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LOW LEVELS OF SELENIUM APPLICATION ATTENUATE LOW TEMPERATURE STRESS IN SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH.] SEEDLINGS

SALWA MOHAMED ABBAS

Department of Biological Science & Geology, Faculty of Education, Ain Shams University, Roxy Cairo, Egypt
Corresponding author's e-mail: salwa_morsy2001@yahoo.com

Abstract

Physiological responses to chilling were investigated in sorghum plants to identify the mechanisms of chilling tolerance. The experiment was conducted in the botany laboratory of Faculty of Education Ain Shams University. Sorghum (*Sorghum bicolor* L.) seeds were soaked in different concentrations (0, 3, 6 and 12 mg L^{-1}) of sodium selenate for 6 h before sowing. During the germination period seedlings were exposed to 4ºC or 8ºC for 7d. and allowed to recover at 25ºC for 3 days. Selenate at lower concentrations (3 and 6 mg L^{-1}) enhanced the growth and levels of chlorophylls (Chl), anthocyanine, sugar, proline(Pro), ascorbic acid(Asc) and enzymatic activities. However, high levels of selenate (12 mg L^{-1}1) exerted toxic effects. The levels of non-enzymatic antioxidants (ascorbic acid) were increased by selenate while the levels of carotenoids (Car) were decreased. Low selenate (3 and 6 mg L^{-1}) reduced lipid peroxidation as measured by malondialdehyde (MDA). The activities of enzymatic antioxidants showed different responses to selenate, guaiacol peroxidase, (GPX), in seedlings they were enhanced by low level of selenate (3 and 6 mg L^{-1}) while ascorbic acid peroxidase (APX) increased only at (12 mg $L^{-1)}$ of sodium selenate. The results showed that both low selenate (3 and 6 mg L^{-1}) especially 3 mg L^{-1} induced cold tolerance in the seedlings. This study explained the effects of low selenate level for young seedlings under cold stress.

Introduction

Low temperature affects germination, seedling growth, early leaf development and overall crop growth and productivity. On the cellular level, chilling can affect membranes and their lipid composition (Zhang *et al*., 2010). In addition to cold specific damages, chilling may impair water absorption by the roots and water transport to the shoot, leading to the physiological effects similar to drought stress (Zhang *et al*., 2010). Antioxidant capacity increases during cold acclimation in several plants as defense mechanism to low temperature (Foyer & Noctor, 2005).

Selenium (Se) is a trace element that can function as an essential nutrient for humans and animals or as an environmental toxicant; the boundary between the two is narrow and depends on its chemical form, concentration, and other environmentally regulating variables. Terry *et al.*, (2000) observed stimulating effect of low concentration of Se on plant growth and yield. Although Se is not classified as a micronutrient for higher plants, numerous studies have shown that at low concentrations, Se exerts a beneficial effect on growth and stress tolerance of plants by enhancing their antioxidative capacity and thereby improve growth and yield (Kong *et al*., 2005). Oxidative stress describes a condition when the generation of reactive oxygen species (ROS) in a system exceeds. Excess ROS can damage cellular lipids, proteins or DNA, thus inhibiting signal transduction pathways (Pennanen *et al*., 2002). Exogenous Se (low concentration) can reduce the intensity of peroxide processes of membrane lipids and affect the activity of redox enzymes and thereby change the redox state of the cell, thereby increasing stress tolerance (Kong *et al*., 2005). Moreover, positive influence of Se on changes in the activity and permeability of the cellular membrane found, and this may be one of the earliest symptoms of the influence of Se on plants (Filek *et al*., 2008). Selenium-like heavy metals-can modify uptake and accumulation of minerals that are important for metabolism. Selenium metabolism closely connected with the metabolism of nitrogenous substances in plants but particularly with amino acids. However, (Rios *et al*., 2009) showed the effect of different application rates of selenate on the production and detoxification of H_2O_2 in lettuce plant in non-stressed condition. The application of selenite triggered a higher foliar concentration of H_2O_2 and a higher induction of lipid peroxidation , in comparison to that observed after the selenate application indicate that the selenate form of Se is less toxic than selenite. In addition, the plants treated with selenate induced higher increases in enzymes that detoxify H_2O_2, especially ascorbate peroxidase (APX) and glutathione peroxidase (GPX), as well as an increase in the foliar concentration of antioxidant compounds such as ascorbate (AsA) and glutathione (GSH). Chu *et al*., (2010) observed that Se treatments with 1.0 mg kg^{-1} significantly reduced Malondialdehyde (MDA) content and the rate of O_2^- production in wheat seedlings grown under cold stress. Additionally, Se treatments significantly increased contents of anthocyanins, flavonoids and phenolic compounds of seedlings subjected to cold stress ,which have the ability to scavenge free radicals and inhibit membrane peroxidation of seedlings. In addition, the effects of Se on catalase (CAT) activity in seedlings exposed to cold stress were also reported by (Chu *et al*., 2010).

The study aimed to alleviate the adverse effects of low temperature for young seedlings subjected to cold stress by application of selenate at low rates.

Materials and Methods

Plant growth parameters: Good quality seeds of Sorghum (*Sorghum bicolor* L.) were surface sterilized with 1.0% sodium hypochloride. The experiment conducted in the lab of Biological Science Department. Seeds were presoaked in distilled water (control) or in

aqueous solutions of Se (0, 3, 6 and 12 mg Se L^{-1} in the form of sodium selenate, Na_2SeO_4) for 6 h at room temperature. After pre-treatment, the solutions decanted off and the seeds washed with distilled water and air-dried. Fifteen seeds from every application arranged in 15 cm Petri dishes covered with two sheets of filter paper moistened with 10 mL of distilled water. Following sowing, germination experiments carried by placing, Petri dishes in an incubator at 22±1ºC for 3 d. Three-d-old-seedlings exposed to low temperature stress by placing them in a cold room at a temperature of 4ºC and 8°C, for 10 d. After exposure to cold stress, the seedlings placed back to the climatic chamber at 22ºC, where they have recovered for 48 hrs. At the end of experiment (15 d), the lengths of the radical and shoot in mm, and fresh weights of the seedlings (g/seedling) from 20 seeds recorded. The dry weights measured by drying the seedlings at 75ºC, to give a constant weight.

Photosynthetic pigments determination: Chlorophyll a, b, and carotenoids in the second leaf of the sorghum seedlings extracted in 80% water acetone. The pigments were determined spectrophotometrically after centrifugation of extract at 3000 rpm for 5 min and estimated by the method of Metzner *et al*., (1965).

Estimasion of anthocyanin: Anthocyanin content estimated according to the method of Krizek *et al*., (1993). Leaf samples were homogenized in 10mL of acidified methanol (HCl: methanol, 1:99, v/v). The homogenate was centrifuged at 18 000 g for 30 min at 4°C, and then the supernatant was filtered through Whatman No1 to remove particulate matter and was stored in darkness at 5°C for 24 h. The amount of anthocyanin was determined from the absorbance at 550 nm.

Determination of Soluble Sugars (TSS): Soluble carbohydrates (TSS) extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600g. The supernatant evaporated until completely dried then dissolved in a known volume of distilled water to be ready for determination of soluble carbohydrates (Homme *et al*., 1992). TSS were analyzed by reacting of 0.1 ml of ethanol extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for 10 minutes and reading the cooled samples at 625 nm using Spekol SpectrocololourimeterVEB Carl Zeiss (Yemm & Willis,1994).

Electrolyte leakage: The plasma membrane intactness estimated through the leakage of electrolytes, described by Sun *et al*., (2006). Fresh leaves (0.3 g) placed in tubes, containing 30 ml bidistilled water and kept for 2 h in water bath at 30°C for measuring the initial conductivity (EC_1). The final electrolyte conductivity (EC_2) measured after boiling the plant samples for 15 min. The leakage percentage was calculated as (EC_1/EC_2) x100 %.

Lipid peroxidation: Malondialdehyde (MDA) was measured using the 2-thiobarbituric acid (TBA) reaction (Heath & Packer, 1968). The plant homogenate (extracted with 0.1% trichloroacetic acid) mixed with potassuim phosphate buffer (pH 7.0) and the TBA-reagent (0.5% thiobarbituric acid in 20% trichloroacetic acid, w/v) in a ratio 1:1:2 (v/v) and the reaction developed for 30 min in boiling water bath. The levels of TBA-conjugated substances was calculated using the extinction coefficient of 155 mM cm–1 from the data read at 532 nm after applying the correction read at 600 nm (for non-specific absorption).

Determination of ascorbic acid: Ascorbic acid was determined as described by (Mukherjee & Choudhuri, 1983). 4 ml of the extract was mixed with 2 ml of 2% dinitrophenyl-hydrazine (in acidic medium) followed by the addition of 1 drop of 10% thiourea (in 70% ethanol). The mixture was boiled for 15 min in a water bath and after cooling to room temperature, 5 ml of 80% (v/v) H_2SO_4 was added to the mixture at 0°C (in an ice bath). The absorbance was recorded at 525 nm using spectrophotometer.

Measurement of proline content: Determination of the free proline (Pro) levels done according to, (Bates *et al*., 1973). Proline extracted in 3% (w/v) aqueous sulfosalicylic acid. The reaction mixture contained supernatant, acetic acid and ninhydrin reagent in a ratio 1:1:1 (v/v) and was boiled for 1h in water bath. Proline quantity estimated from the data received reading at 520 nm.

Enzyme activities: For the analyses of guaiacol peroxidase 0.2g plant tissue was homogenized in 100 mM potassium phosphate buffer (pH 7.0), containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.5% insoluble polyvynil pyrrilidone (the tissue/ buffer ratio of 1:5, w/v). After centrifugation, the guaiacol peroxidase activity was estimated by the method of Polle *et al.*, (1994).

To analyze the activity of APX, plant tissue was homogenized in 50mM potassium phosphate buffer (pH 7.0), containing 5mM EDTA, 2mM sodium ascorbate, 0.5% insoluble pyrrilidone (Nakano & Asada, 1981), and centrifuged at 16 000 x g for 5 min. The reaction medium contained 50mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 0.5 mM sodium ascorbate and 6 mM H_2O_2. The oxidation of sodium ascorbate in the presence of APX started by adding of 9.7 M hydrogen peroxide and followed at 290 nm for 30 sec.

Results

Growth parameters: The results obtained indicate that all growth parameters (Plumule length, radicals length, fresh and dry weights of sorghum seedlings) under low temperature condition (4ºC and 8ºC) were increased in response to low concentrations of Se (3and 6 mg L^{-1}) as compared to controls (untreated with Se). On the other hand, application of high Se concentration (12 mg L^{-1}), significantly reduced these parameters (Table 1). It is obvious that under cold stress, low levels of Se induced increases in growth parameters and biomass production of sorghum seedlings.

Table 1. Changes in certain growth criteria of sorghum (*Sorghum bicolor* L.) seedlings grown under cold stress conditions after 13 days of germination as influenced by seed soaking in various concentrations of selenium prior to germination.

Cold stress	Selenium concentrations of (mg L^{-1})	Plumule length (cm)	Radicle length (cm)	Fresh wt. (g/plant)	Dry wt. (g/plant)
	(Control)	4.50 ± 0.05	5.76 ± 0.06	0.08 ±0.04	0.013 ± 0.08
4 ºC	0	3.92 ** ± 0.06	4.41 ** ± 0.04	0.06 ** ± 0.01	0.012 ** ± 0.07
	3	5.53 ** ± 0.04	6.87 ** ± 0.06	0.10 ** ± 0.05	0.016 ** ± 0.02
	6	5.32 ** ± 0.03	6.21 ** ± 0.07	0.09 ** ± 0.04	0.015 ** ± 0.03
	12	3.21 ** ± 0.05	4.05 ** ± 0.09	0.06 ** ± 0.06	0.012 ** ± 0.07
L.S.D. at:	0.05	0.14	0.17	0.00	0.00
	0.01	0.20	0.25	0.00	0.00
8 ºC	0	4.32 ± 0.04	5.00 ** ± 0.02	0.07 ** ± 0.02	0.013 ± 0.04
	3	7.58 ** ± 0.09	8.41 ** ± 0.08	0.11 ** ± 0.05	0.018 ** ± 0.02
	6	6.24 ** ± 0.08	7.52 ** ± 0.07	0.10 ** ± 0.04	0.017 ** ± 0.09
	12	3.76 ** ± 0.07	4.32 ** ± 0.03	0.06 ** ± 0.08	0.013 ± 0.05
L.S.D. at:	0.05	0.23	0.25	0.00	0.00
	0.01	0.33	0.35	0.00	0.00

Values ± SD are mean from 20 independent experiments. **, Highly significant change

Effect of Se on photosynthetic pigments: Exposure of sorghum plants to low Se concentrations (3 mg L^{-1}) induced a significant increase in photosynthetic pigments contents at 4ºC (Table 2) compared to untreated control. On the other hand, a significant reduction in carotenoid contents was observed in sorghum leaves exposed to all treatments of selenate. High concentrations of Se (12 mg L^{-1}) highly significant reduced the amount of pigments content in sorghum seedlings grown under cold stress as compared with controls.

Soluble sugar contents: The results revealed that, exposure of sorghum seedlings to 3 and 6 mg L^{-1} Se induced significant increase in soluble sugar content during cold stress, while higher Se concentration (12 mg L^{-1}) reduced their content as compared to controls (Fig. 2). The results revealed that the accumulation of soluble sugars was higher in plants grown at 8ºC than at 4ºC.

Anthocyanins: The anthocyanines are one of the flavonoids groups, located in the vacuoles of the epidermal and mesophyll cells throughout the plant kingdom. In our experiment addition of sodium selenate (3-and6 mg L^{-1}) increased the anthocyanin contents in sorghum seedlings under cold stress as compared with the control whereas the high concentration of sodium selenate (12 mg L^{-1}) reduced their contents (Fig. 1).

Electrolyte leakage: The results showed that electrolyte leakage in Se-treated of sorghum plants leaves under cold stress highly significantly decreased at concentrations of 3 and 6mg L^{-1} as compared with control and untreated seedlings (Fig. 3). However, high concentration of Se (12 mg L^{-1}) resulted in increase in electrolyte leakage.

Lipid peroxidation: MDA taken as an indicator for the degree of lipid peroxidation , as MDA produced by peroxidation of unsaturated fatty acids in plant cell membrane. (Fig. 4) Malondialdehyde (MAD) increased in sorghum seedlings under cold stress as compared to control. The high concentration of Se (12 mg L^{-1}) showed highly significant increase in this content. On the other hand, high significant decreases in MDA content were observed at the lower concentrations of Se as compared to control.

Ascorbic acid: The obtained results revealed that the content of (Asc) increased in Se-treated sorghum seedlings (3and6 mg L^{-1}) under cold stress as compared with control (Fig. 5). While, the high concentration of Se (12 mg L^{-1}) lead to reduction in ascorbic acid content as compared to 0 mg L^{-1}Se (cold treatment only) but is higher than in control.

Proline content: Proline content was higher in sorghum seedlings subjected to cold stress as compared to the control (Fig. 6). Application of low concentrations of Se (3 and 6 mg L^{-1}) induced additional increase in the concentration of Pro in cold-stressed sorghum seedlings. At a higher level (12 mg L^{-1}), however, Se exerted a reverse effect where Pro. content of the seedlings decreased compared with untreated with Se.

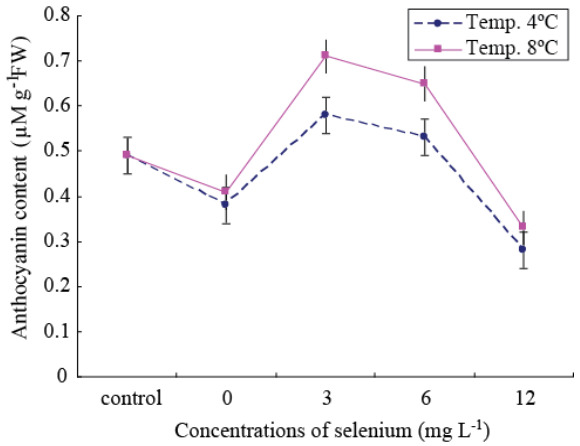

Fig. 1. Anthocyanins content of sorghum seedlings grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

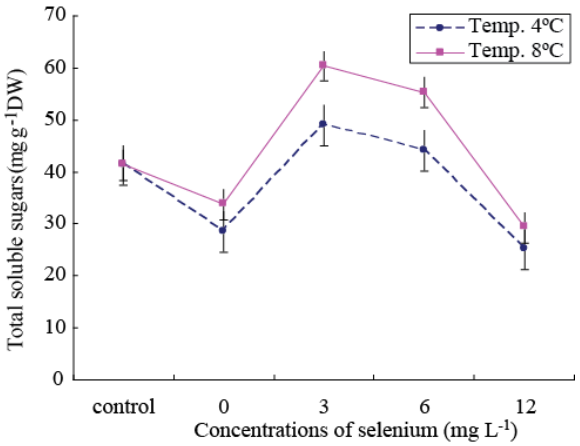

Fig. 2. Total soluble sugars content of sorghum seedlings grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

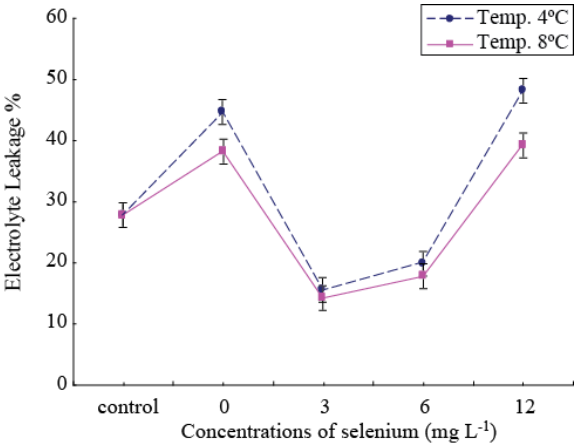

Fig. 3. Estimation of electrolyte leakage (EL) of sorghum plumules grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

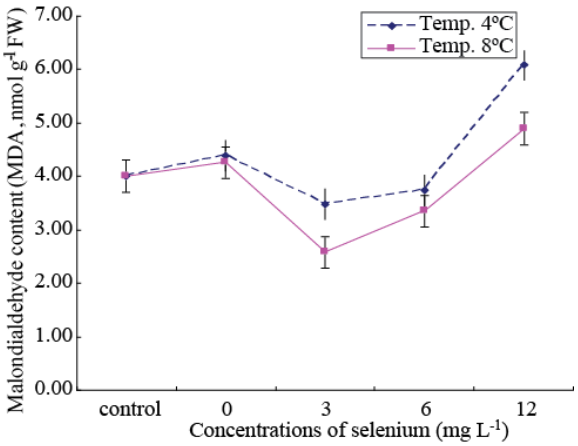

Fig. 4. Malondialdehyde (MDA) content of sorghum seedlings grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

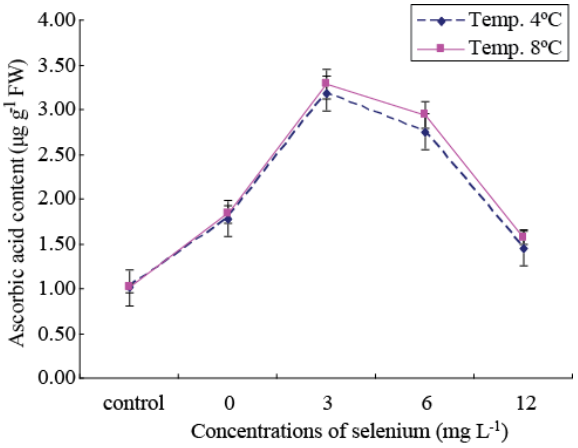

Fig. 5. Ascorbic acid content of sorghum seedlings grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

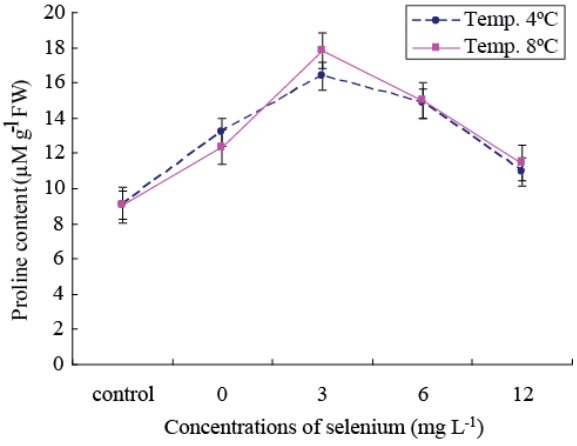

Fig. 6. Proline content of sorghum seedlings grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

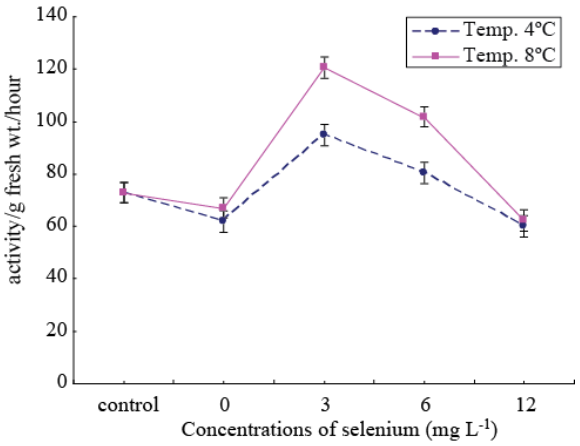

Fig. 7. Changes in enzyme activity of peroxidase of sorghum seedlings grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

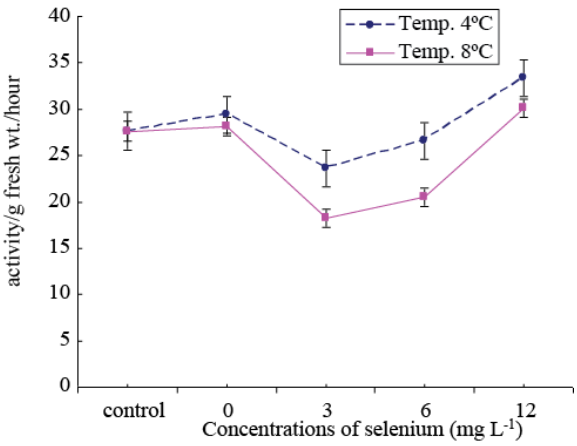

Fig. 8. Changes in enzyme activity of ascorbic acid peroxidase of sorghum seedlings grown under cold stress conditions as influenced by seed soaking in selenium concentrations prior to germination. Error bars indicate ± SE.

Table 2. Changes in photosynthetic pigments of the plumules of sorghum (*Sorghum bicolor* L.) seedlings grown under cold stress conditions after 10 days of germination as influenced by seed soaking in various concentrations of selenium prior to germination.

Cold stress	Concentrations of selenium (mg L^{-1})	Chlorophyll (a) Mg/fresh wt.	Chlorophyll (b) Mg/fresh wt.	Chlorophylls (a+b)	Carotenoids Mg/fresh wt.	Total pigments Mg/fresh wt.
	(Control)	0.33 ± 0.06	0.58 ± 1.05	0.91 ± 0.03	0.74 ± 0.05	1.65 ± 0.06
4 ºC	0	0.29 * ± 0.03	0.55 * ± 0.08	0.84 * ± 0.05	0.59 ** ± 0.04	1.43 ** ± 0.03
	3	0.49 ** ± 0.01	0.50 ** ± 0.07	0.99 * ± 0.04	0.53 ** ± 0.03	1.52 ** ± 0.01
	6	0.31 ± 0.02	0.51 ** ± 0.09	0.82 ** ± 0.07	0.59 ** ± 0.09	1.41 ** ± 0.06
	12	0.19 ** ± 0.04	0.53 ** ± 0.05	0.72 ** ± 0.06	0.58 ** ± 0.08	1.30 ** ± 0.04
L.S.D. at:	0.05	0.03	0.02	0.05	0.02	0.07
	0.01	0.05	0.03	0.08	0.03	0.10
8 ºC	0	0.30 ± 0.05	0.41 ** ± 1.12	0.71 ± 0.09	0.75 ± 0.07	1.46 ± 0.02
	3	0.21 * ± 0.07	0.37 ** ± 1.03	0.58 ** ± 0.04	0.28 ** ± 0.05	0.86 ** ± 0.07
	6	0.31 ± 0.03	0.52 ± 0.08	0.83 ± 0.06	0.60 ** ± 0.03	1.43 * ± 0.04
	12	0.30 ± 0.09	0.53 ± 0.07	0.83 ± 0.07	0.57 ** ± 0.06	1.39 * ± 0.06
L.S.D. at:	0.05	0.12	0.10	0.22	0.08	0.21
	0.01	0.17	0.15	0.32	0.12	0.31

Values ± SD are mean from 3 independent experiments.*, Significant change. **, Highly significant change.

Antioxidants: The results revealed an increase in activity of peroxidase (POD) in plants, treated withes Se at (3 and6 mg L^{-1}) under cold stress, whereas this activity was, reduced at (12 mg L^{-1}) as compared to control. In addition, the activity of peroxidase (POD) showed higher in its activity at 8ºC than at 4ºC increase. The results revealed that the activity of ascorbic acid peroxidase (Apx) increase in Se-untreated sorghum seedlings under cold stress (0 mg L^{-1}) and at high concentration of Se (12 mg L^{-1}) compared to control (Figs. 7& 8).

Discussions

Growth parameters: Exogenous application of Se acted differently under different abiotic stress in plants as studied by many researchers. The chilling effect manifested by

physiological perturbations, generally called low-temperature injury (Zhang *et al*., 2010). The chilling stress below 15°C often happens in the rice delayed growth period (Zhang *et al*., 2010). Low temperature affects germination, seedling growth, early leaf development and overall maize crop growth and productivity, maize exposed to low temperature during its early development resulting in poor photosynthetic performance, which is associated with retarded plant development (Leipner *et al.,* 1999). The enhanced production of (ROS) is responsible for peroxidation of membrane lipids, photosynthetic pigments, protein and nucleic acids (Kayani *et al.,* 2010). Recent researches have demonstrated that Se not only able to promote growth and development of plants, but also increase resistance and antioxidant capacity of plants subjected to various stresses , it can scavenge reactive oxygen species (Djanaguiraman *et al*., 2005). Se affected plant growth promotion might be the result of increased starch accumulation in chloroplasts and that protected cell content (Hawrylak-Nowak *et al*., 2010). Se at low concentration, could act as an antioxidant enhancing growth seedlings, whereas at higher concentration it could act as a growth-inhibiting agent (Turakainen, 2007). Se supply improved the recovery of potato plants from light and chilling stress (Chu *et al.,* (2010). Plants treated with Se and subjected to low temperature generally grew better than plants grown without the addition of Se were (Hawrylak-Nowak *et al.,* 2010). The extent of plant damage caused by exposure to low temperature depends on factors such as the developmental stage (Lyons *et al*., 2009).

Photosynthetic Pigments: Low temperature is one of the most important factors that limit photosynthetic activity. It has reported that Chl *a* and *b* content decreased in plants when plants subjected to cold treatment environment stress may lead to the decrease of the chlorophyll level (Wu *et al*., 2006). Aghaee *et al*., (2011) showed that higher contents of Chl in leaves of rice seedlings, under stress were associated with tolerance to chilling. However, chilling temperature around the leaves could disrupt key processes in photosynthesis, including thylakoid electron transport, carbon assimilation and stomatal control (Allen & Ort, 2001). In addition, cold stress can affect photosynthesis rates by inhibiting the light and dark reactions of photosynthesis and change in the activities of several enzymes of photosynthetic carbon assimilation.

The increase in Chl *a* and Chl *b* contents of sorghum seedlings attributed to Se effect on protection of chloroplast enzymes and thus increasing the biosynthesis of photosynthetic pigments (Pennanen *et al.*, 2002). High concentration of Se-induced reduction in photosynthetic pigments content observed in this study is consistent with the result of (Padmaja *et al*., 1995).

Anthocyanins: Plants accumulate a large number of metabolites namely flavonoid, isoflavonoids and anthocyanins. These compounds act as osmoprotectants, in response to environmental stress, it is accumulating under drought stress and at cold temperatures (Chu *et al*., 2010). Anthocyanins are reported mainly involved in photoprotection at low temperatures (Hatier & Gould 2008). Se treatments significantly increased contents of anthocyanins, and flavonoids of seedlings subjected to cold stress which have the ability to scavenge free radicals and inhibit membrane lipid peroxidation of seedlings (Chu *et al*., 2010). Antioxidant compounds content (anthocyanins, flavonoids, and phenolic compounds) increased by different selenium treatments in plants under cold stress (Liang *et al.,* 2009).

Total soluble sugars: Seedlings adapt to stress environment, associated with maintaining osmotic homeostasis by metabolic adjustments that lead to the accumulation of metabolically compatible compounds such as soluble sugar, membrane stability (Chang *et al.,* 2001). Cold acclimation is associated with multiple mechanisms that include changes accumulation of substantial amounts of compatible solutes, such as soluble sugars (Chinnusamy *et al*., 2007). Accumulation of soluble sugars reported in many plant species under diverse abiotic stress conditions that might indicate that osmolyte could increase the tolerance of plants to stress conditions in some degrees (Xiong & Zhu, 2002). Soluble sugars increased sharply right after transferring the cultures of strawberry to the low temperature (Xiao *et al*., 2009). Long-term acclimation to the cold and winter survival in herbaceous plants strongly correlated with the maintenance of soluble carbohydrate reserves at low temperature (Strand *et al.,* 2003).

Beneficial effects of Se were reported in terms of plant protection against abiotic stress, plant protection against ROS (Ríos *et al.,* 2009). Pennanen *et al.,* (2002) have showed that plant growth promoted by Se is due to the increased starch accumulation in chloroplasts.

Electrolyte leakage: Electrolyte leakage has thought to be an important index of the physiological functions of the cell (Zhou *et al*., 2005). In addition, prolonged exposure to low temperatures increased the leakage of solutes in mung bean seedlings, such as soluble sugars and free amino acids (Chang *et al*., 2001). Autocatalytic peroxidation of membrane lipids can trigger by AOS, resulting in loss of membrane semipermeability, one of the primary mechanisms of stress injury. Zhou *et al*., (2005), who reported that the membrane system of *Stylosanthes guianensis* damaged under chilling stress due to the induction of oxidative damage related to the imbalance of ROS production. It has been shown that plant membrane damage during chilling is related to the peroxidation of membrane lipid due to the stress-induced accumulation of free radicals (Stanisława *et al*., 2011).

Low concentrations of Se decrease electrolyte leakage as compared to control. Therefore, this indicated that the reduced electrolyte leakage may a direct consequence of Se treatment

Lipid peroxidation: MDA is a common product of lipid peroxidation and a sensitive diagnostic index of oxidative injury. In the present study, MDA content is an expression of lipid peroxidation increased by low temperature treatment. The accumulation of MDA used as an indicator of lipid peroxidation (Wu *et al*., 2006). Adaptation of plant cells to low temperature is associated with maintaining osmotic homeostasis by metabolic adjustments that lead to the accumulation of metabolically compatible compounds such as soluble sugar, malondialdehyde (MDA) and proline. The adaptation of plant cells to low temperature is based on their ability to maintain saturation of fatty acids in membrane lipids, thus modifying membrane fluidity

(Szalontai *et al.,* 2003). Low level of Se significantly diminished lipid peroxidation that measured as malondialdehyde (product of lipid peroxidation). The decrease in lipid peroxidation by Se is attributed to its effect on the activity of antioxidant enzymes and/or the increased levels of water-soluble ascorbic acid and glutathione (Pennanin *et al*., 2002).

Ascorbic acid: Ascorbic acid carries out a number of non-antioxidant functions in the cell. It has implicated in the regulation of the cell division, cell cycle progression (Smirnoff *et al*., 2001). Ascorbate act as antioxidant in the non-enzymatic detoxification of ROS (Ruth *et al*., 2004). The higher activities of defense enzymes and higher content of antioxidant under stress were associated with tolerance to chilling (Huang & Guo, 2005). The antioxidant defense system of plants includes the low molecular weight (water-soluble) substances (Wu *et al.,* 2004). Exogenous Se can affect the activity of redox enzymes and thereby change the oxidation-reduction status of the leaves increasing its stress tolerance (Vikhreva *et al*., 2002).

Proline content: Many reports indicated the accumulation of Pro during biotic abiotic stresses. The higher level of free Pro in cold-stressed plants has suggested as a factor conferring chilling tolerance. In contrast, Pro accumulation has also considered as a symptom of injury rather than an indicator of low temperature tolerance (Hawrylak-Nowak *et al*., 2010). The present results supported by that of (Chen & Li 2002), who reported most of the accumulated Pro in maize cells, during a prolonged chilling treatment. The effect of Se on the Pro level could be due to its effect on one of Pro metabolism enzymes (Xiong & Zhu, 2002).

Antioxidative enzymes: One of the biochemical changes occurring when plants subjected to low temperature stress is the production of ROS, which are highly reactive, and in the absence of any protective mechanism, they can disrupt normal metabolism through oxidative damage to lipids, protein and nucleic acids (Ya & Yong, 2011). Antioxidative are the most important components in the scavenging system of ROS. Plants respond to oxidative stress through increasing the enzymatic and non-enzymatic antioxidants (Cao *et al.,* 2004 & Waqas *et al.,* 2011).

There are changes in the activities of enzymes and the contents of antioxidants involved in antioxidant metabolism in response to low temperature stress (Wu *et al*., 2004). Plant itself could induced its defensive system to protect against free radical when suffered abiotic stresses, as shown by the transient increases of SOD, CAT and APX activities (Hong *et al*., 2011).

Peroxidases (POD): The activities of some antioxidant enzymes increase during stress treatment, and the types of enzymatic activities that increase are dependent on the form stress imposed(Foyer, & Noctor 2011). The enzymes whose activities increase during stress treatment may play an important role in defense against that particular stress. SOD and POD activities increased and then decreased gradually with the duration of cold treatment time in rice seedlings (Foyer & Noctor, 2011). Wu *et al*., (2004) reported expression of acidic POD bands with different band intensities that are responsible for tolerance to freezing stress of 15 olive cultivars.

Ascorbate peroxidase (APX): APX is one of the most important enzymes of the AsA-GSH cycle and plays a vital role in plant defense against oxidative stress by catalyzing the conversion of H_2O_2 to H_2O. The results indicate that higher APX activity enhances H_2O_2-scavenging capacity and protects sorghum from lipid peroxidation, thereby increasing spikelet fertility under cold stress (Sato *et al*., 2011). The protective role of Se on the cold stress in sorghum seedlings could be due to, reduction of oxygen radicals, osmotic regulation by synthesis of osmoregulatory compound, and increasing the biosynthesis of enzymatic and non-enzymatic antioxidants(Sandalio *et al*., 2001). The beneficial effect of Se in plants subjected to stress conditions has in most cases attributed to increased antioxidant activity (Jincehng *et al*., 2012). Moreover, the protective effect of Se on plants has demonstrated as a decrease in lipid peroxidation and an increase in the activity of antioxidant enzymes thereby improving stress resistance. Se attributed to the activation of antioxidant defense system of plants (Sandalio *et al*., 2001).

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