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Full Length Research Paper

Influence of different soaking times with selenium on growth, metabolic activities of wheat seedlings under low temperature stress

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Low temperature (LT) is one of the most important factors limiting the growth, development and distribution of wheat plants in temperate regions. Selenium often acts as an antioxidant in plants and this study hypothesize that selenium application can partly alleviate LT-induced oxidative stress and negative impacts of LT on wheat (*Triticum aestivum* L.) plant. Wheat seeds were soaked in aqueous solutions of selenium (5 mg Se L^{-1}) for 5, 10 and 15 h. Then, the seeds were germinated at 3 or 5°C for 14 days and allowed to recover at 22°C for three days. The results show that low temperature stress inhibited the growth, chlorophylls, soluble sugars and antioxidant enzyme activities and increased oxidant production and membrane damage. Soaking the seeds in selenium solution for different times was feasible in enhancing the growth, chlorophylls, anthocyanin, sugars, proline contents and enzymatic activities and decreased membrane damage by enhancing antioxidant defense coupled with the appearance of novel protein bands. Se induced the lowering of respiratory potential measured as electron transport system (ETS) activity of mitochondria. However, prolonged soaking in selenium (15 h) exerts toxic effects. These positive effects of Se are, however, dependent on the period of soaking.

Key words: Selenium, cold stress, wheat seedlings, antioxidants, protein electrophoresis, respiratory potential.

INTRODUCTION

Many crops encounter cold stress in growing periods, which brings greater loss to agricultural production. Low temperature stress induces considerable changes in the biochemistry and physiology of plants which exhibit symptoms of injury when exposed to low non-freezing temperatures (Lynch, 1990). Low temperatures act as an abiotic stress factor that has a strong impact on the survival, growth, reproduction and distribution of plants. Low temperature affects germination, seedling growth, early leaf development and overall crop growth and productivity. The general metabolic activity of individual organisms can be assessed from terminal electron transport system (ETS) activity in mitochondria. Under stress condition, organisms increase their need for energy. The ability to cope with stress in vital plants is therefore related to respiratory potential (ETS activity) of certain tissues. When stress is too immense, the antioxidant metabolism is defeated. Strong stress causes reduced vitality of tissues that reflects in lower respiratory potential (Germ and Gaberščik, 2003).

Wheat is one of the most important cereal crops of the world. In most areas of the world, wheat is a principal food. Germination and growth before emergence is normally controlled by soil temperature. Under stress conditions of different regions, low moisture and cold stress are limiting factors during germination. The rate and degree of seedling establishment are extremely important factors in determining both yield and time of maturity. Seed germination is a major problem of wheat (*Triticum aestivum* L.) production because it is influenced by many environmental factors but the availability of soil moisture has a major effect on germination and subsequent emergence.

Searching for suitable ameliorants or stress alleviant is one of the tasks of plant biologists. Recent researchers have identified several beneficial effects of selenium (Se)

in plants. Positive effects of Se on plants are mainly exhibited (Yao et al., 2009). Selenium is an element whose deficiency causes the decrease in defense mechanisms of living organisms. Se plays a role in antioxidative mechanisms in plants (Ekelund and Danilov, 2001). Earlier studies have indicated that Se maintains antioxidative defence systems and enhances sugar and starch accumulation. Se has been recognized as an integral component of different enzymes such as glutathione, peroxidase and thioredoxin reductase. Chemically, it is similar to sulphur (S), leading to nonspecific replacement of S by Se in proteins and other sulphur components (Nowak et al., 2004). This results in non-functional proteins and enzymes. In plants, Se functions as an antioxidant (Hartikainen et al., 2000). Plants take up Se from the soil primarily as selenate (SeO_4^{2-}) or selenite (SeO_3^{2-}) (Ellis and Salt, 2003). Se can increase the tolerance of plants to UV-induced oxidative stress, delay senescence and promote the growth of ageing seedlings (Xue et al., 2001). Exogenous selenium (low concentration) can reduce the intensity of peroxide processes of membrane lipids and affect the activity of redox enzymes, and thereby change the oxidation-reduction status of the cell, thus increasing stress tolerance (Vikhreva et al., 2002; Salwa, 2012). Moreover, positive influence of selenium on changes in the activity and permeability of the cellular membrane has been found and this may be one of the earliest symptoms of the influence of selenium on plants (Filek et al., 2008). Additionally, selenium treatments significantly increased the contents of anthocyanins, flavonoids and phenolic compounds of seedlings subjected to cold stress which have the ability to scavenge free radical**s** and inhibit membrane lipid peroxidation of seedlings. Also, the effects of selenium on peroxidase (POD) and catalase (CAT) activities in seedlings exposed to cold stress were also reported by Chu et al. (2010). Although Se is an essential trace nutrient important to humans and most other animals as an antioxidant, it is toxic at high concentrations due to incorporation of Se in place of sulphur in amino acids, with subsequent alteration of protein three-dimensional structure and impairment of enzymatic function (Amweg et al., 2003).

Thus, the aim of the present study was to determine whether the presoaking in selenium for various soaking times could protect wheat seedlings from injuries caused by low temperature stress.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of wheat (*T. aestivum* L.) which were of good quality and had high germination rate of 100% under optimum conditions were used. The seeds were surface sterilized with 1.2% sodium hypochloride solution for 30 s and rinsed several times with deionised water. Wheat seeds were divided into three groups:

i) The first group was soaked in distilled water without selenium to serve as control (optimal temperature without selenium).
ii) The second group of the seeds was soaked in distilled water without selenium to serve as untreated seeds (cold stressed without selenium)
iii) The third group of seeds was soaked in aqueous solutions of selenium (5 mg Se L^{-1} in the form of sodium selenate) for 5, 10 and 15 h, hence, such group consisted of three sets of the seeds. Each set represented a defined soaking time.

After pretreatment, the solutions were decanted off and the seeds were washed with distilled water and dried. The seeds from every application were arranged in 15 cm Petri dishes covered with two sheets of filter paper moistened with 10 ml of distilled water. Following sowing, germination experiments were carried by placing the seeds of the first group in an incubator at 22±1°C until the end of the germination experiment. Three-day-old-seedlings of the second group were divided into two sets; the first set was placed in a cold room at a temperature of 3°C for 14 days, while the other one was placed in a cold room at a temperature of 5°C for 14 days then both sets of this group were placed back to the climatic chamber at 22°C, where they recovered for three days. Three-day-old-seedlings of the third group were treated in a similar manner as that of the second group. The seedlings from all groups were collected at 20 days after sowing. 10 seedlings per treatment were taken at the end of the experimental period to investigate the effects of presoaking in selenium at different soaking times and/or lower temperatures on some growth aspects, molecular and physiological changes of wheat seedlings as follow:

Growth parameter

Shoot length, root length, fresh weight of seedlings, and dry weight of seedlings were obtained by drying the material in oven at 80°C until constant weight.

Chlorophyll determination

Chlorophyll were extracted from the leaves and estimated by the method of Maclachlam and Zalik (1963).

Total soluble sugars (TSS)

Total soluble sugars (TSS) were extracted by overnight submersion of dry tissue in 10 ml of 80% (v/v) ethanol at 25°C with periodic shaking, and centrifuged at 600 *g*. The supernatant was evaporated till 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 0.1 ml of ethanolic extract with 3.0 ml freshly prepared anthrone (150 mg anthrone + 100 ml 72% H_2SO_4) in boiling water bath for ten minutes and reading the cooled samples.

Determination of free proline

Free proline content of fresh seedling tissues was determined according to the method described by Petters et al. (1997). The proline content was expressed as $\mu g.g^{-1}$(FW).

Lipid peroxidation

The level of lipid peroxidation was measured by determination of malondialdhyde (MDA) in fresh seedling tissues as described by Heath and Packer (1968). One gram of fresh seedling tissue was

homogenized in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10000x *g* for 5 min, and then 4 ml of thiobarbuteric acid (0.5% in TCA 20%) was added to 1 ml of the supernatant. The mixture was heated to 95°C for 30 min, and then quickly cooled in ice bath. The contents were centrifuged at 10000x *g* for 15 min and the absorbance of supernatant was measured at 532 nm. The MDA content was calculated using extinction coefficient of 155 mM^{-1} cm^{-1}. MDA content expressed as µmol.g^{-1} fresh weight (FW).

Measurement of electrolyte leakage

The electrolyte leakage was measured according to the method described by Kong et al. (2005). 20 leaf discs from each subgroup were excised and rinsed thoroughly with double distilled water to remove contamination caused by sampling. Samples were then transferred to tubes with 20 ml double distilled water. The electrical conductivity (E0) of the solution was immediately measured using an electrical conductivity meter (DDSJ-308A, Shanghai) at 25°C. The tubes were incubated at 30°C for 30 min, and the electrical conductivity (E1) measured again. Subsequently, the tubes were placed in boiling water bath for 20 min and the electrical conductivity (E2) read after the tubes had cooled to 25°C. The electrolyte leakage percentage (EL%) of leaf cells was calculated in accordance to the following equation:

EL% = (E1 - E0) / (E2 - E0) × 100

Anthocyanin

Anthocyanin content was estimated according to the method of Krizek et al. (1993). Leaf samples were homogenized in 10 ml of acidified methanol (HCl: methanol, 1:99, v/v). The homogenate was centrifuged at 18000 *g* for 30 min at 4°C, and then the supernatant was filtered through Whatman No. 1 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. Anthocyanin content was expressed as µmol/g FW and the concentration of anthocyanin was calculated using the extinction coefficient of anthocyanin ε = 33 000/mol^2 cm.

Ascorbic acid

Ascorbic acid was determined as described by Mukherjee and Choudhuri (1983). 4 ml of the extract was mixed with 2 ml of 2% dinitrophenyl-hydrazine (in acidic medium) followed by the addition of one 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 at 625 nm using Spekol Spectrocololourimeter VEB Carl Zeiss (Yemm and Willis 1994).

Antioxidant enzymes

Catalase (CAT) (EC 1.11.1.6) activity was measured as described by Chandlee and Scandalios (1984), with some modifications. We homogenized 0.5 g of frozen plant material in a prechilled pestle and mortar with 5 ml of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing 1 mM phenylmethylsulfonyl fluoride (PMSF). The extract was centrifuged at 4°C for 20 min at 12,500 rpm. The supernatant was used for enzyme assay. The assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH 7.0), 400 µl of 15 mM H_2O_2, and 40 µl of enzyme extract. The decomposition of H_2O_2 was followed by a decline in absorbance at 240 nm.

Polyphenol oxidase (PPO; EC 1.10.3.1) activity was assayed as described by Kumar and Khan (1982). The assay mixture for PPO contained 2 ml of 0.1 M phosphate buffer (pH 6.0), 1 ml of 0.1 M catechol, and 0.5 ml of enzyme extract. This was incubated for 5 min at 25°C, after which, the reaction was stopped by adding 1 ml of 2.5 N H_2SO_4. The absorbency of the purpurogallin formed was read at 495 nm. To the blank, 2.5 N H_2SO_4 was added at zero time to the same assay mixture. PPO activity was expressed in U mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

ETS activity

The respiratory potential of mitochondria was measured in plants via terminal ETS activity (Packard, 1971; Kenner and Ahmed, 1975). The ETS acts as a bridge between the oxidizing organic matter and O_2. Plant material was homogenised in an ice-cold homogenisation buffer and sonicated with an ultrasound homogeniser (40W, 4710, Cole-Parmer, Vernon Hills, IL, USA). The homogenate was then centrifuged (8500× *g*, 4 min, 0°C) in a top refrigerated ultracentrifuge. 1.5 ml of substrate solution and 0.5 ml of iodo-nitro-tetrazolium-chloride (INT) were added to triplicates of the supernatant (0.5 ml) and incubated at 20°C for 40 min. During the incubation, INT instead of oxygen was reduced to formazan. After stopping the reaction with a stopping solution (formaldehyde and phosphoric acid, 1:1), the formazan absorption at 490 nm was determined. ETS activity was measured as the rate of tetrazolium dye reduction, and converted to equivalent oxygen as described by Kenner and Ahmed (1975).

Electrophoretic of protein patterns

Electrophoretic protein profile of wheat leaves were analyzed according to SDS-PAGE technique (Laememli, 1970) which relates polypeptide maps, molecular protein markers, and percentage of band intensity using gel protein analyzer version 3 (MEDIA-CYBERNE TICE,USA).

Statistical analysis

The data shown are mean values ± SD. The data were subjected to statistical analysis of variance and the value of LSD was calculated whenever F values were significant at 5% level of probability (Snedecor and Cochran, 1982).

RESULTS

Changes in growth parameters

The obtained results show that the low temperature stress alone inhibited the growth of wheat seedlings as compared to control (22°C). Increase in growth rate such as shoot length, root length, fresh and dry weights of seedlings after low temperature stress was higher in selenium-treated seedlings compared to the untreated ones (3 and 5°C). Moreover, stronger effect of selenium on all growth parameters was observed as a result of soaking the seeds for 5 h at 3 and 5°C, the latter temperature giving the highest values in all growth parameters over the untreated seeds by 164.25, 163.1,

Table 1. Effect of different soaking times in selenium prior to sowing on growth parameters of wheat (*Triticum aestivum* L.) seedlings grown under cold stress conditions.

Treatment	Shoot length (cm)	Root length (cm)	Fresh wt. of seedlings (g)	Dry wt. of seedlings (g)
22°C (control)	10.08±0.11	9.52±0.23	0.15±0.08	0.031±0.03
3°C	9.56±0.15	8.63±0.18	0.14±0.05	0.026±0.02
3°C + 5 h in Se	13.10± 0.23	10.67±0.17	0.17±0.03	0.032±0.04
3°C + 10 h in Se	12.93±0.16	10.54±0.27	0.16±0.02	0.032±0.06
3°C + 15 h in Se	9.33±0.25	7.43±0.20	0.13±0.04	0.021±0.05
5°C	9.79±0.28	8.89±0.17	0.14±0.06	0.030±0.09
5°C + 5 h in Se	16.08±0.16	14.50±0.16	0.20±0.04	0.035±0.08
5°C + 10 h in Se	13.33±0.19	12.45±0.11	0.17±0.08	0.033±0.07
5°C + 15 h in Se	9.51±0.22	7.77±0.15	0.13±0.06	0.025±0.06
LSD at 5%	0.92	0.82	0.01	0.003

Data are means ± SD (n=5).

Table 2. Effect of different soaking times in selenium prior to sowing on chlorophyll, total soluble sugars and proline contents of wheat (*Triticum aestivum* L.) seedlings grown under cold stress conditions.

Treatment	Chlorophyll content (mg/g FW)	Total soluble sugars content (mg/g DW)	Proline content (µg/g FW)
22°C (Control)	10.13±0.10	58.76±0.67	10.2±0.42
3°C	8.16±0.13	48.25±0.54	14.16±0.55
3°C + 5 h in Se	12.22±0.11	69.78±0.62	21.38±0.34
3°C + 10 h in Se	11.31± 0.17	61.26±0.49	18.56±0.62
3°C + 15 h in Se	9.75±0.11	35.26±0.51	10.04±0.47
5°C	8.29±0.18	51.16±0.72	15.10±0.81
5°C + 5 h in Se	12.29±0.20	80.53±0.65	22.61±0.56
5°C + 10 h in Se	11.94±0.16	75.41±0.43	19.55±0.71
5°C + 15 h in Se	10.00±0.19	39.37±0.81	10.71±0.52
LSD at 5%	0.78	4.95	1.44

Data are means ± SD (n=5).

142.8 and 116.7% for shoot length, root length, fresh weight of seedlings and dry weight of seedlings, respectively (Table 1). The lowest biomass production which occurred in wheat seedlings was observed at 3°C by soaking the seeds in selenium for 15 h before germination.

Chlorophyll content

Table 2 shows chlorophyll content of young wheat seedlings at various soaking times in Se and grown at lower temperatures. Chlorophyll content significantly decreased when wheat seedlings were exposed to low temperatures (3 and 5°C). The effect of Se treatment on chlorophyll content was related to the soaking time in Se and the degree of the low temperature used. A longer soaking time in Se (15 h) did not affect chlorophyll content. However, at the shortest soaking time in selenium (5 h), a significant increase of chlorophyll content in wheat seedlings was detected at 3 and 5°C.

Soluble sugar content

An increase in total soluble sugar content was observed in wheat seedlings soaked in Se for 5 and 10 h before sowing and exposed to cold stress; more so, the longest soaking time (15 h) reduced this content as compared to control (Table 2). The results reveal that, accumulation of soluble sugars was higher in seedlings grown under 5°C.

Proline content

Proline content was increased in wheat seedlings previously soaked in selenium for 5 and 10 h and grown at both temperatures of cold stress (Table 2). This

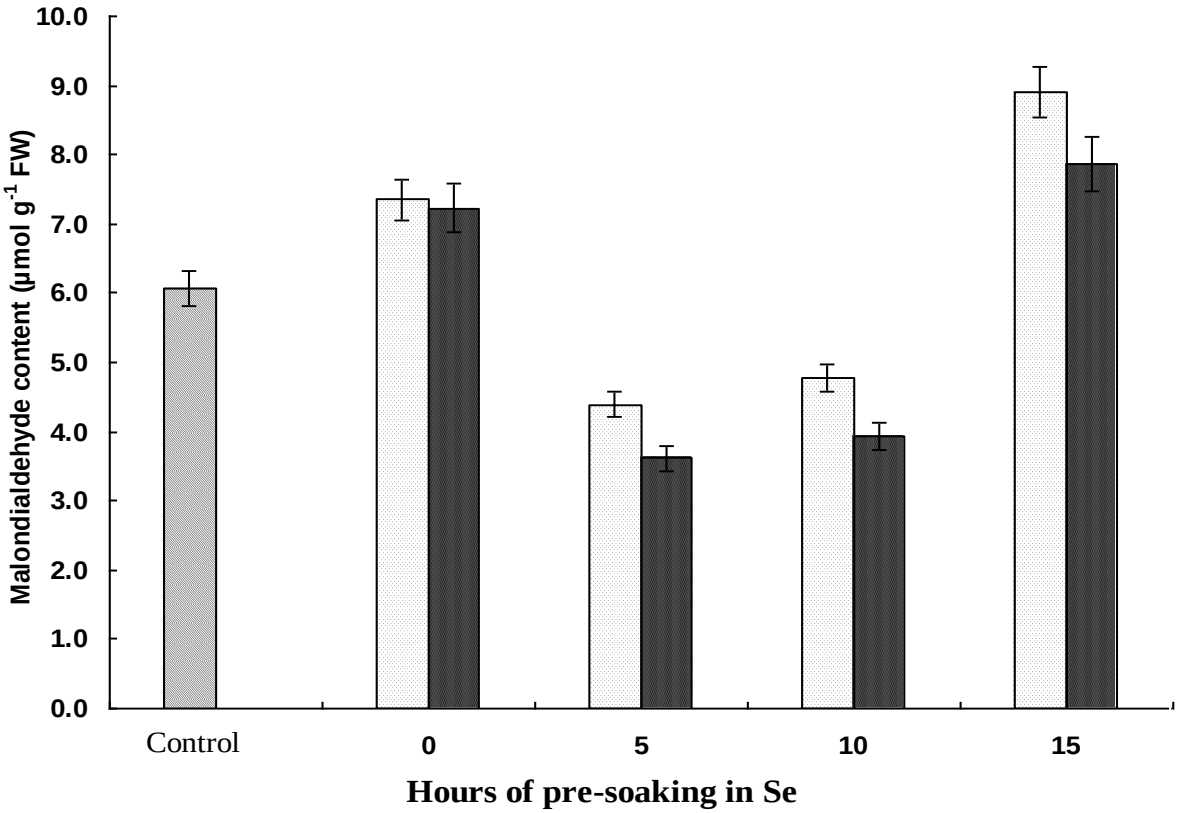

Figure 1. Effect of different soaking times in selenium prior to sowing on malondialdehyde (MDA) content of wheat seedlings grown under cold stress conditions. The results are expressed as mean ± SD with three replications; white and black bars indicate cold stress (3°C) and cold stress (5°C), respectively.

content reached its maximum value due to seeds being soaked for 5 h in selenium and grown at 5°C. On the other hand, our results showed that proline content was non-significantly changed in wheat seedlings soaked for 15 h in selenium and exposed to cold stress.

Changes in oxidative damage

Lipid peroxidation

An increase of MDA accumulation following low temperature stress was observed in both selenium-untreated seedling and the seedlings previously soaked in selenium for 15 h (Figure 1). The MDA content in selenium-treated seedlings for lower soaking times (5 and 10 h) was reduced below that observed in control.

Electrolyte leakage

The effect of low temperature on plasma membrane intactness is shown in Figure 2. Levels of electrolyte leakage of wheat seedlings grown under cold stress (3 and 5°C) were significantly increased while presoaking the seeds in selenium for lower soaking time (5 and10 h) provoked a strong decrease in plasma membrane leakage estimated in leaves of the produced seedlings. By contrast, the longer soaking time in selenium (15 h) showed high levels of electrolyte leakage as compared with control.

Changes in non-enzymatic antioxidants

Anthocyanins

Application of low temperature stress to the untreated-selenium seedlings decreased anthocyanins content below the control (22°C). Presoaking the seeds in selenium for 5 and 10 h partly overcame the negative effects of both temperatures. Whereas, the increase in the soaking time in selenium before germination (15 h) reduced anthocyanins content, particularly at 3°C (Figure 3).

Ascorbic acid

Exposure of the untreated-selenium seedlings to low temperatures alone did not affect ascorbic acid content, but this content was increased in the seedling presoaked in selenium for 5 and 10 h and grown under cold stress particularly at 5°C. By soaking the seeds in selenium for

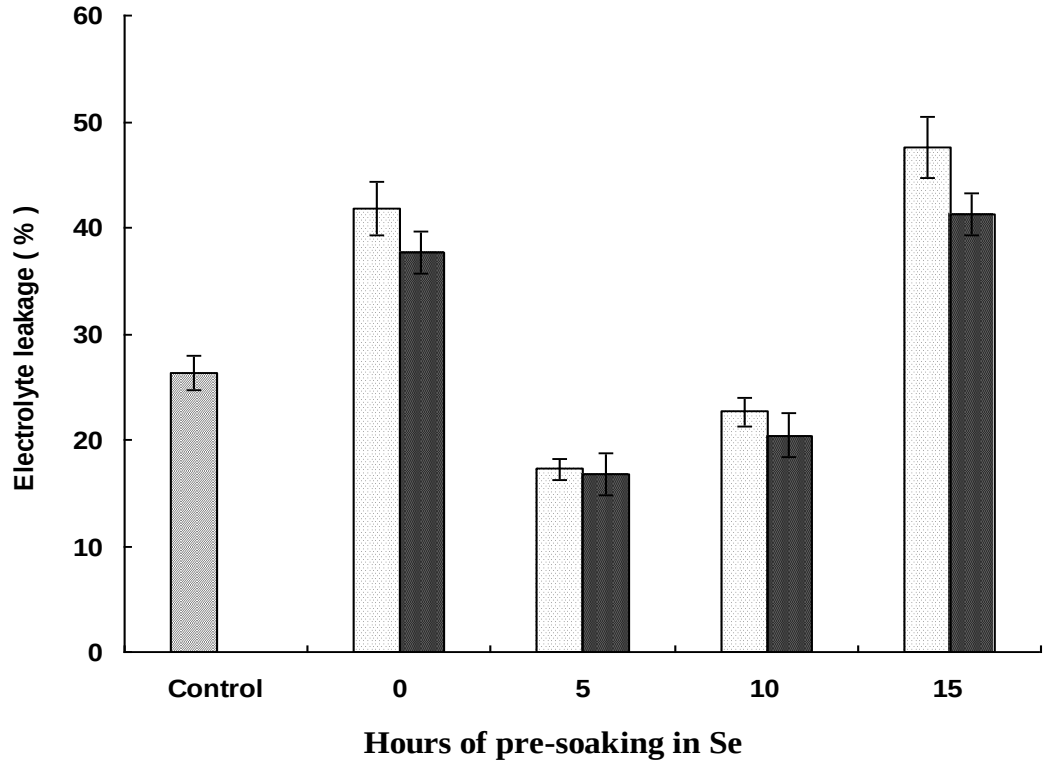

Figure 2. Effect of different soaking times in selenium prior to sowing on electrolyte leakage (EL) of wheat seedlings grown under cold stress conditions. The results are expressed as mean ± SD with three replications; white and black bars indicate cold stress (3°C) and cold stress (5°C), respectively.

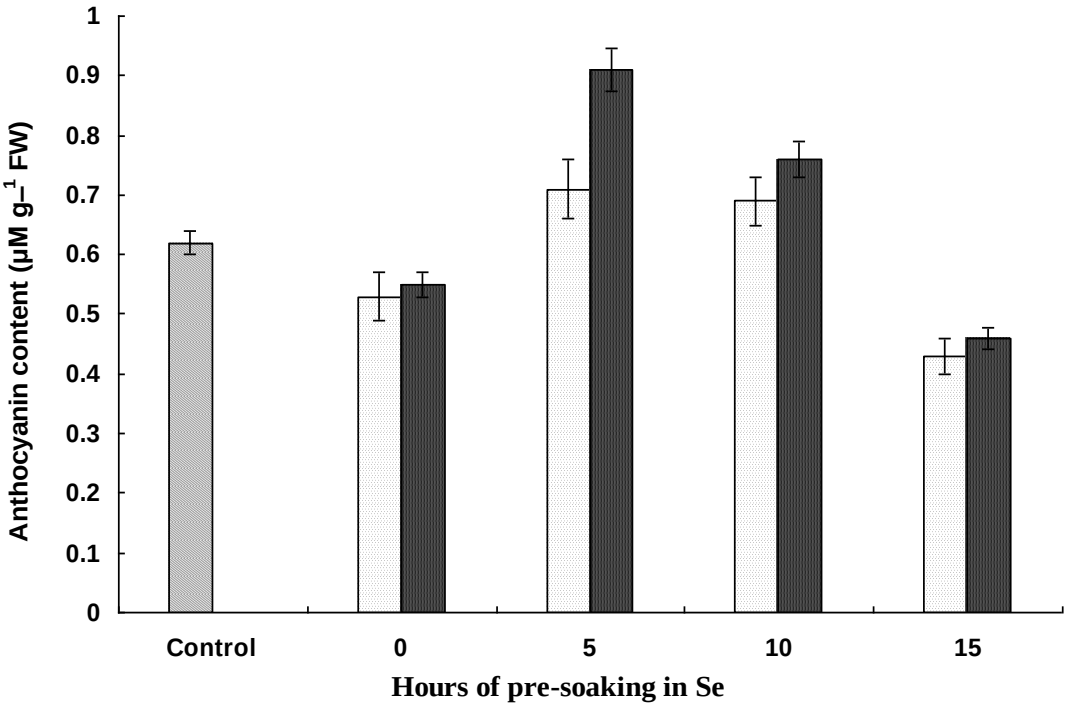

Figure 3. Effect of different soaking times in selenium prior to sowing on anthocyanin content of wheat seedlings grown under cold stress conditions. The results are expressed as mean ± SD with three replications; white and black bars indicate cold stress (3°C) and cold stress (5°C), respectively.

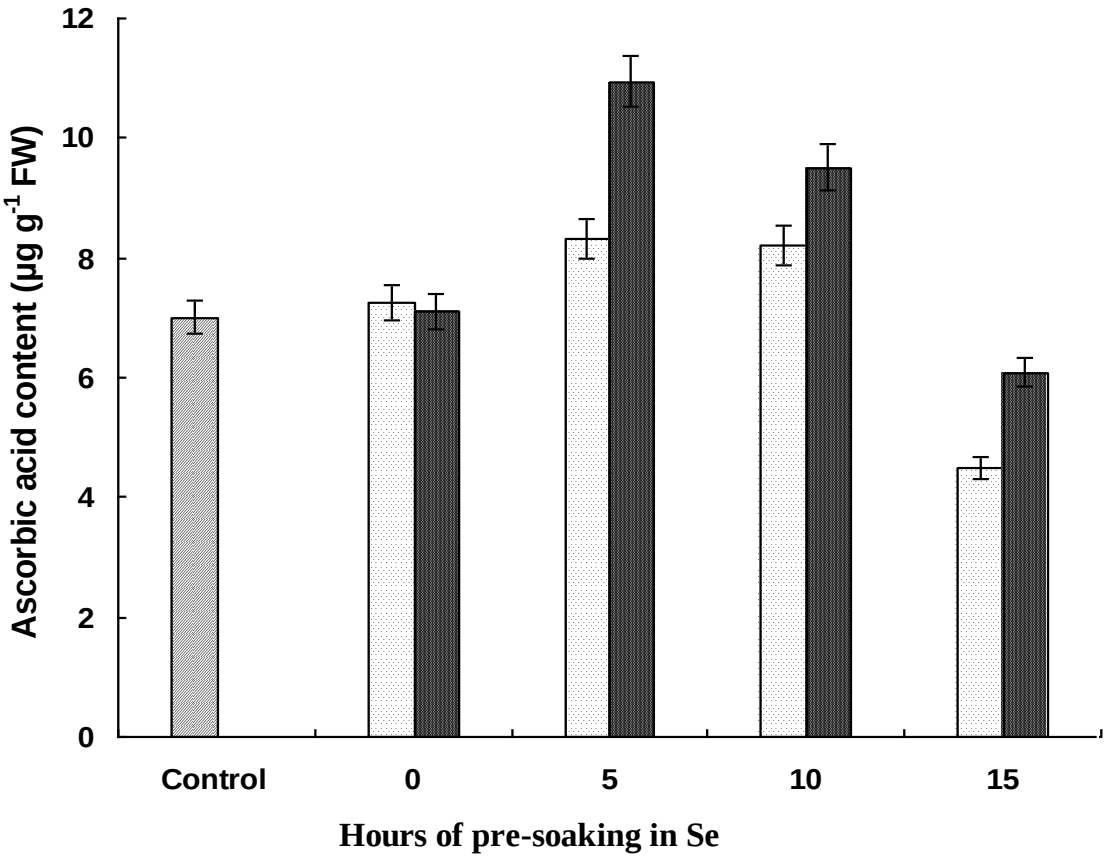

Figure 4. Effect of different soaking times in selenium prior to sowing on ascorbic acid content of wheat seedlings grown under cold stress conditions. The results are expressed as mean ± SD with three replications; white and black bars indicate cold stress (3°C) and cold stress (5°C), respectively.

15 h, the ascorbic acid content was decreased at both temperatures (Figure 4).

Changes in enzymatic antioxidants

The results of the present work show that, the activities of the antioxidant enzymes (CAT and PPO) in wheat seedlings grown under 3 or 5°C of low temperature were significantly decreased (Figures 5 and 6). Application of selenium for shorter soaking time (5 and 10 h) increased the activities of CAT and PPO of the seedling at both temperatures as compared with those of the control seedlings or seedlings that treated with selenium for longer soaking time (15 h), the magnitude of such response was much more pronounced at 5°C.

ETS activity

Figure 7 shows that terminal electron transport system activity was higher in the seedlings grown under low temperature stress alone. Se application lowered ETS activity in the seedlings under both temperatures particularly at the shorter soaking time in selenium before sowing.

Protein electrophoresis

The changes in protein electrophoretic pattern of wheat seedlings presoaking in selenium for various soaking times and grown under cold stress conditions (3 and 5°C) are shown in Figure 8; these results are analyzed and recorded in Table 3. In the control wheat seedlings, the separation of 10 protein bands was apparent, their molecular weights ranged between 271.76 and 11.43 kDa. Exposure of wheat seedlings to cold stress conditions throughout their growth showed an increase in the number of protein bands to 12 bands. These results indicate that the seedlings of wheat plants grown under cold stress were characterized by disappearance of certain bands and the appearance of new ones as compared with that of the control seedlings. In the present study, cold stress, in general, induced synthesis of a new set of protein bands (six bands) at molecular weights 55.10, 37.14, 35.71, 24.57, 13.29 and 7.14 kDa at 3°C and also six bands at molecular weights 55.10,

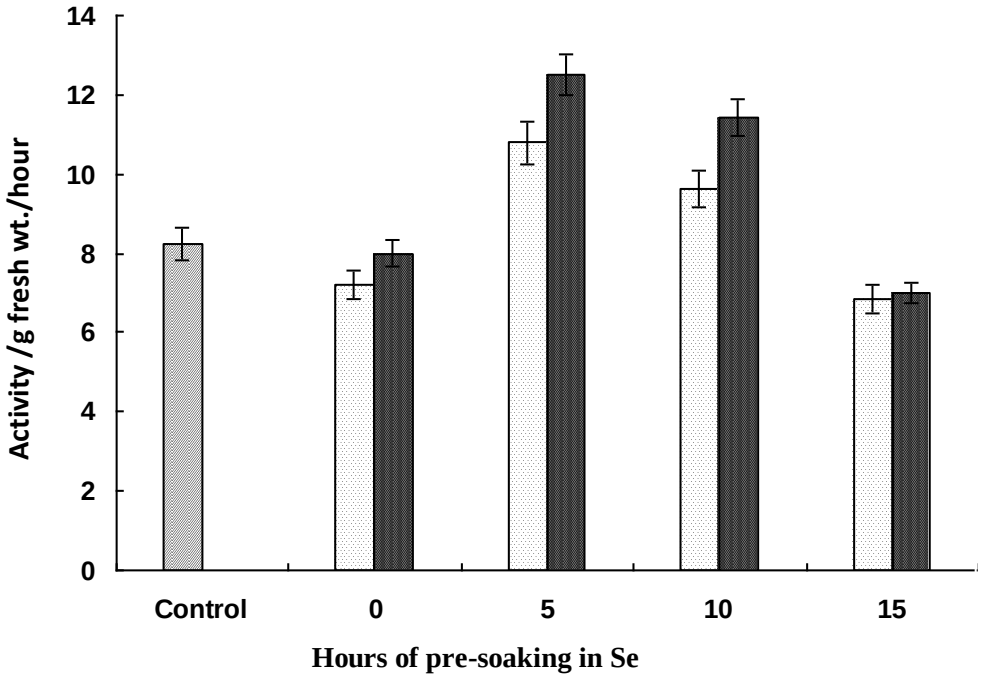

Figure 5. Effect of different soaking times in selenium prior to sowing on enzyme activity of catalase of wheat seedlings grown under cold stress conditions. The results are expressed as mean ± SD with three replications; white and black bars indicate cold stress (3°C) and cold stress (5°C), respectively.

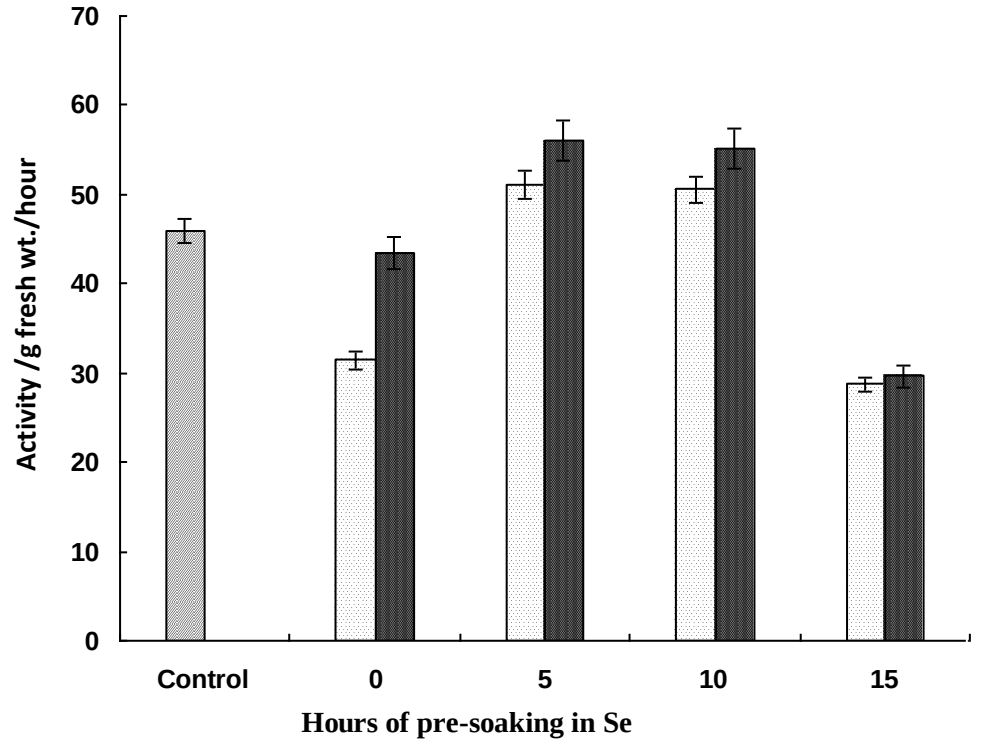

Figure 6. Effect of different soaking times in selenium prior to sowing on enzyme activity of polyphenol-oxidase of wheat seedlings grown under cold stress conditions. The results are expressed as mean ± SD with three replications; white and black bars indicate cold stress (3°C) and cold stress (5°C), respectively.

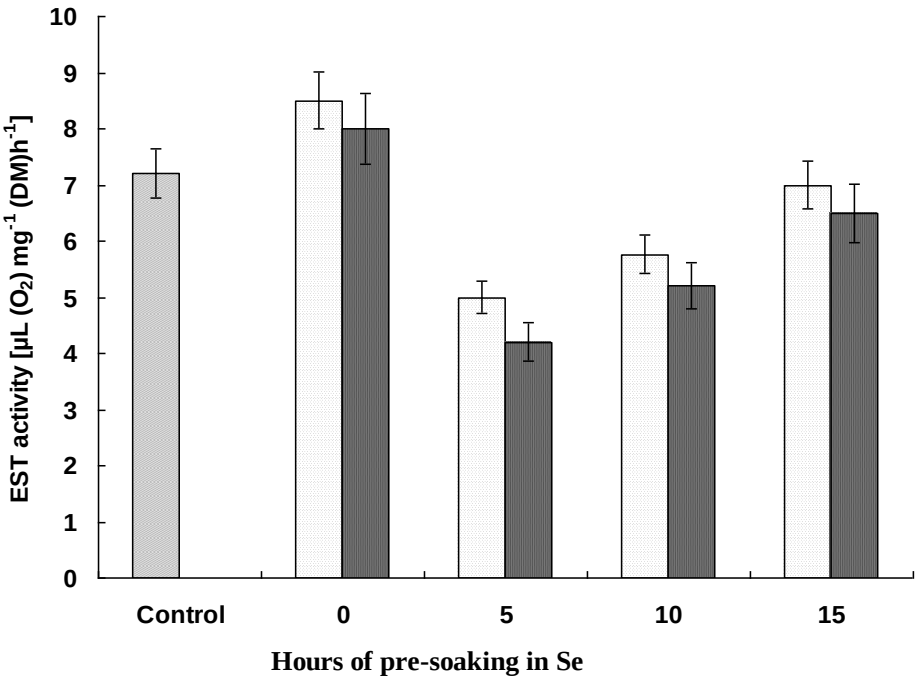

Figure 7. Effect of different soaking times in selenium prior to sowing on terminal electron transport system (ETS) activity of wheat seedlings grown under cold stress conditions. The results are expressed as mean ± SD with three replications; white and black bars indicate cold stress (3°C) and cold stress (5°C), respectively.

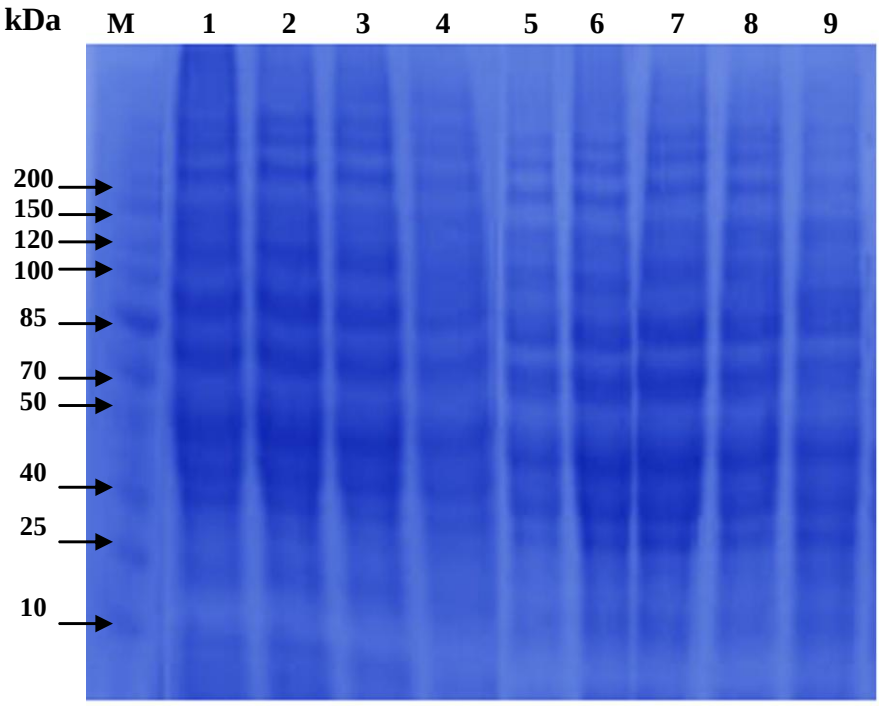

Figure 8. Electrophoretic banding profiles of protein extracted from the leaves of wheat seedlings grown under cold stress as influenced by seed soaking in selenium for various hours prior to germination. M, Marker protein; lane 1, control; lane 2, 3°C; lane 3, 3°C + 5 h in Se; lane 4, 3°C + 10 h in Se; lane 5, 3°C + 15 h in Se; lane 6, 5°C; lane 7, 5°C + 5 h in Se; lane 8, 5°C + 10 h in Se; lane 9, 5°C + 15 h in Se.

Table 3. Effect of different soaking times in selenium prior to sowing on electrophoretic pattern of wheat (*Triticum aestivum* L.) seedlings grown under cold stress conditions.

Molecular weight (kDa)	Control	3 ºC				5 ºC			
		3ºC	Pre-soaking time in Se (hours)			5ºC	Pre-soaking time in Se (hours)		
			5	10	15		5	10	15
271.76	+	+	+	+	+	+	+	+	+
182.56	+	+	+	+	+	+	+	+	+
131.23	+	+	+	+	+	+	+	+	+
106.25	+	+	+	+	+	+		+	+
94.44	+						+		
65.68	+	+	+	+	+	+	+		
59.28	+		+	+	+			+	+
55.10		+	+	+	+	+	+	+	+
49.05	+					+			+
37.14		+	+	+	+	+	+	+	+
35.71		+	+	+	+	+	+	+	
30.57	+	+			+				+
27.14			+	+	+		+	+	+
24.57		+				+		+	+
13.29		+				+			
11.43	+		+						
7.14		+		+					
4.33						+	+	+	+
Total number of bands	10	12	11	11	11	12	10	11	12
Number of new bands		6	4	4	5	6	5	6	5

37.14, 35.71, 24.57, 13.29 and 4.33 kDa at 5°C. At the meantime, exposure of the wheat seedlings to cold stress conditions induced disappearance of some protein bands at molecular weights 94.44, 59.28 and 11.43 kDa at both temperatures. Moreover, the protein band at molecular weight 49.05 kDa disappeared at lower temperature of cold stress (3°C). Also, the band at molecular weight 30.57 kDa disappeared at 5°C.

It is worth mentioning that selenium treatments induced the appearance of new protein band at molecular weight 27.14 kDa. This band disappeared when plants were subjected to both temperatures of cold stress. The protein bands that appeared at molecular weights 55.10, 37.14 and 35.71 kDa referred to the effect of both cold stress levels and presoaking-selenium treatments. While the band that appeared at 13.29 kDa appeared in response to cold stress treatments only. In addition, the protein band which had the lowest molecular weight of 4.33 kDa, characterized the plants grown under 5°C of cold stress.

DISCUSSION

The chilling effect is manifested by physiological perturbations, generally called low-temperature injury (Zhang et al., 2010). The chilling stress below 15°C causes heavy seed loss and delayed growth period (Sharifi, 2010). Exposure of plants to chilling enhanced the production of reactive oxygen species (ROS) in plant cells. The enhanced production of reactive oxygen radicals is responsible for peroxidation of membrane lipids, photosynthetic pigments, protein and nucleic acids. Recent researches have demonstrated that selenium was not only able to promote growth and development of plants, but also increased resistance and antioxidant capacity of plants subjected to various stresses including low temperature stress. It can scavenge reactive oxygen species (Djanaguiraman et al., 2005). Our results demonstrate that wheat seedlings treated with selenium exhibited higher increases in their growth parameters under cold stress. Such results indicate that Se addition increased plant biomass production. Similar results were observed and previously reported by Djanaguiraman et al. (2005) who reported that selenium was able to promote growth and development of plants by increasing resistance and antioxidant capacity of plants subjected to various stresses including low temperature stress. Selenium, which affected plant growth promotion, might be due to the increase in starch accumulation in

chloroplasts and the protected cell content (Xue et al., 2001). In a recent study, it had been found that plants treated with Se and subjected to low temperature generally grew better than plants grown without the addition of Se (Hawrylak et al., 2010). Low temperature is one of the most important factors that limit photosynthetic activity (Wu et al., 2006). 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 (Salwa, 2012). The obtained results suggest that Se has a promoting influence on chlorophyll content and this effect depends on soaking time in selenium and its content in the seedlings before germination. This may be because Se could stimulate the respiration rates and the flow of electrons in respiratory chain, and accelerate the chlorophyll biosynthesis (Germ et al., 2005). Moreover, increase in chlorophyll contents of wheat seedlings may be attributed to selenium effect on protection of chloroplast enzymes and thus, increasing the biosynthesis of photosynthetic pigments (Pennanen et al., 2002). On the other hand, the reduction or non significant change in chlorophyll content observed in this study due to longer soaking time (15 h) in Se is consistent with the result of Padmaja et al. (1995). Seedlings adapt to stress environment with maintaining osmotic homeostasis by metabolic adjustments that lead to the accumulation of metabolically compatible compounds such as soluble sugar (Chinnusamy et al., 2007). At an optimal level, selenium appears to promote tuber growth of potato tubers, and rapidly expanding leaves in lettuce and ryegrass leaves are strong sinks for carbohydrates (Xue et al., 2001).

Several studies indicated that proline accumulated during biotic and abiotic stress is considered as a signal/regulatory compound able to activate multiple physiological or molecular mechanisms. The higher level of free proline in cold-stressed plants has been suggested as a factor conferring chilling tolerance. In contrast, proline accumulation has also been considered as a symptom of injury rather than an indicator of low temperature tolerance (Hawrylak et al., 2010). It is clear from the presented results that chilling effectively enhanced the proline accumulation under chilling stress conditions. Positive correlations between the accumulation of endogenous proline and improved cold tolerance have been found in maize (Zhou et al., 2002). The effect of selenium on the proline level could be due to its effect on proline metabolism enzymes. High proline synthesis in stressed plants could favor a better recovery of these plants and could also be a good defense mechanism for survival.

MDA is a common product of lipid peroxidation and a sensitive diagnostic index of oxidative injury. In this respect, increase in lipid peroxidation was reported in many plants under various environmental stresses. The accumulation of MDA is often used as an indicator of lipid peroxidation (Wu et al., 2006). 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. The decrease in lipid peroxidation by selenium may be attributed to its effect on the activity of antioxidant enzymes and/or the increased levels of water soluble ascorbic acid and glutathione (Pennanen et al., 2002). Electrolyte leakage has been thought to be an important index of the physiological functions of the cell. Adversities such as drought, salinity, high, and low temperatures initially damage the structure of the cell membrane, thereby affecting its function, leading to an increase in membrane permeability, and resulting in leakage of intracellular contents. 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). Electrolyte leakage and lipid peroxidation level in mung bean plant increased under chilling conditions. Freezing stress which causes extracellular ice-crystal formation, freeze-induced dehydration and concentration of cell sap has major mechanical impacts on cell walls and plasma membranes and leads to cell rupture (Margesin et al., 2007). The reduced electrolyte leakage which was observed in selenium-treated seedlings in our study may be a direct consequence of Se treatment. The electrolyte leakage and the MDA content were significantly lower in the embryonic axes of seeds soaked in selenite than in seeds soaked in water (Stanisława et al., 2011). Mervi et al. (2003) showed that selenium-treated lettuce plants were somewhat more tolerant and the integrity of membranes was improved during oxidative stress (low temperature).

Plants accumulate a large number of metabolites such as flavonoid, isoflavonoids, anthocyanins and ascorbic acid as osmoprotectants in response to environmental stress. All these compounds have been reported to exhibit a wide range of protective functions in plants under stress (Salwa, 2012). They are involved in defense against environmental stresses like ultraviolet radiation, drought and cold temperatures. Several reports demonstrated that anthocyanins are mainly involved in photoprotection at low temperatures. Our obtained results revealed that selenium treatments significantly increased anthocyanins and ascorbic acid contents of the seedlings subjected to cold stress which have the ability to scavenge free radicals and inhibit membrane lipid peroxidation of seedlings (Chu et al., 2010). Moreover, ascorbic acid carries out a number of non-antioxidant functions in the cell. It has been implicated in the regulation of the cell division, cell cycle progression, and plays a key role in antioxidant defenses.

Selenium treatment at its lower soaking time caused a significant elevation in the specific activities of antioxidant enzymes (CAT and PPO) of selenium–treated wheat seedlings which indicate that selenium can increase the

tolerance of plants against oxidative stress. Superoxide dismutase (SOD) and CAT activities increased and then decreased gradually with the duration of cold treatment time in rice seedlings (Foyer and Noctor, 2011). Recent researches have demonstrated that Se was able to promote growth and development of plants and increase antioxidant capacity of plants subjected to stresses (Peng et al., 2002). The protective role of Se in the cold stress could be due to reduction of oxygen radicals and osmotic regulation by synthesis of enzymatic and non-enzymatic antioxidants.

Increased ETS activity observed in our results indicates that plants are under stress because plants need more energy during cold stress in order to develop essential structural components. The author suggests that this may be a result of decreasing ability of the cells to fully use their potential enzymic capacity or it may be the direct consequence of a decrease in the respiratory enzymatic capacity of the leaf. However, the present results are in accordance with Valkama et al. (2003) who reported on a decreased density of mitochondria in barley in response to Se. This could be attributed to alteration of mitochondrial division. According to the present results, it can be assumed that both temperatures, together with Se application, imposed stress on the plants which led to a lower respiratory potential. This is a physiologically significant phenomenon because of the claims of many authors that energy demands are increased during stress (Germ and Gaberščik, 2003). On the other hand, it has been reported that ETS activity was lowered in stress caused by Pb (Vodnik et al., 1999) or unsuitable growth conditions (Urbanc-Bercic and Gaberscik, 2001). Combined UV-B and Se treatment of seeds probably imposed a strong stress on the plants which could not be overcome. The synergistic impact of both (low temperatures and selenium) was evident, indicating that stress was a factor connected with the changes in the respiratory potential of the cell.

The outcome of the obtained results clearly indicate that soaking the grains of wheat plants for different times in selenium before germination led to the appearance of new protein bands which varied according to the soaking times. The existence of such newly formed protein bands in treated wheat seedlings might be explained, based on the potentiality of Se for triggering the expression of specific genes along DNA molecule in the target cells; a process which appears to play a significant role in regulating a cascade of biochemical reactions which might determine the ultimate appearance of growth patterns of the produced seedlings. This, accompanied by a persistent effect, carried over to the progeny *via* alteration of DNA- binding protein receptors mechanism, might amplify the signal-transduction pathway. This suggestion is reinforced by the findings of Abdel-Hamid (2002). In the present work, the appearance of new protein bands due to the application of selenium could be attributed to the fact that selenium has chemical properties similar to those of sulphur and can be incorporated into proteins in place of cysteine and methionine. Insertion of selenocysteine and selenomethionine into protein could interfere with the formation of disulphide bridges, leading to slight structural changes which might lead to changes in the protein activity.

Conclusions

The results of this study indicate that presoaking the seeds of wheat plants in selenate for suitable soaking times could provide an ecological adaptation for young seedlings subjected to stress conditions by the increase in antioxidant levels and enzymes' activities.

REFERENCES

Abdel-Hamid M (2002). Effect of seed soaking in paclobutrazole on growth parameters productivity, photosynthetic pigments, ion content of faba bean. Chemical composition and protein profile of the harvested seed. Egypt. J. Biotech. 11:48-70.

Amweg EL, Stuart DL, Weston DP (2003). Comparative bioavailability of selenium to aquatic organisms after biological treatment of agricultural drainage water. Aquat. Toxicol. 63:13-25

Chandlee JM, Scandalios JG (1984). Analysis of variants affecting the catalase development program in maize scutellum. Theor. Appl. Gen. 69:71-77.

Chang MS, Chen S, Lee L, Chen Y (2001). Cold-acclimation and root temperature protection from chilling injury in chilling-sensitive mung bean (*Vigna radiata* L.) seedlings. Bot. Bull. Acad. Sin. 42:53-60.

Chinnusamy V, Zhu JH, Zhu JK (2007). Cold stress regulation of gene expression in plants. Trends Plant Sci. 12:444-451.

Chu I, Yao X, Zhang Z (2010). Responses of wheat seedlings to exogenous selenium supply under cold stress. Biol. Trace Elem. Res. 136:355-363.

Djanaguiraman M, Devi DD, Shanker AK, Sheeba A, Bangarusamy U (2005). Selenium- an antioxidative protectant in soybean during senescence. Plant Soil 272:77-86.

Ekelund NGA, Danilov RA (2001). The influence of selenium on photosynthesis and "light-enhanced dark respiration" (LEDR) in the flagellate *Euglena gracilis* after exposure to ultraviolet radiation. Aquat. Sci. 63:457-465.

Ellis DR, Salt DE (2003). Plants, selenium and human health. Curr. Opin. Plant Biol. 6:273-279.

Filek M, Keskinen R, Hartikainen H, Szarejko I, Janiak A, Miszalski Z, Golda A (2008). The protective role of selenium in rape seedlings subjected to cadmium stress. J. Plant Physiol. 165:833-844.

Foyer CH, Noctor G (2011). Ascorbate and glutathione: the heart of the redox hub. Plant Physiol. 155:2-18.

Germ M, Gaberščik A (2003). Dihalni potencial – kazalnik stresa pri rastlinah. Zb. Bioteh. Fak. Univ. Ljublj. Kmet. 81(2):335-339.

Germ M, Kreft I, Osvald J (2005). Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (*Cucurbita pepo* L.). Plant Physiol. Bioch. 43(5):445-448.

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

Hawrylak N, Matraszek BR, Szymańska M (2010). Selenium modifies the effect of short-term chilling stress on cucumber plants. Biol. Trace Elem. Res. 86:13-15.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts. Arch. Biochem. Biophys. 125:189-198.

Homme PM, Gonzalez B, Billard J (1992). Carbohydrate content, frutane and sucrose enzyme activities in roots, stubble and leaves of rye grass (*Lolium perenne* L.) as affected by sources/link modification

after cutting. J. Plant Physiol. 140:282-291.
Kenner RA, Ahmed SI (1975). Measurements of electron transport activities in marine phytoplankton. Mar. Biol. 33:119-127.
Kong L, Wang M, Bi D (2005). Selenium modulates the activities of antioxidant enzymes, osmotic homeostasis and promotes the growth of sorrel seedlings under salt stress. Plant Growth Regul. 45:155-163.
Krizek DT, Kramer GF, Upadhyaya A, Mirecki RM (1993). UV-B Response of cucumber seedling grown under metal halid and high pressure sodium/deluxe lamps. Plant physiol. 88:350–358.
Kumar KB, Khan PA (1982). Peroxidase and polyphenol oxidase in excised ragi (*Eleusine coracana* cv. PR 202) leaves during senescence. Indian J. Exp. Bot. 20:412-416.
Laememli UK (1970). Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nat. 227:680-685.
Lynch DV (1990). Chilling injury in plants: the relevance of membrane lipids, in: F. Katterman (ed.), Environmental Injury to Plants, Academic Press, New York pp.17–34.
Maclachlam S, Zalik S (1963). Extraction and estimation of chlorophyll. Can. J. Bot. 41:1053.
Margesin R, Neuner G, Storey KB (2007). Cold-loving microbes, plants, and animals – fundamental and applied aspects. Naturwissenschaften 94:77–99.
Mervi S, Marja T, Helinä H (2003). Selenium effects on oxidative stress in potato. Plant Sci. 165(2):311-319.
Mukherjee SP, Choudhuri MA (1983). Implications of water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Plant Physiol. 58:166-170.
Nowak J, Kaklewski K, Ligocki M (2004). Influence of selenium on oxidoreductive enzymes activity in soil and in plants. Soil Biol. Biochem. 36:1553-1558.
Packard TT (1971). The measurement of respiratory electrontransport activity in marine phytoplankton. J. Mar. Res. 29:235-243.
Padmaja K, Somasekharaiah BV, Prasad ARK (1995). Inhibition of chlorophyll synthesis by selenium: involvement of lipoxygenase mediated lipid peroxidation and antioxidant enzymes. Photosyn. 31:1–7.
Peng XL, Liu YY, Luo S (2002). Effects of selenium on lipid peroxidation and oxidizing ability of rice roots under ferrous stress. J. Northeast Agric. Univ. 19:9-15.
Pennanen A, Xue T, Hartikainen H (2002). Protective role of selenium in plant subjected to severe UV irradiation stress. J. Appi. Bot. 76:66-76.
Petters W, Piepenbrock M, Lenz B, Schmitt JM (1997). Cytokinine as a negative effector of phosphoenolpyruvate carboxylase induction in *Mesembryanthemum crystallinum*. J. Plant Physiol. 151:362-367.
Salwa MA (2012). Effects of low temperature and selenium application on growth and the physiological changes in sorghum seedlings. J. Stress Physiol. Biochem. 8(1):268-286.
Snedecor GW, Coshran WC (1982). Statistical methods Oxford and I.B.H. Publishing G.7th ed. Iowa, State Univ. Press, Iowa USA. p. 275.
Sharifi P (2010). Evaluation on Sixty-eight Rice Germplasms in Cold Tolerance at Germination Stage. Rice Sci. 17(1):77-81.
Stanisława P, Ewelina R, Ewa K (2011). The protective role of selenium in recalcitrant *Acer saccharium* L. seeds subjected to desiccation. J. Plant Physiol. 168:220-225.
Urbanc-Bercic O, Gaberscik A (2001). The influence of water table fluctuations on nutrient dynamics in the rhizosphere of common reed (*Phrogmites australis*). Water Sci. Technol. 44:245-250.
Valkama E, Kivimaenpaa M, Hartikainen H, Wulff A (2003). The combined effects of enhanced UV-B radiation and selenium on growth, chlorophyll fluorescence and ultrastructure in strawberry (*Fragaria xananassa*) and barley (*Hordeum vulgare*) treated in the field. Agric. Meteorol. 120:267-278.
Vikhreva VA, Balakhnina TI, Gins VK (2002). Effect of selenium on intensity of peroxide processes and enzyme activity in Caucasian goat's rue leaves under extreme growing condition. Russian Agric. Sci. 2:1–4.
Vodnik D, Gaberscik A, Gogala N (1999). Lead phytotoxicity in Norway spruce (*Picea abies* (L.) Karst.): The effect of Pb and zeatin-riboside on root respiratory potential. Phyton. 39:155-159.
Wu XX, Zhu YL, Zhu WM (2006). Physiological effects of exogenous nitric oxide in tomato seedlings under NaCl stress. Sci. Agric. Sinica 39(3):575-581.
Xue T, Hartikainen H, Piironen V (2001). Antioxidative and growth-promoting effect of selenium in senescing lettuce. Plant Soil 27:55-61.
Yao X, Cho J, Wang G (2009). Effects of selenium on wheat seedlings under drought stress. Biol. Trace Elem. 130:283-290.
Yemm EW, Willis AJ (1994). The respiration of barley plants. IX. The metabolism of roots during assimilation of nitrogen. New Phytolo. 55:229-234.
Zhang S, Jiang H, Peng S, Korpelainen H, Li C (2010). Sex-related differences in morphological, physiological, and ultrastructural responses of *Populus cathayana* to chilling. J. Exp. Bot. 1:12.
Zhou BY, Li YB, Chen JZ, Ji ZL, Hu ZQ (2002). Effects of low temperature stress and ABA on flower formation and endogenous hormone of litchi. Acta Hortic. Sinica 29:577-578.