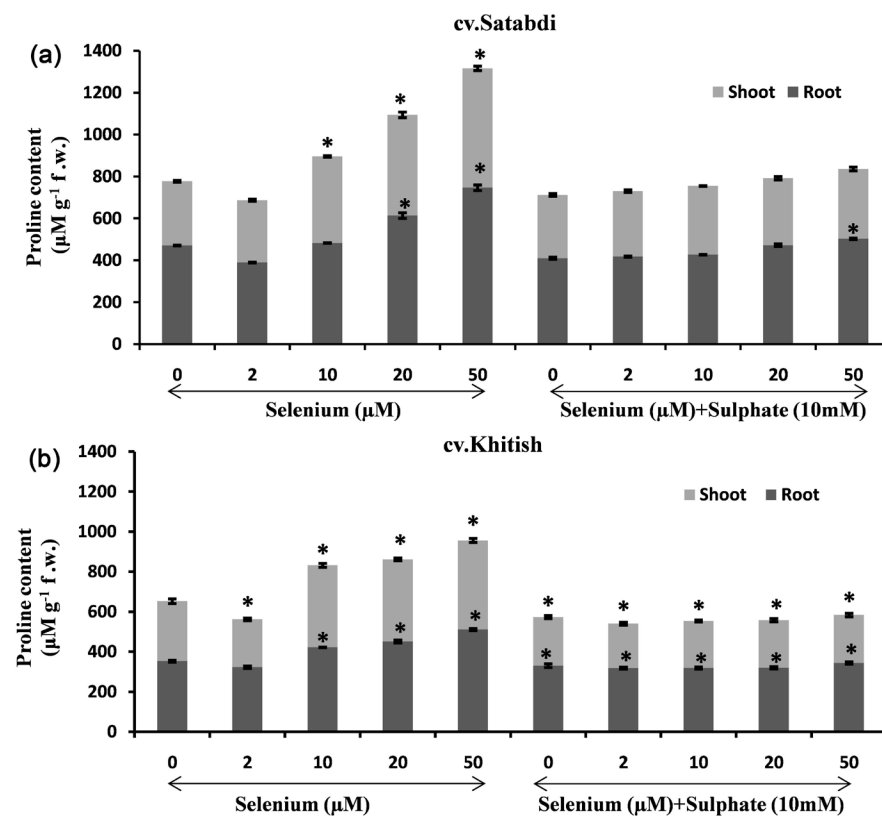

Figure 6. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the proline content of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the proline content of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

Simultaneous treatment with selenium and 10 mM sulphate declined proline contents in root and shoot of both the rice cultivars. Though application of 2 μM selenate and sulphate reduced proline contents by about 11% in root of cv. satabdi, small increment occurred in shoot over water control. On the contrary in 2 μM selenate and sulphate treated cv. khitish, the proline contents decreased by about 10% in root and doubly in shoot with respect to control. The proline contents which decreased by about 9% in root registered an increase by about 7% in shoot of 10 μM selenium and sulphate treated test seedlings of cv. satabdi. Further combined application of 20 μM, 50 μM selenate along with sulphate increased proline contents on an average, by about 3% in root and 6% in shoot of cv.. satabdi which was much less in comparison to treatment with selenate only. In cv. khitish the proline contents were inhibited on an average by about 7% in roots and 21% in shoots of test seedlings exposed to 10 μM, 20 μM and 50 μM selenate along with sulphate over water control.

3.5.2. Effect of on Total Peroxide (H_2O_2) Content

Selenium exposure caused a steep increase in total peroxide contents in root and shoot of both cultivars. A stimulatory effect occurred in 2 μM selenate treated rice seedlings of cv. satabdi where the total peroxide contents increased on an average by about 12% with respect to control. The total peroxide contents further increased by about 43%, 49% and 73% in roots and by about 46%, 63% and 68% in shoots of cv. satabdi under 10 μM, 20 μM and 50 μM selenate treatment respectively. Although the total peroxide level in roots of cv. khitish was initially inhibited by about 10% under 2 μM selenate treatment, it registered a stimulatory effect of about 8% in shoot under same concentration. The level of total peroxide also increased by about 2%, 33% and 39% in roots and by about 21%, 40% and 50% in shoots of 10 μM, 20 μM and 50 μM selenate treated test seedlings of cv. khitish respectively over water control (Figure 7(a) and Figure 7(b)).

Co-application of sulphate and selenate altered the effect caused by selenate treatment alone and decreased the total peroxide contents in both test cultivars. Exposure to 2 μM selenate and sulphate increased the total peroxide contents in root and shoot by about 12% and 9% respectively in cv. satabdi whereas in cv. khitish it diminished by about 9% in root but increased by equal amount in shoot over water control. The total peroxide recorded very little increase in its content in root whereas it increased on an average, by about 29% in shoot of cv. satabdi under 10 μM and 20 μM selenate and sulphate treatment. Similarly in cv. khitish the total peroxide contents increased by about 7% and 18% in shoot but decreased by about 30% and 23% in root under 10 μM and 20 μM selenate and sulphate treatment respectively. The total peroxide contents also increased in root and shoot by about 24% and 42% in cv. satabdi and by about 11% and 32% in cv. khitish respectively when jointly treated with 50 μM selenate and 10 mM sulphate but the increment was much less compared to treatment with 50 μM selenate alone.
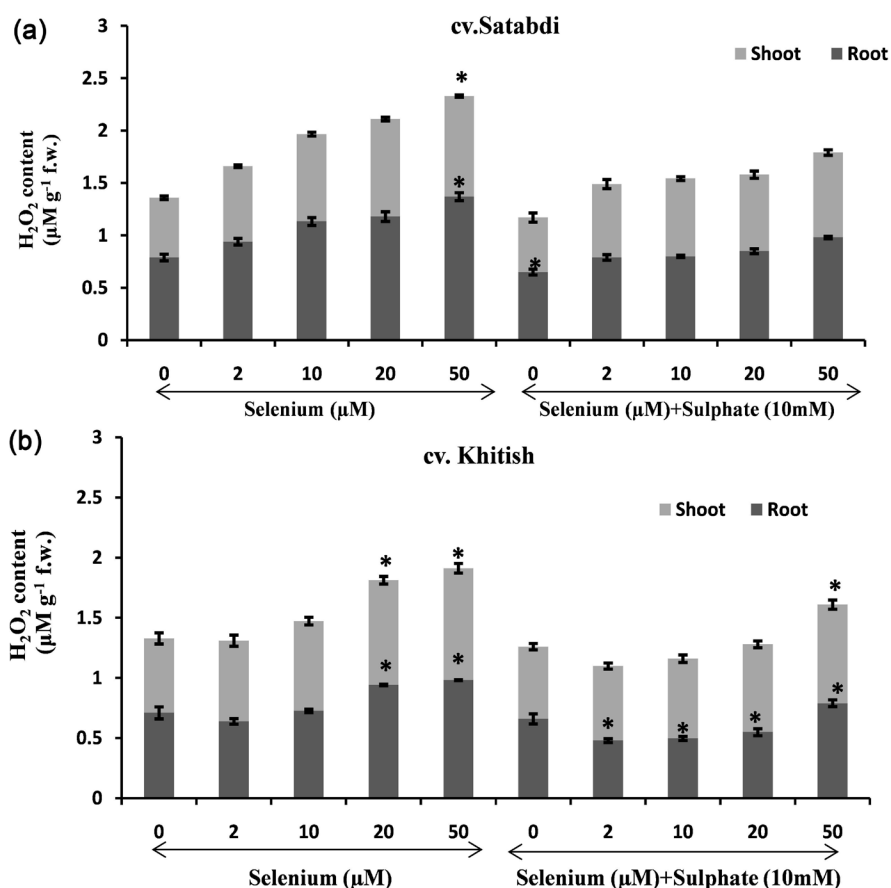
Figure 7. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the H_2O_2 content of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the H_2O_2 content of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

3.5.3. Effect on Malondialdehyde (MDA) Content

Selenate at low concentration acted as an antioxidant, inhibiting lipid peroxidation, whereas at higher concentrations, it acted as a pro-oxidant, enhancing lipid peroxidation as measured by the accumulation of malondialdehyde contents. Low concentration of selenate (2 μM) diminished lipid peroxidation on an average by about 14% in roots and very little in shoots of cv. satabdi and by about 13% in roots and 5% in shoots of cv. khitish with respect to water control. The decrease in MDA contents were more pronounced in roots than in shoots of both cultivars. In cv.satabdi, loss of MDA content followed a descending order where it initially decreased very little at 10 μM selenate, then increased by about 10% and 23% in roots of 20 μM and 50 μM selenate treated rice seedlings respectively over control. In shoot the MDA level linearly increased by about 11%, 17% and 28% under 10 μM, 20 μM and 50 μM of selenate treatment in cv.satabdi. The MDA contents of roots and shoots of cv. khitish declined by about 10% and 4%

respectively under 10 μM selenate treatment whereas very little or negligible effect was observed in 20 μM selenate treated rice seedlings of cv. khitish. However, the MDA contents registered a dramatic increase by about 33% and 24% in roots and shoots respectively of same cultivar under 50 μM selenate treatment (Figure 8(a) and Figure 8(b)).

Combined selenate and sulphate treatment ameliorated the effect of selenate only and apprehended the rise in MDA level in seedlings of both test cultivars. Joint application of 2 μM selenate and sulphate significantly decreased MDA level in root and shoot by about 25% and 6% in cv. satabdi and by about 15% and 6% in cv. khitish respectively. Further the increase in MDA level initially narrowed very little under combined 10 μM selenate-sulphate treatment but it increased by about 10% and 16% under 20 μM and 50 μM selenate-sulphate treatment respectively in roots of cv.satabdi. Similar trend occurred under same condition in roots of cv. khitish where the MDA level were enhanced by about 9%, 12% and 14% respectively over water control.Under 10 μM, 20 μM and 50
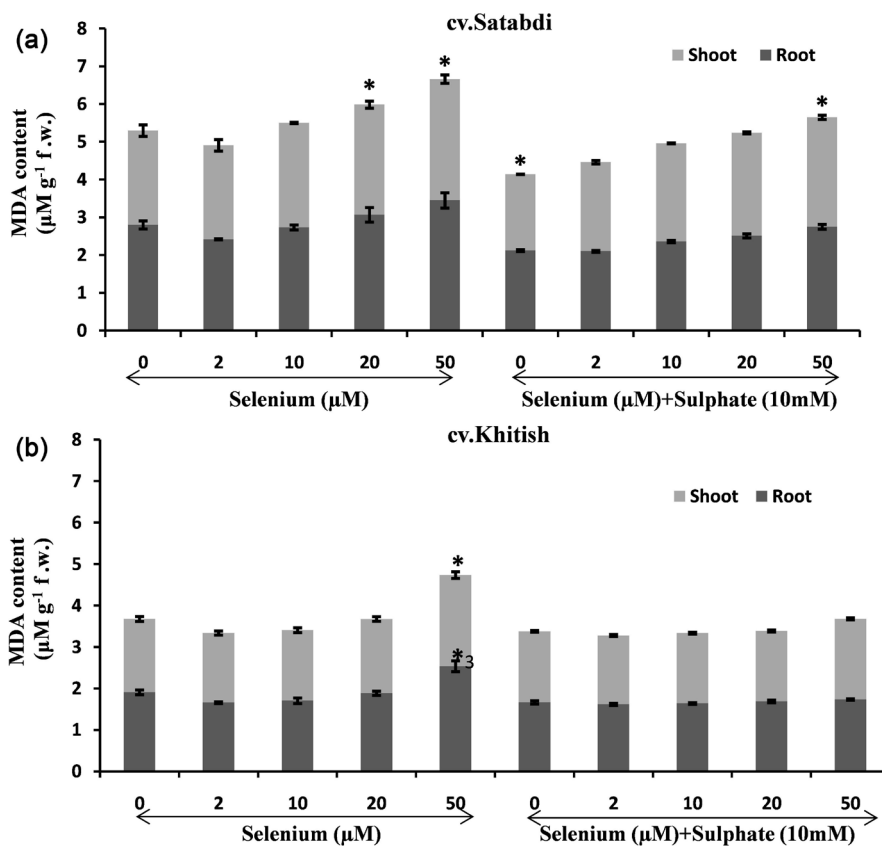

Figure 8. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the MDA content of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the MDA content of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

µM selenate and 10 mM sulphate treatments the lipid peroxidation contents also increased by about 4%, 9% and 16% in shoots of cv. satabdi and decreased initially by about 4% and 3% and then increased by about 10% in shoots of cv. khitish respectively over control.

## 3.6. Effect on Starch and Carbohydrate Contents

3.6.1. Starch

In both root and shoot of the test seedlings, the starch content decreased with increasing selenate treatment. Initially at 2 µM selenate concentration the starch level increased by about 23% and 11% in root and shoot of cv. satabdi and on an average by about 5% in seedlings of cv. khitish respectively over control. Under 10 µM, 20 µM and 50 µM selenate treatment, the starch level gradually decreased by about 13%, 18% and 22% in roots and by about 5%, 11% and 12% in shoots of cv. satabdi respectively. In cv. khitish, the starch content also decreased by about 10%, 12% and 15% in roots and by about 7%, 9% and 11% in shoots under similar condition with respect to control (**Figure 9(a)** and **Figure 9(b)**).

Joint application of said concentrations of selenate with 10 mM sulphate altered the effect caused by selenate alone in the starch content of both the test cultivars. Combined sulphate and 2 µM selenate had a negligible promotive effect on roots and inhibitory effect on shoots of cv. satabdi whereas in cv. khitish, the starch content were overall inhibited slightly with respect to water control. Subsequent addition of 10 µM, 20 µM and 50 µM selenate sulphate solutions
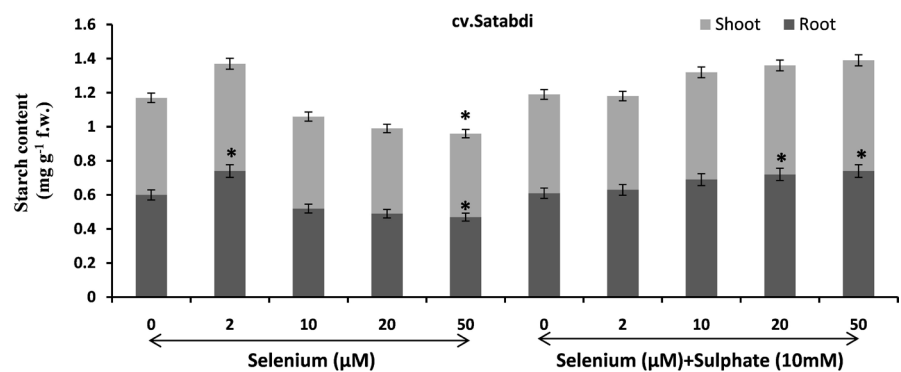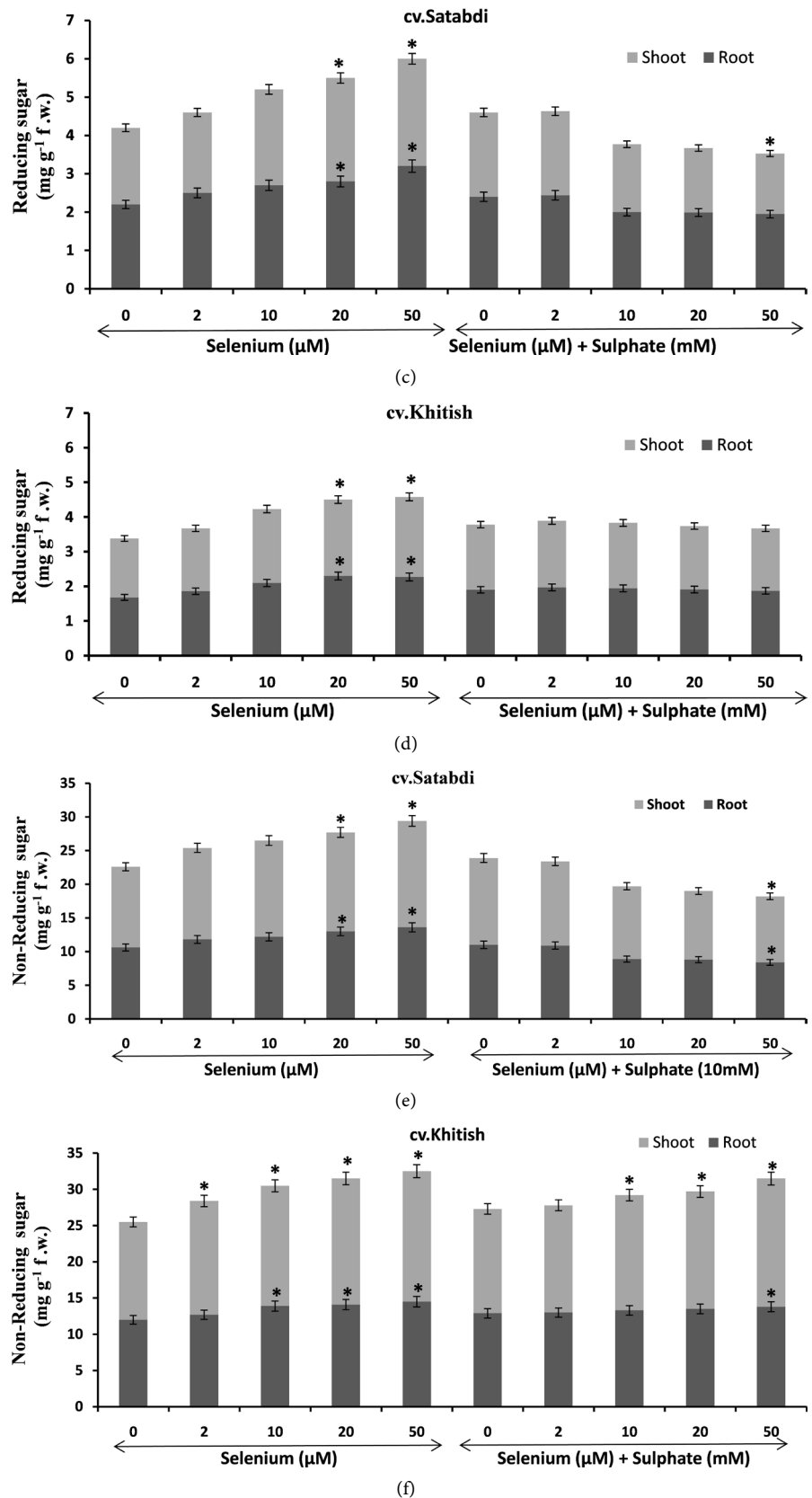

(a)
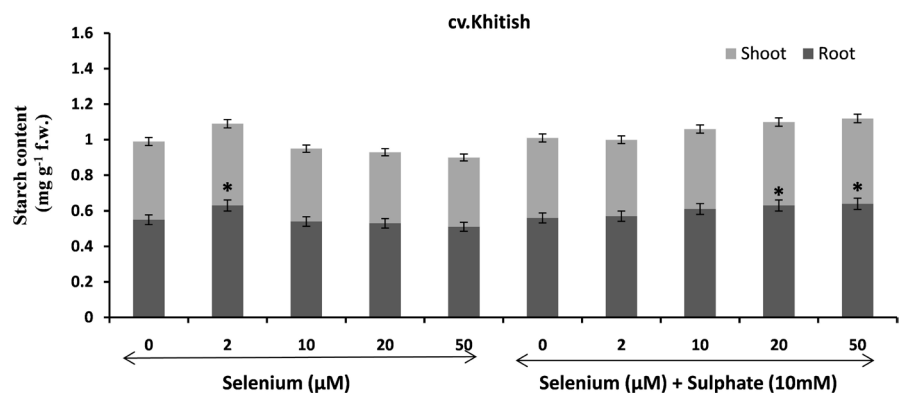
(b)

Figure 9. (a) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the starch content of rice (cv. satabdi) seed-

lings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the starch content of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (c) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the reducing sugar content of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (d) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the reducing sugar content of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (e) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the non reducing sugar content of rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (f) Effect of various concentrations of selenate and/or sulphate (10 mM) applied either alone or in their combination on the non reducing sugar content of rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control.

promoted starch content on an average by about 15%, 20% and 23% in roots and by about 11%, 12% and 14% in shoots of cv. satabdi. In cv. khitish co-application of 10 μM, 20 μM and 50 μM selenate sulphate solutions increased starch content by about 2%, 5% and 7% in roots and by about 2%, 7% and 9% in shoots respectively over water control.

3.6.2. Reducing Sugar
A stimulatory effect on the reducing sugar content was observed in both roots and shoots of the test cultivars with increase in selenate concentrations. The reducing sugar content increased by about 14% and 9% in roots and shoots of cv. satabdi and by about 11% and 7% in roots and shoots of 2 μM treated seedlings of cv. khitish respectively. Addition of 10 μM, 20 μM and 50 μM selenate to test solutions further increased the reducing sugar contents significantly in cv. satabdi by about 23%, 27% and 46% in roots and by about 25% 35% and 40% in shoots over control. Similarly it increased considerably by about 25%, 36% and 35% in roots and by about 25%, 29% and 36% in shoots of cv. khitish rice seedlings under 10 μM, 20 μM and 50 μM selenate treatment respectively over water control.

Simultaneous treatment with sulphate and selenate showed an ameliorative effect on the seedlings of test cultivars. At 2 μM selenate and sulphate, the reducing sugar increased very little in seedlings of cv. satabdi whereas in cv. khitish it increased by about 17% in root and by about 13% in shoots over water control.Joint application of 10 μM, 20 μM and 50 μM selenate along with sulphate decreased the reducing sugar level by about 9%, 10% and 11% in roots and by about 12%, 16% and 21% in shoots of cv. satabdi respectively. However, the reducing sugar content increased in cv. khitish under similar selenate

sulphate treatment by about 16%, 14% and 11% in roots and 11%, 8% and 6% in shoots respectively over control but the increment were less compared to treatment with said concentrations of selenate only (Figure 9(c) and Figure 9(d)).

3.6.3. Non-Reducing Sugar

The non reducing sugar content was enhanced in seedlings of both cultivars with increase in selenate concentrations. The non reducing sugar content were increased by about 12% and 13% in roots and shoots of cv. satabdi as against 6% in roots and 16% in shoots of 2 μM treated seedlings of cv.khitish respectively. Further in cv.satabdi, the non reducing sugar level increased by about 15%, 23% and 28% in roots and by about 19%, 23% and 32% in shoots under 10 μM, 20 μM and 50 μM selenate treatment respectively over control. On a similar note the non reducing sugar level also increased in cv.khitish by about 16%, 18% and 21% in roots and by about 23%, 29% and 33% in shoots treated with 10 μM, 20 μM and 50 μM selenate respectively over water control (Figure 9(e) and Figure 9(f)).

The influence of selenate applied singly on the test seedlings were reversed by combined treatment with sulphate and said concentrations of selenate in both cultivars. At 2 μM selenate and sulphate, the non reducing sugar increased on an average by about 12% in the test seedlings of cv. satabdi whereas in cv. khitish it increased by about 8% in root and about 3% in shoots over water control. Joint application of 10 μM, 20 μM and 50 μM selenate individually along with sulphate decreased the non reducing sugar content by about 16%, 17% and 21% in roots and by about 10% 15% and 18% in shoots of cv. satabdi respectively. However, in cv. khitish, the non reducing sugar contents increased by about 11%, 13% and 15% in roots and by about 18%, 20% and 31% in shoots under above mentioned selenate sulphate doses but the increment were less compared to seedlings treated with selenate alone with respect to water control.

3.7. Effect on Mineral Nutrients

3.7.1. Selenium

Compared to the control seedlings, accumulation of Se considerably increased in roots of test seedlings under 2 μM selenate treatment which were about 4.3 mg·Kg^{-1} D.W.in cv. satabdi and 2.7 mg·Kg^{-1} D.W.in cv. khitish. On the contrary, in shoots, similar treatment reduced the uptake of selenate by approximately half in both test cultivars with respect to control. Thereafter, a dose dependent steep incline in selenium level were observed in the rice seedlings treated with higher selenate concentrations. In both cv. satabdi and cv. khitish, the selenium concentration in roots increased 1000 fold in 2 μM and 10 μM treated seedlings followed by double that amount in 20 μM and considerably more in 50 μM treated test seedlings over control. Similar trend were observed in shoots where the selenium level significantly increased with increase in selenate concentration. In shoots, the selenium content linearly increased under 2 μM, 10 μM and 20 μM selenate treatments by about 3 mg·Kg^{-1} D.W., 15 mg·Kg^{-1} D.W. and 23 mg·Kg^{-1}

D.W. respectively in cv. satabdi and by about 2 mg·Kg⁻¹ D.W., 14 mg·Kg⁻¹ D.W. and 21 mg·Kg⁻¹ D.W. respectively in cv. khitish. Maximum Se accumulation in shoots occurred in 50 μM selenate treated rice seedlings which were about 31 mg·Kg⁻¹ D.W. in cv. satabdi and about 33 mg·Kg⁻¹ D.W. in cv. khitish (Figure 10(a) and Figure 10(b)).

However, presence of sulphur in the medium seems to inhibit Se uptake in test seedlings of both cultivars. Co-application of selenium with sulphate reduced the accumulation of Se in roots of both cultivars with respect to water control. The Se level narrowed down in roots of rice seedlings jointly treated with 2 μM, 10 μM, 20 μM, 50 μM selenate and sulphate in contrast to selenium applied singly by about 6 mg·Kg⁻¹ D.W., 20 mg·Kg⁻¹ D.W., 45 mg·Kg⁻¹ D.W. and 46 mg·Kg⁻¹ D.W. respectively in cv. satabdi and by about 4 mg·Kg⁻¹ D.W., 20 mg·Kg⁻¹ D.W., 40 mg·Kg⁻¹ D.W and 118 mg·Kg⁻¹ D.W. respectively in cv. khitish. However, in shoots, the Se content initially increased under 2 μM selenate and sulphate treatment with respect to its single application followed by a steady decline in its level under combined 10 μM, 20 μM and 50 μM selenate and sulphate treatment in both cv. satabdi and cv. khitish.

3.7.2. Sodium

The Na content was associated with a marked increase in its level in roots and shoots of both cultivars treated with selenate. The Na content significantly increased by about 60% in roots and by about 115% in shoots of cv. satabdi under 2 μM selenate treatment. Similar effect was observed in 2 μM selenate treated rice seedlings of cv. khitish where the Na level increased by about 51% in roots and 92% in shoots with respect to water control. A significant rise in Na content by about 60%, 61% and 63% in roots and by about 120%, 127% and 142% in shoots of cv. satabdi were observed under the influence of 10 μM, 20 μM and 50 μM selenate treatment respectively. Similar promotive effect were observed in cv. khitish where the Na content increased by about 57%, 66% and 76% in roots and by about 95%, 108% and 104% in shoots over water control.
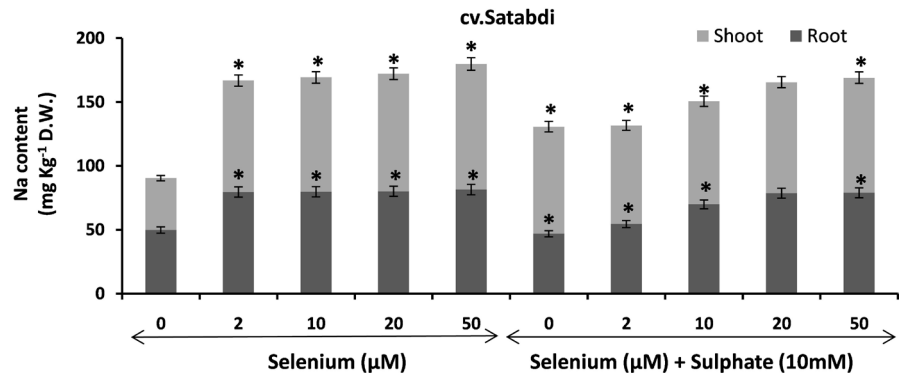
Combined application of selenate and sulphate exhibited stimulatory effect on Na accumulation in the tissues of both test cultivars but at a lesser degree than observed in test seedlings treated with selenate alone. The Na contents increased by about 55% and 77% in roots and shoots respectively of cv. satabdi under 2 μM selenate and sulphate treatment. However, in cv. khitish, the level of Na ions initially decreased in roots by about 11% but registered a sharp increase by about 73% in shoots of 2 μM selenate and sulphate treated rice seedlings. A linear increment in Na content by about 70%, 78% and 79% in roots and about 81%, 87%, and 90% in shoots of cv. satabdi were observed when treated with 10 μM, 20 μM and 50 μM selenate and sulphate respectively over control. In cv. khitish, the Na content also increased by about 23%, 34% and 43% in roots and by about 89%, 103%, and 96% in shoots of test seedlings under similar treatment with respect to control (Figure 10(c) and Figure 10(d)).

(a)

(b)
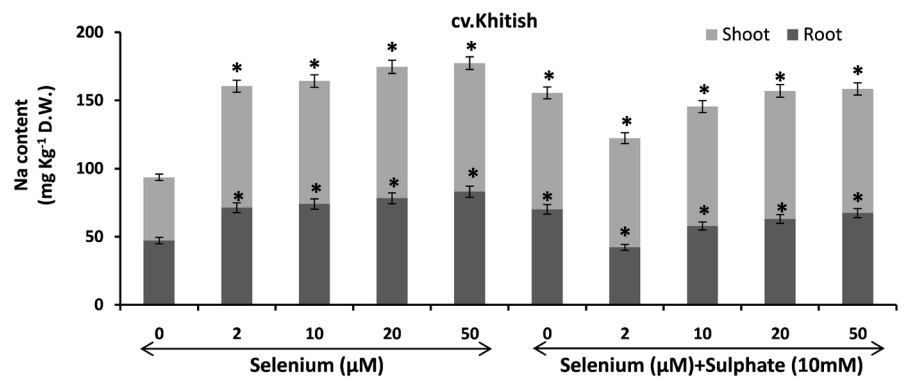
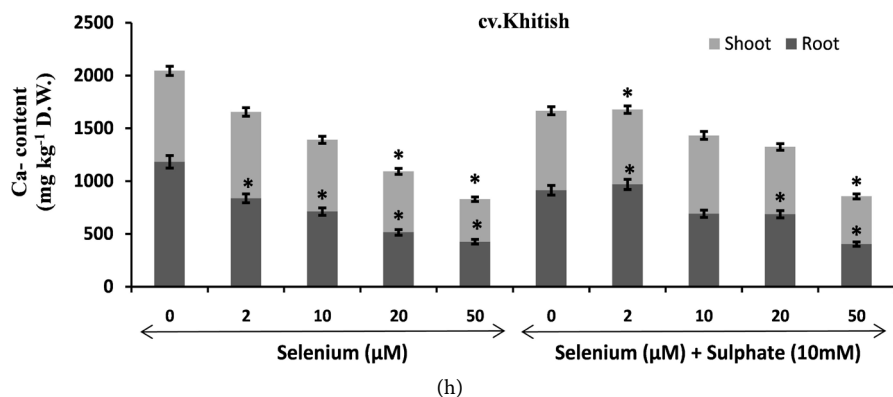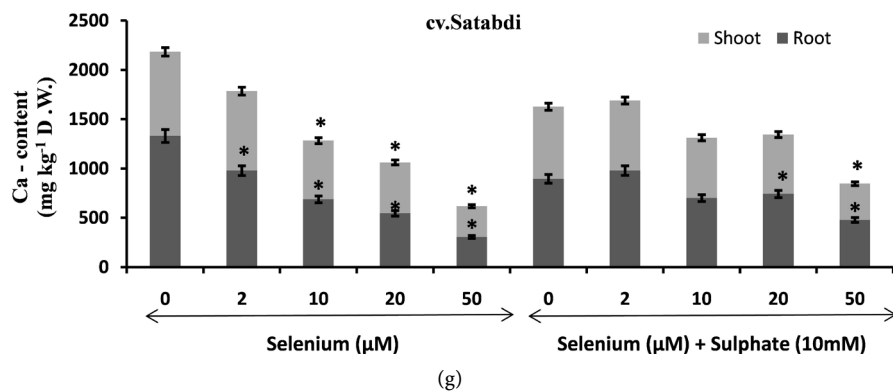(c)

(d)

(e)

(f)
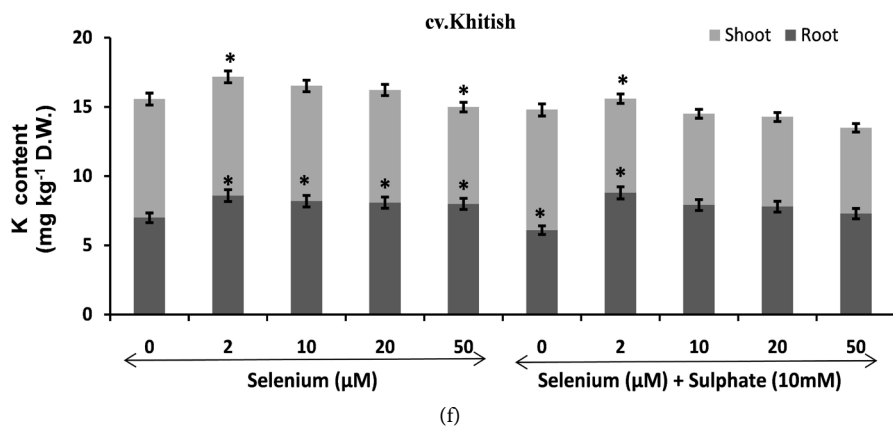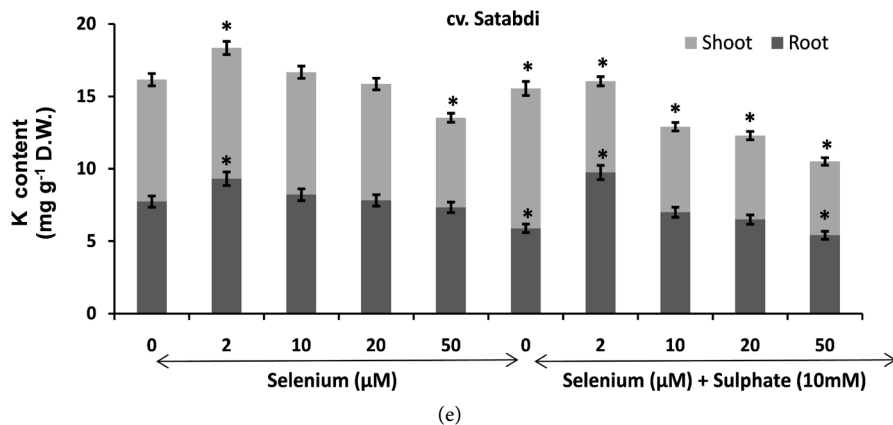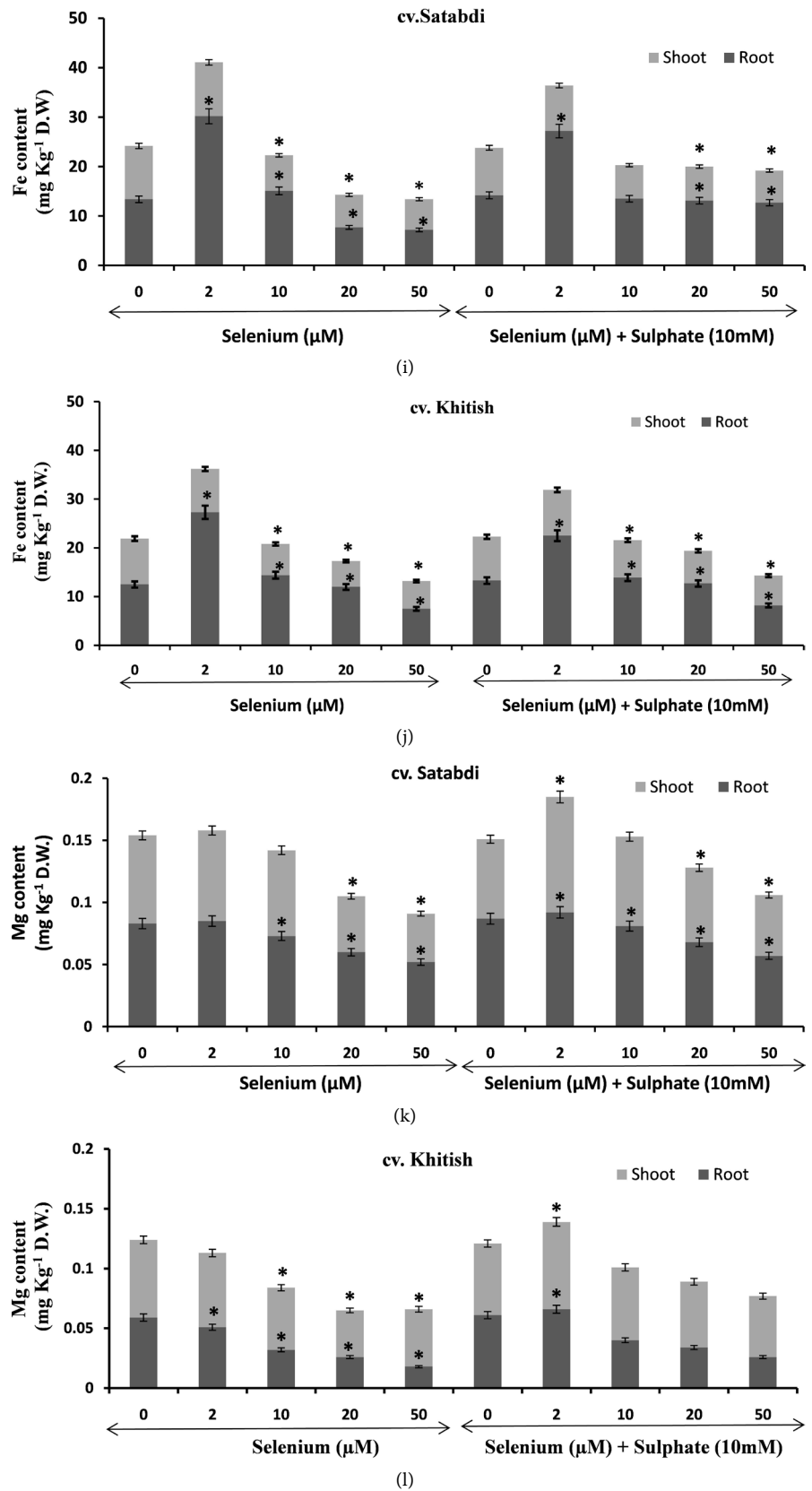

(g)

(h)

(i)

(j)

(k)

(l)
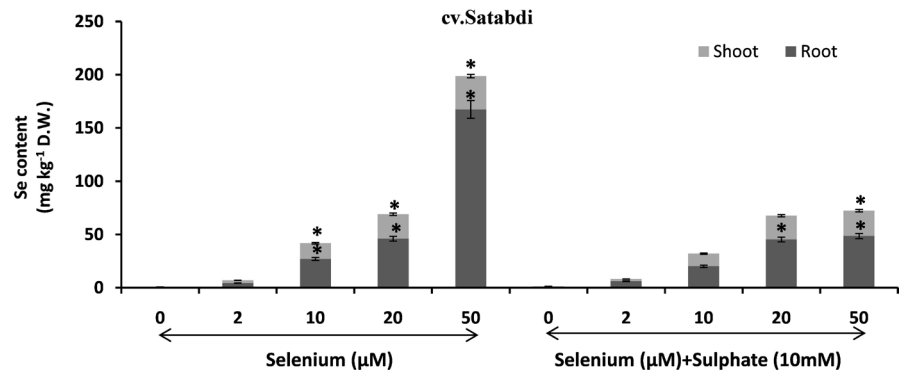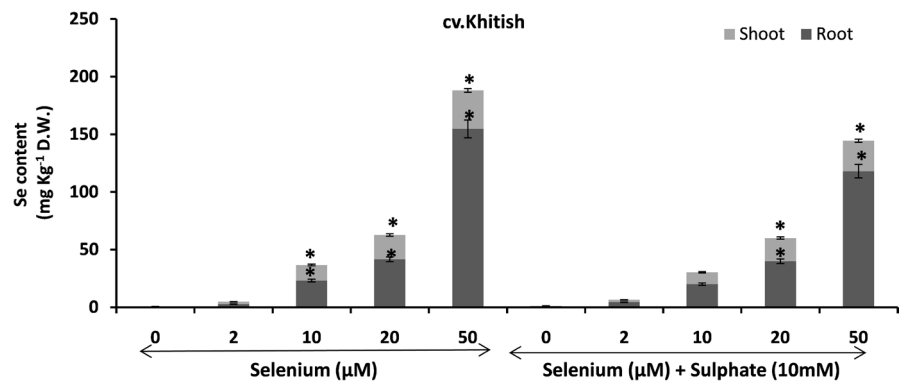
Figure 10. (a) Effect of selenate and sulphate applied singly or in combination on selenium content of twenty-one days old rice (cv. satabdi) seedlings. Values are the mean

± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (b) Effect of selenate and sulphate applied singly or in combination on selenium content of twenty-one days old rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (c) Effect of selenate and sulphate applied singly or in combination on sodium content of twenty-one days old rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (d) Effect of selenate and sulphate applied singly or in combination on sodium content of twenty-one days old rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (e) Effect of selenate and sulphate applied singly or in combination on potassium content of twenty-one days old rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (f) Effect of selenate and sulphate applied singly or in combination on potassium content of twenty-one days old rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (g) Effect of selenate and sulphate applied singly or in combination on calcium content of twenty-one days old rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (h) Effect of selenate and sulphate applied singly or in combination on calcium content of twenty-one days old rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (i) Effect of selenate and sulphate applied singly or in combination on iron content of twenty-one days old rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (j) Effect of selenate and sulphate applied singly or in combination on iron content of twenty-one days old rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (k) Effect of selenate and sulphate applied singly or in combination on magnesium content of twenty-one days old rice (cv. satabdi) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, *indicates statistically significant at p ≤ 0.05 respectively compared to control. (l) Effect of selenate and sulphate applied singly or in combination on magnesium content of twenty-one days old rice (cv. khitish) seedlings. Values are the mean ± SE of three repeats with two replicas in each treatment, * indicates statistically significant at p ≤ 0.05 respectively compared to control.

3.7.3. Potassium

Elevation in the level of K ions at low concentration followed by its loss at higher concentrations of selenate was observed in roots and shoots of both cultivars with respect to water control. The K ions increased by about 20% in roots and 7% in shoots of cv. satabdi and by about 23% in roots and negligibly in roots of cv. khitish under 2 μM selenate treatment. Subsequently the K content increased in roots of cv. satabdi by about 6% and 1% in 10 μM and 20 μM selenate respectively till it was inhibited by about 5% under 50 μM selenate treatment. Further the negligible decline of about 1% and 5% in K content in 10 μM and 20 μM treated shoots of cv. satabdi respectively reached a maximum inhibition of 28% under 50 μM selenate treatment. In cv. khitish, the stimulatory effect on the K

contents in roots narrowed down by about 17%, 16% and 14% whereas in shoots the K level decreased by about 2%, 3% and 16% in rice seedlings under 10 μM, 20 μM and 50 μM selenate treatment respectively over control.

Simultaneous administration of selenate and 10 mM sulphate adversely affected the level of K ions in roots and shoots of both test cultivars. At 2 μM selenate sulphate treatment the K contents increased by about 26% in roots and decreased by about 27% in shoots of cv.satabdi. Thereafter the K contents linearly decreased by about 9%, 16% and 30% in roots and by about 30%, 32% and 41% in shoots of cv. satabdi treated with 10 μM, 20 μM and 50 μM selenate along with 10 mM sulphate respectively. Similarly, in cv. khitish the stimulatory effect on K ions in roots decreased by about 26%, 13%, 11% and 4% and in shoots by about 19%, 22%, 25% and 26% in seedlings treated with 2 μM 10 μM, 20 μM and 50 μM selenate and sulphate respectively over control (Figure 10(e) and Figure 10(f)).

3.7.4. Calcium

A strong inhibitory effect on Ca content were observed at higher selenate concentrations in both roots and shoots of the test cultivars. The Ca content decreased by about 26% and 29% in roots and by about 6% and 5% in shoots of 2 μM treated rice seedlings of cv. satabdi and cv. khitish respectively. Further a steep decline by about 48%, 59% and 77% in roots and by about 30%, 40% and 64% in shoots of cv. satabdi test seedlings were observed when treated with 10 μM, 20 μM and 50 μM selenate solution over water control. Similarly in cv. khitish, the Ca content were inhibited by about 40%, 56% and 64% in roots and by about 14%, 33% and 53% in shoots of rice seedlings treated with 10 μM, 20 μM and 50 μM selenate respectively (Figure 10(g) and Figure 10(h)).

The effect caused by selenate applied singly were partially ameliorated in both roots and shoots of test cultivars by joint application of said concentrations of selenate and 10 mM sulphate. The Ca content decreased by about 26% and 18% in roots and by about 17% and 18% in shoots of cv. satabdi and cv. khitish treated with combined solution of sulphate and 2 μM selenate respectively. Co-application of selenate (10 μM, 20 μM and 50 μM) and sulphate also decreased the Ca content considerably by about 47%, 44% and 64% in roots and by about 28%, 29% and 57% in shoots of cv. satabdi respectively. Similar trend was reflected in cv. khitish where the Ca content decreased by about 42%, 42% and 66% in roots and about 21%, 26% and 48% in shoots respectively over control.

3.7.5. Iron

Selenate treatments decreased Fe content in both root as well as shoot of both the test cultivars. Under 2 μM selenate, the Fe content increased on an average by about 63% in cv. satabdi compared to an increment of about 57% on an average in cv. khitish over control. The Fe content declined by about 1%, 42% and 46% in roots of cv. satabdi under 10 μM, 20 μM and 50 μM selenate treatments respectively over control. In cv. khitish, Fe content increased by about 11% un-

der 10 μM selenate treatment. However, under 20 μM and 50 μM selenate treatments the Fe content declined by about 4% and 40% respectively over control. Similarly in shoot, Fe content declined by about 37%, 36% and 40% in cv. satabdi and by about 5%, 32% and 44% in cv. khitish respectively under said treatments over control.

The effect of selenate treatment was ameliorated to certain extent on application of said concentrations of selenate and sulphate jointly in the test cultivars. At 2 μM selenate and sulphate treatment, Fe content in root increased by about 103% and 80% in cv. satabdi and cv. khitish respectively over control. Similar treatments in shoot revealed a decrease of about 15% in the said content in cv. satabdi while in cv. khitish the Fe content equalled the control. Application of sulphate combined with 10 μM selenate in cv. satabdi enhanced Fe content in roots by about 13% but caused a decline of about 2% and 5% under 20 μM Se + SO$_4$ and 50 μM Se + SO$_4$ doses. In shoots, however, Fe content declined by about 33%, 39% and 43% under identical treatments. In cv. khitish roots, joint treatment of sulphate with selenate enhanced Fe content under 10 μM Se + SO$_4$ and 20 μM Se + SO$_4$ doses by about 8% on an average while under 50 μM Se + SO$_4$, the Fe content declined by about 34% with respect to control seedlings. In shoots, the Fe content decreased by about 18%, 29% and 35% under the stated doses over control (Figure 10(i) and Figure 10(j)).

3.7.6. Magnesium

The Magnesium content decreased with increase in selenate concentrations in both roots and shoots of cv. satabdi and cv. khitish. In 2 μM selenate treated root of cv. satabdi, the Mg content declined by about 14% while it increased by about 2% in root of cv. khitish under similar treatment. Thereafter, in roots the Mg content declined significantly by about 46%, 56% and 70% in cv. satabdi and by about 12%, 28% and 37% in cv. Khitish under 10 μM, 20 μM and 50 μM selenate treatments with respect to control. Co-application of sulphate along with selenate enhanced Mg contents in both cultivars in comparison to seedlings treated with selenate only. In roots, the Mg contents declined by about 32%, 42% and 56% in cv. satabdi under 10 μM Se + SO$_4$, 20 μM Se + SO$_4$ and 50 μM Se + SO$_4$ treatments over control while in cv. Khitish the contents declined by about 2%, 18% and 31% under similar treatments. However, under 2 μM Se + SO$_4$ treatment, increments of about 3% and 11% were noted in cv. satabdi and cv. khitish roots respectively over control (Figure 10(k) and Figure 10(l)).

In cv. satabdi shoot, Mg content declined by about 3%, 37% and 45% under 10 μM, 20 μM and 50 μM selenate treatments respectively over control. However, in cv. khitish the said decline was by about 20%, 26% and 40% under similar treatments compared to control. Under 2 μM selenate treatment in cv. satabdi, Mg content increased by about 3% in contrast to a decline of about 5% in cv. khitish over control. Co-application of sulphate along with selenate enhanced Mg contents when compared to the individually selenate treated seedlings in both the cultivars. The Mg contents enhanced by about 31% in cv. satabdi while in cv.

khitish an increment of about 12% was documented under 2 μM Se + SO$_4$ treatment. Under 10 μM Se + SO$_4$ treatment an increase in the said content by about 1% was noted in cv. satabdi while a decline of about 16% and 31% occurred under 20 μM Se + SO$_4$ and 50 μM Se + SO$_4$ treatments. In cv. khitish however, a decline by about 6%, 15% and 22% occurred under 10 μM Se + SO$_4$, 20 μM Se + SO$_4$ and 50 μM Se + SO$_4$ treatments over control.

4. Discussion

Stress in any form restrict development and may cause lethal changes in living organisms. Interaction with contaminants such as metalloids or heavy metals leads to accumulation of ROS or singlet oxygen species that influences the integrity of the cells. In order to counteract such damages, both animals and plants have developed an antioxidant system comprising of enzymatic viz., SOD, CPX, CAT and non enzymatic antioxidants. Bio fortification by enhancing essential nutrient elements in crop plants viz., rice, wheat and maize has been documented to prevent malnutrition, especially in children and also fortifies the plant cells from various stresses.

Sodium selenate (Na$_2$SeO$_4$) used in the present study acts as an antioxidant at low concentration (≤10 μM selenate) and prevents or eliminates build up of harmful ROS. However, at higher concentrations (≥10 μM selenate) it behaves as a prooxidant and induces ROS formation leading to oxidative stress (7). Our study similarly revealed the nutritive role of selenate (2 μM) in contrast to its higher concentrations (≥10 μM) where symptoms of necrosis and reduction in growth were noticed in both the test cultivars with prominent effects in cv. satabdi in comparison to that of cv. khitish. Such promotive influence of selenate at low concentration (2 μM) compared to higher doses (≥10 μM) was evident from the phenotypic variations in contrast to higher doses where growth was restricted. Decline in growth was accompanied by apical browning, necrosis and chlorosis along with a decline in water content in both the cultivars. Our results are in line with studies conducted by Simaei *et al.* and Hawrylak-Nowak in tomato and lettuce respectively [46] [47]. However, the degree of decline in growth was much more in cv. satabdi compared to that of cv. khitish.

Effects of selenate in the test cultivars could be modulated by the presence or absence of sulphate in the treatment solution. Uptake of selenium in the plant tissue is regulated by sulphate transporters since the chalcogens, selenium and sulphur compete for the same transport pathway for entry to the cells. Application of sulphate with selenate helped to overcome the detrimental effects of selenate alone on growth and water content of the tested cultivars probably due to the antagonistic effects of sulphate and selenate ions for entry into the plant tissue.

Accumulation of Se in the plant tissues of the test cultivars showed a directly proportional relationship with increase in Se concentrations. The results obtained in our study corroborates with those obtained in other agricultural crops such as in *Triticum aestivum* by Broadley *et al.* [48] and by Freeman *et al.* [49] in

Stanleya pinnata. Further, the test seedlings treated with both selenate and sulphate showed less uptake leading to lower Se content over control which may be the result of competition for preferential entry between sulphate and selenate primarily through sulphate tranporters in plants [49].

Photosynthetic pigments like chlorophyll and carotenoids are adversely affected by high Se concentrations [47] [50] [51]. In the current study, the total chlorophyll contents including chlorophyll a and chlorophyll b declined with increase in Se concentrations in the test seedlings. Such negative influence of Se on porphobilinogen synthesis was also observed in *Sinapis alba* L. by Fargasova *et al.* [52]. However, the assimilatory pigments like carotenoids showed a dose dependent effect on test seedlings and registered an increase with increase in concentrations of selenate. The increase in carotenoid contents may well act to protect the plant tissue from oxidative stress. Similar increase in carotenoid levels under high Se concentrations were obtained in rocket plants by Khattab [9]. Combined application of sulphate and selenate ameliorated the effect of selenate on the photosynthetic pigments to some degree and increased chlorophyll a and chlorophyll b contents of rice seedlings of both test cultivars. This may be attributed to beneficial effect of low concentration of Se as well as its chalcogen nature with sulphate which might restrict entry of Se salt at higher concentrations thus protecting the chloroplast enzymes and increasing the biosynthesis of photosynthetic pigments. Previous studies have established that the intensity of fluorescence emission bands or band ratios is directly related to the development of plants under stress [53]. The fluctuations in chlorophyll contents due to stress is the result of changes occurring in the chlorophyll apparatus which in turn might alter the fluorescence emission and prevent from fully converting the harvested light energy to its chemical form [54]. A reduction of the pigment content in the case of increasing levels of heavy metals and metalloids was previously observed in *Arabidopsis thaliana* by Baek [55]. Study on stress induced in maize plant under the influence of Ni found that it not only disrupts the photosynthetic apparatus by destroying mesophyll tissue thereby decreasing the chlorophyll contents [56], it also severely affects the electron transport chain and its intermediates. Inhibitory effects of increasing Ni concentrations on Hill activity of spinach leaves were also studied by Boisvert *et al.* [57]. All these evidences support our findings in which Se also exerted a similar influence on the rate of Hill reaction in both the test cultivars.

Selenium exerted a dose dependent effect on the plant tissues of the test cultivars which in turn enhanced the activities of antioxidant enzyme like SOD but not CPX and CAT in the rice seedlings [58]. There were considerable increase in level of oxidative stress markers like Proline, H_2O_2 and MDA with increase in selenate concentrations in the test seedlings. Similar observations were recorded in different crop species where the level of antioxidants and oxidative stress markers increased in order to withstand ROS formation during salt stress [58] [59] [60] [61]. The results obtained in our study are further supported by recent

evidences where exogenous supplementation of Na_2SeO_4 was found to uplift the activity of various antioxidant enzymes such as SOD, CPX and CAT thereby protecting the plants from oxidative stress [62] [63] [64] [65].Our observations are also in agreement with previous studies in salinity stressed seedlings of rapeseed and *Anethum graveolens* where supplementary selenium in test solutions led to considerable increase in activity of SOD, CPX and CAT [18] [66].

The level of non toxic, low molecular weight compatible solutes also increases due to salinity stress in plants. The accumulation of such solutes helps to protect the plants from cellular damage induced during salinity stress by preventing or alleviating ROS formation and maintaining membrane integrity of the cells [67]. Proline is a major compatible solute which counteract the harmful effects of ROS by acting as a molecular chaperone and binding with the metal ions [68]. Results obtained in our study corroborates with such views as the proline level increased substantially with increase in concentrations of applied selenate. Similar results were also shown to occur where exogenous application of selenate improved proline concentrations in canola [69] and cucumber seedlings [47] under various abiotic stresses [68].

Abiotic stresses leads to photo-oxidative reactions causing significant increase in level of superoxide radicals and H_2O_2 content in the cells. Such accumulation of superoxide radicals and H_2O_2 in turn degrades the membranes integrity of thylakoids and chloroplasts resulting in chlorophyll degradation [69]. Increase in H_2O_2 levels due to salinity stress promotes cell shrinkage, chromatin condensation, DNA fragmentation and apoptosis in cells [70]. In our study, there was significant elevation in H_2O_2 contents which coincides with the decrease in chlorophyll level in the test seedlings of both cultivars under the influence of higher selenate concentrations. The results also indicates that supplementing the test solutions with both sulphate and Na_2SeO_4 effectively reduced the damage caused by selenate alone by preventing the degradation of chloroplasts and increasing the chlorophyll contents in the test cultivars. Peroxidation of lipids measured by MDA levels in plant cells are strong indicators of oxidative stress [71]. In our study, the MDA levels in rice seedlings consistently increased when treated with higher concentrations of selenium compared to the control plants. The opposite effect were observed in test seedlings treated with both sulphate and selenate. This suggest that combined application of sulphate and selenate helped in reducing MDA level in treated seedlings by lowering lipid peroxidation thereby protecting the membranes by improving the activity of the antioxidant enzymes.

Accumulation of carbohydrate products occurs in plant tissues to combat various stress conditions [72]. Sucrose and starch are the end products of the photosynthetically fixed carbon in plant cells. Starch acts as a temporary storage form of fixed carbon in the chloroplast and is finally stored in the cereal grains [73]. Sucrose, after synthesis in cytosol is transported to different organs of the seedlings for various metabolic activities [74]. Studies made by Dubey [75] and Devi *et al.*, [76] have shown that soluble sugar increases under salt and cadmium

stress. According to Couee *et al.*, 2006 [77] such increase in the level of soluble sugars acts as a counteractive way to maintain homeostasis within plant cells. These views are supported by results obtained in our study on the two cultivars of rice in which the total sugar content including reducing and non reducing sugars were increased with increase in Se concentrations. The present result also corroborates to the fact that starch level concomitantly decreased with rise in soluble sugar contents in both cultivars. Inhibitory effect on starch mobility may have caused the reduction in starch content. Our observations are supported by studies made by Rahoui *et al*, 2010 [78] in cadmium treated seedlings of *Vicia faba*.

Reportedly production of ROS induces depolarization in cell membrane resulting in modified translocation of ions such as efflux of chloride and potassium ions and influx of calcium ions. Studies have shown that under salinity stress, plant cells accumulate more Na ions leading to oxidative damages due to disturbances occurring in ionic balance and metabolic activities. The status of K ions also poses as a limiting factor during salinity stress [79]. Increase in Na ions lowers the relative water content (RWC) in salinity stressed plants as it causes sodicity restricting root growth which in turn reduces the water mobility, hydraulic conductivity and functionality of aquaporins, thus minimizing the transport of water to the aerial parts [80]. According to Fu *et al.* [81] differential expression of Na and K transporters is also responsible for the differences observed in Na/K homeostasis and salt tolerance between cereals like barley and rice. Further research is required to completely understand and elucidate the mode of action of sodium selenate in this area. In the current study, rice seedlings treated with Na_2SeO_4 showed accumulation of Na ions and K ions with increase in selenate concentrations which was amended by co-application with sulphate thus exhibiting better development and growth in the test seedlings. Combined sulphate and selenate application may have reduced the sequestering of said cations and diminished their toxic effects on the rice seedlings compared to control (seedlings grown in solution without selenate or sulphate). Similar observations were made by Rios *et al.* [82] where Ca concentration in lettuce plants treated with selenium salt decreased but K concentration increased. Increase in K level was also observed in wheat plant treated with selenium salt [83]. In plants, Ca is involved in cell wall strengthening and cell division, and is the second messenger in osmoregulation [84]. Iron is considered to be essential in normal growth and development in plants. Iron is also an essential component and an important co factor for enzymes like superoxide dismutase, peroxidase and catalase and is invariably involved in chlorophyll biosynthesis and photosynthesis [85]. Study made by Feng and Wei [86] suggest that high concentrations of selenium induces maximum Fe absorption compared to lower Se concentrations which shows reduced absorption of Fe ions. Similar result has been observed in our experiments where the Fe contents decreased linearly with increase in concentrations of selenate which also correlates to degradation of

chlorophyll synthesis observed as more etiolated aerial parts in rice seedlings treated with high selenate levels. The influence of Selenium in plants is conspicuous through modifications in the concentrations of certain ions as a result of possible alterations in their absorption or due to changes occurring in the permeability coefficient of cellular membranes [87]. Therefore, the variable changes in level of Na, K, Ca and Fe observed in our study could possibly be due to these ions being involved in the regulation of cell membrane potential in the rice seedlings of both cultivars treated with selenate.

5. Conclusion

Sodium selenate (Na_2SeO_4) acts as both antioxidant and prooxidant depending on the concentrations applied. Lower concentration of selenate (2 μM) had a promotive effect and increased the nutritional capacity in the test cultivars enhancing growth and metabolism specifically in cv. satabdi. However, higher concentrations of selenate (≥10 μM) led to gradual reduction in growth along with decrease in pigment contents, fluorescence intensity and Hill activity in the test cultivars. The oxidative stress markers and antioxidative enzymes were concomitantly altered along with considerable changes in ion contents. Accumulation of sugars occurred in the test seedlings in response to the oxidative stress incurred due to selenate treatments. However, the response was differential in the two rice cultivars cv. satabdi and cv. khitish, changes being more pronounced in cv. satabdi. Khitish being a comparatively tolerant variety succumbed to less damage than cv. satabdi. Application of sulphate (10 mM) along with different test concentrations of selenate partially or almost completely ameliorated the toxic effects caused by selenate alone in the test seedlings. Combined sulphate and selenate was effective in countering the oxidative stress generated in the test seedlings under the influence of high selenium concentrations leading to better growth and metabolic activities in the test cultivars cv. satabdi and cv. khitish. The ameliorative influence was more pronounced in the sensitive variety cv. satabdi than in cv. khitish.

Acknowledgements

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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