JTUSCI

Available online at www.taibahu.edu.sa

ISSN: 1658-3655

Abdel Kader et al / JTUSCI 5: 14-24 (2011)

Journal of Taibah University for Science

Chilling-induced oxidative stress and polyamines regulatory role in two wheat varieties

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Received August 11th 2011 Revised September 25th 2011 Accepted October 22nd 2011

Abstract

Fifteen-day-old seedlings of two wheat varieties (Side 1 & Gimmiza 7) were randomly separated into three equal groups to investigate the effect of chilling stress on their antioxidant defense system. Moreover, the possible role of polyamines (PAs) pretreatment during alleviating chilling injuries. The first group was kept in a green house at 21/15°C (day/ night) and 10: 14h light/dark (-ve control). Second seedlings group were chilled at 5°C for 6 or 9 hours (+ve control). The Seedlings of the third group were divided into three sub-groups and sprayed with 0.5, 1.0 and 2.0mM spermidine (Spd). After 12 hours of polyamine pre-treatments, the seedlings were incubated at 5°C for 6 or 9 hours. At the end of the chilling period, the treated plants were transferred to the pre-experimental conditions in the greenhouse where they recovered for 10 days. Chilling stress induced a significant increase of lipid peroxidation, membrane leakage and hydrogen peroxide level, while Spd treatments resulted in a significant decrease. In addition, endogenous PA level increased in response to chilling stress. Activities of catalase, peroxidase and ascorbate peroxidase declined after the exposure to chilling whereas glutathione and free ascorbate increased. Spd treatments alleviated the injury caused by chilling stress by preventing the decrease in the activity of the antioxidant enzymes. It can be concluded that the stress protection caused by spermidine treatment probably contributes to the enhancement of the activity of the free-radical scavenging systems. Moreover, it was clear that the Sids-1 cultivar was more tolerant to chilling stress than Gemmieza-7cultivar.

Key Words: Antioxidants; Chilling; Gemmieza-7cultivar; Oxidative stress; Sids-1 cultivar; Wheat.

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Introduction

Many agronomically important plants of tropical or sub-tropical origin are exposed to low temperatures during their cultivation in temperate climate. They usually encounter these low temperatures during autumn as young plantlets during the developmental stages particularly those susceptible to chilling stress. Moreover, they experience not only chilling, but also considerable day/night temperature fluctuations as well. At this time of the year low temperatures most frequently occur at night or early morning and the temperature during the day often strongly increases [1]. If growth and development took place at sub-optimum temperatures, various types of physiological damage would occur. Some of these effects only become visible during the warmer period after chilling [2].

These chilling injuries may include the following symptoms: 1) a decrease of the plant growth rate and leaf elongation. 2) stomatal conductance and photosynthetic performance [3], 3) changes in protein content [4]. 4) enzyme activities [5].5) an increase in the production of active oxygen species (AOS) (6) as well as of enzymatic and non-enzymatic antioxidants [7], 6) changes in membrane structure and lipid composition [8], 7) cellular leakage of electrolytes and amino acids, a diversion of electron flow to alternate pathways [9].8) cellular and sub-cellular structure changes (Kutýk *et al.* [10]). Moreover, in line of this, Abdel-Kader [11] examined the effect of different chilling periods on *Lupinus termis* seedlings. It was found that exposure of *Lupinus* seedlings to 4ºC for 2, 4 and 6h induced irreversible chilling injury through the increase in lipid peroxidation and electrolyte leakage.

Zhang *et al*. [12] studied the effects of low, non-freezing temperature on morphological, physiological, and ultrastructural traits of leaves of males and females *Populus cathayana* Rehd. They found that chilling (4°C) significantly inhibited H_2O_2 content, and ascorbate peroxidase (APX) activity in both male and female plants, whereas peroxidase (POD) and glutathione reductase (GR) activities decreased and thiobarbituric acid reactive substance (TBARS) content increased only in females. Moreover, activities of catalase, ascorbate peroxidase and glutathione reductase were decreased in the case of chilling sensitive rice cultivar [13], cucumber [14] and *Stylosanthes guianesis* [15] under chilling stress conditions. The redox active thiol group GSH that may be involved in the regulation of the cell cycle and may act as a defense compound against oxidative stress. It is strongly implicated in chilling tolerance, particularly in maize [16-20].

Apart from its function as a primary antioxidant, ascorbate (AsA) can also act as a secondary antioxidant [21]. Ascorbate could be able to reduce superoxide to hydrogen peroxide and also reacts with singlet oxygen at a relatively fast rate. During the mitogen-activated protein (MAP) reaction, hydrogen peroxide is quenched by ascorbate peroxidase when using ascorbate as a hydrogen donor. Furthermore, ascorbate seems to recycle α-tocopheroxyl radicals to α -tocopherol [22] and inhibits the peroxy radical-initiated oxidation of methyl linoleate [23].

A numerous growing evidences have suggested that Pas are not only involved in the regulation of plant developmental and physiological processes [24-25], but also play important roles in modulating the defense response of plants to diverse environmental stresses [26-28], including salt (29-30), metal [31], oxidative stress (32-33), drought [34] and chilling stress [35,36].Therefore, genetic manipulation of crop plants with genes encoding enzymes of polyamine biosynthetic pathways may provide better stress tolerance to crop plants. Furthermore, the exogenous application of PAs is also another option for increasing the stress tolerance potential in plants [37].

PAs such as spermidine (Spd) and spermine (Spm) occur ubiquitously in plants, together with their diamine precursor putrescine (Put) [38]. It has been found that chilling-tolerant plants increase their endogenous PA levels in response to chilling effect to a greater extent than chilling-sensitive ones [39]. Shen *et al.* [40] have shown that, during chilling process, Spd content in leaves markedly increases in cold-tolerant cucumber cultivars, but not in sensitive cultivars. Besides, He *et al.* [41] have shown in spinach, a cold-tolerant plant, that inhibition of Spd synthesis increased photoinhibition effect. These findings indicate the involvement of PAs in the chilling tolerance of plants [27].

PAs have been reported as efficient antioxidants for many experimental systems, exerting effect through the protection of cellular components such as cell membranes, nucleic acids and polyunsaturated fatty acids from the oxidative damage [42]. It is believed that these polycationic molecules stabilize cellular membranes, thereby minimizing changes in permeability and loss of fluid [40]. Exogenous polyamines could reduce stress symptoms caused by ozone [43], and increase the tolerance of vegetables to low temperatures [44].

The present study was carried out to investigate the effect of chilling stress on antioxidant defense system of two wheat varieties (Sids1 and Gemmiza7) cultivated in Egypt. It was aimed also to evaluate the possible role of polyamines (PAs) pretreatment in alleviating chilling injuries.

Materials & Methods

1. Plant Material

Two newly released cultivars of wheat (*Triticum aestivum* L.) were selected: Sids-1 and Gemmieza-7. The seeds of both cultivars were surface sterilized with 2.5% sodium hypochlorite for 5 minutes then washed well with distilled water. The seeds were grown in plastic pots (25cm in height and 20cm in diameter) equally filled with peat-moss. Each pot contained typically 20 seeds. All pots were watered up to saturation, kept in a greenhouse under natural

photoperiod (10: 14 light/dark) and a temperature regime of 21/15°C (day/ night). They were irrigated regularly every two days until treatment. Thinning was carried out after 7 days from germination so that ten seedlings of similar growth rates were left per pot. Fifteen days post germination; the seedlings of each cultivar were randomly divided into the following three groups:

1.1.Negative control treatments (5 pots)
The seedlings of both cultivars were left in the greenhouse at 21/15°C (day/ night) and 10: 14 light/dark periods all over the experimental period. Plants were harvested after 25 days post germination.

1.2.Positive control treatments (direct chilling, 10 pots, 5 for each treatment)
Fifteen-day-old seedlings of each cultivar were transferred to a dark incubator at 5°C for 6 and 9 hours. At the end of the chilling period, the treated plants were returned to the pre-experimental conditions in the greenhouse.

1.3.Polyamine treatments (30 pots, 5 for each treatment)
Fifteen-day-old seedlings of the wheat cultivars under investigation were divided into three sub-groups sprayed with 0.5mM, 1.0mM and 2.0mM spermidine (spermidine trihydrochloride, Sigma, analytical grade). After exactly 12 hours of polyamine pretreatments, the seedlings were incubated at 5°C for 6 and 9 hours. At the end of the chilling period, the treated plants were transferred to the pre-experimental conditions in the greenhouse where they have recovered for 10 days (25 days old).

2. Methods
2.1. malondialdehyde (MDA) accumulation
Lipid peroxidation was assayed spectrophotometrically using thiobarbeturic acid-malondialdehyde (TBA-MDA) assay [45] as a measure of lipid peroxidation.

2.2. Electrolyte leakage
Electrolyte leakage was determined according to Dionisio-Sese and Torbita, 1998 [46] using a Chemtrix type 700 portable conductivity meter.

2.3. Determination of hydrogen peroxide level
H_2O_2 concentration of the treated plant's leaf was measured by the FOX I method [47] based on the peroxide-mediated oxidation of Fe^{2+} with xylenol orange.

2.4. Enzymatic antioxidants
Enzyme extracts were prepared by homogenizing wheat leaves in a pre-chilled mortar containing 20 ml chilled extraction phosphate buffer (pH 7.5). Extracts were then centrifuged at 6000 rpm for 20 min at 5°C. Enzyme assay was conducted immediately following extraction. Catalase activity was assayed as described by Aebi [48]. Activity was determined by following the decomposition of H_2O_2 at 240 nm. Peroxidase activity was determined by following the dehydrogenation of guaicol at 436nm [49]. ASPX activity was determined using the method of Nakano and Asada [50]. Activity was determined by following the H_2O_2- dependent decomposition of ascorbate at 290 nm.

2.5. Non-enzymatic antioxidants
Glutathione content was determined spectrophotometrically according to the method described by [51]. Free ascorbate was assayed photometrically following Frank [52] through the reduction of 2, 4-dichlorophenolindophenol (DCPIP). Carotenoids were estimated in the fresh mung bean leaves according to the procedure of Metzner *et al.* [53].
2.6. Analysis of Polyamines
Polyamines were extracted and determined according to Mietz and Karmas [54].
Statistical analyses:

Statistical analyses were performed using SPSS statistical package (SPSS Inc., Version 11.5) and Microsoft Excel professional 2003. Significant differences among treatments were tested by analysis of variance at 0.01 probability level. The data were expressed as mean ±SE (n = 3) of samples.

Results

Chilling stress increased lipid peroxidation, electrolyte leakage and H_2O_2 levels significantly for both cultivars (Figures 1-3, $p<0.01$) at both exposure durations. In addition, prolonged exposure to chilling stress increased chilling injury parameters. The application of Spd decreased the above parameters and this decrease was positively proportional with Spd increasing concentration. The effect of Spd was much more pronounced with Sids 1 than Gemmiza 7.

3.1. Antioxidant enzyme activities
Significant reduction of catalase, peroxidase and ASPX activities were observed at both cultivars subjected to the two chilling periods (fig. 4-6). The lowest levels of peroxidase and ASPX were observed at Gemmiza 7 subjected to 9-h chilling period (Figs. 5& 6). Spd application increased catalase, peroxidase and ASPX activities at both cultivars when compared to positive control.

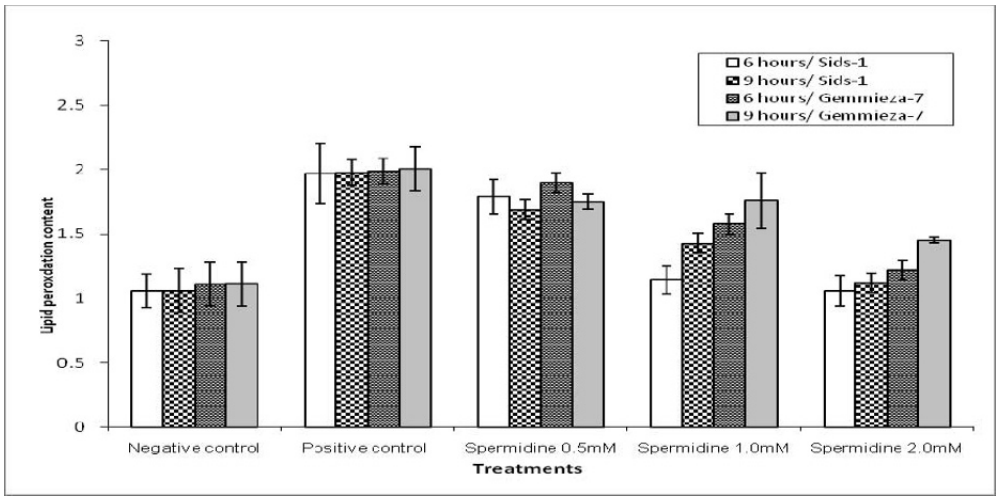

Fig.1. Lipid peroxidation as malondialdehyde content (MDA) expressed as µmole/g fresh wt. in wheat seedlings. Fifteen-days-old seedlings were pretreated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pretreated seedlings were chilled for 6 and 9 hours at 5ºC. Then, half of the treated seedlings were transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

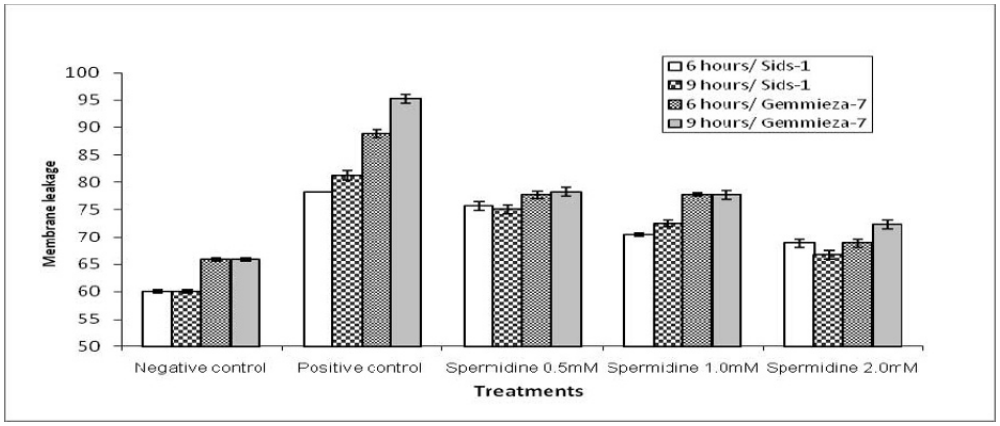

Fig.2. Electrolyte leakage expressed as (%) fresh wt. in wheat seedlings. Fifteen-days-old seedlings pretreated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pretreated seedlings were chilled for 6 and 9 hours at 5ºC. Then transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

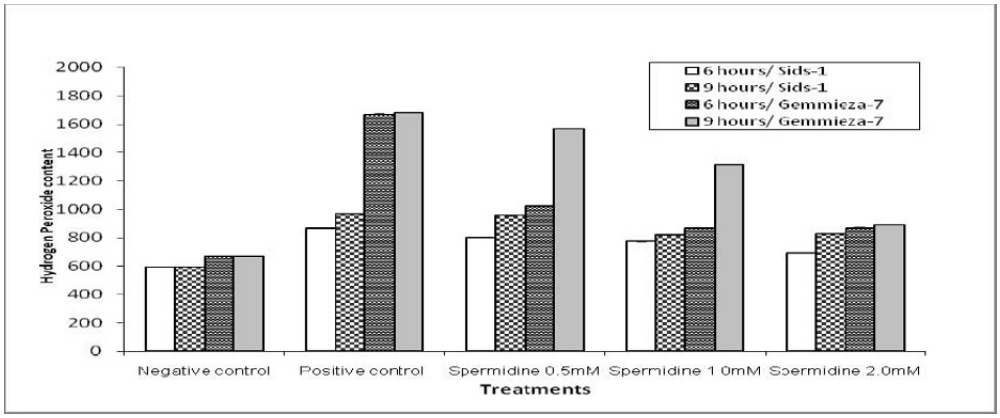

Fig.3. H_2O_2 content expressed as µmole/g fresh wt. in wheat seedlings. Fifteen-days-old seedlings pretreated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pretreated seedlings were chilled for 6 and 9 hours at 5ºC. Then transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

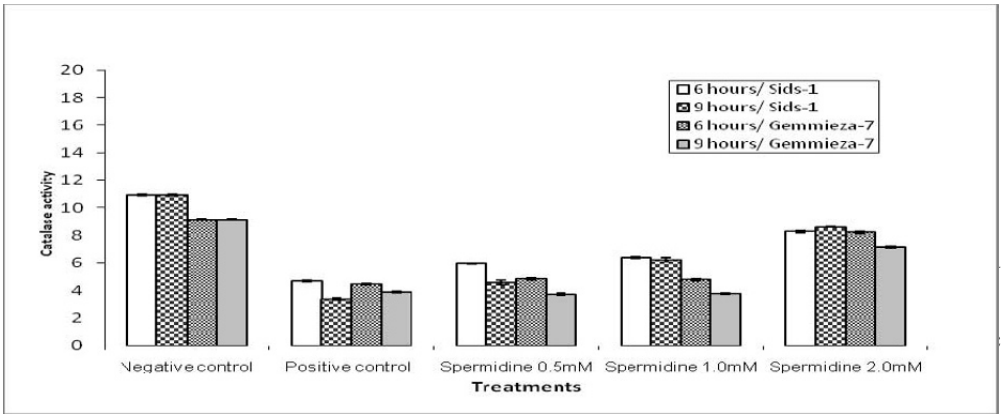

Fig.4. Catalase activity expressed as unit/mg protein in wheat seedlings. Fifteen-days-old seedlings pre-treated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pre-treated seedlings were chilled for 6 and 9 hours at 5ºC. Then transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

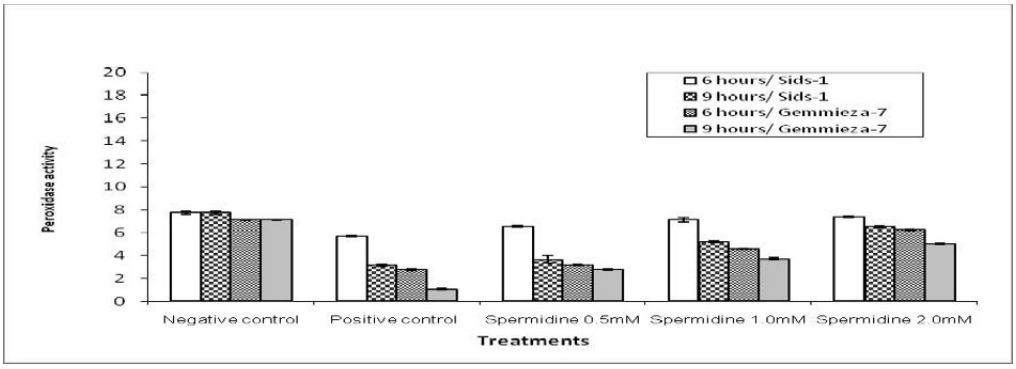

Fig.5. Peroxidase activity expressed as unit/mg protein in wheat seedlings. Fifteen-days-old seedlings pretreated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pretreated seedlings were chilled for 6 and 9 hours at 5ºC. Then transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

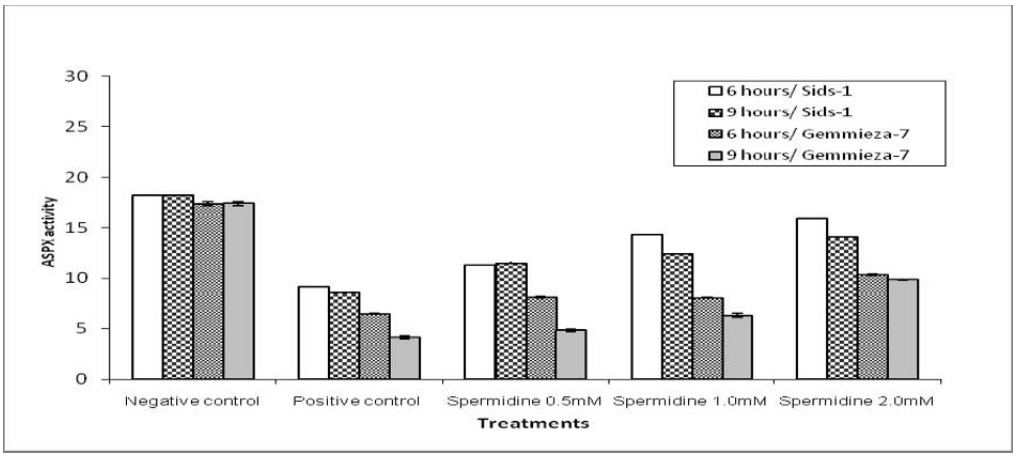

Fig. 6. ASPX activity expressed as unit/mg protein in wheat seedlings. Fifteen-days-old seedlings pre-treated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pre-treated seedlings were chilled for 6 and 9 hours at 5ºC. Then, half of the treated seedlings were transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

3.2. Glutathione (GSH) content

The glutathione content increased significantly in response to chilling stress when compared to negative control (Fig. 7). The increase of glutathione was time dependent. Pre-treatment of Sids-1 seedlings with 1.0mM Spd increased GSH content at both chilling periods in comparison with positive control. In Gemmieza-7 seedlings, 2.0mM Spd achieved the highest glutathione content values in comparison with positive control.

3.3. Free ascorbate content

The data (Fig. 8) show that chilling stress induced significant increase free ascorbate content when compared to the negative control. Application of Spd reduced free ascorbate content in Sids-1 compared to that of the positive control. Spd at 1.0mM concentration was the most effective in the reducing of the ascorbate content.

3.4. Carotenoids content

The data (Fig. 9) showed that the chilling stress induced a significant decrease in the carotenoids content in wheat seedlings when compared to the negative control for both cultivars. Application of Spd increased the carotenoids content for both cultivars. The effect of Spd on the carotenoids was concentration dependent.

3.5. Polyamines content

The data (Table 1) showed that chilling stress induced an increase in the total polyamines content for both wheat cultivars when compared to negative control. In case of Sids-1 cultivar, the percentage of increase was 124.63 and 140.29 % in the seedlings exposed to 5ºC for 6 or 9 hours respectively. In the other cultivar (Gemmieza-7), the increase was 126.61 and 133.59 % under the same conditions. Pre-treatment with different concentrations of Spd increased total polyamines over that of the negative and positive control. The highest increase in total polyamines was observed at 2.0mM Spd in both cultivars under study which were chilled for 9 hours.

Discussion

Chilling, low but non-freezing temperature is one of the most severe abiotic stress factors, restricting plant growth and productivity worldwide. Injuries caused by chilling stress usually include immediate mechanical constraints, activity changes of macromolecules, reduced osmotic potential in the cellular milieu [55], and significant alternation of other cell components [56]. In addition to the ultrastructural changes, chilling also results in a series of physiological, biochemical and molecular modification, such as the photoinhibition of photosystem I (PS I) [57] and increased hydrogen peroxide (H_2O_2) accumulation in chilled leaves [58].

In the present study, electrolyte leakage (Fig. 1) and lipid peroxidation level (Fig. 2) of wheat plants increased under chilling conditions. Zhou *et al*. [15] and [59] found that electrolyte leakage and lipid peroxidation level increased in *Stylosanthes guianensis* and mung bean plants under chilling stress. Several studies indicate that hydroxyl radicals can directly cause cell wall loosening or breakage through oxidation of cell wall polysaccharides and this process occurs *in vivo* through controlled release of hydroxyl radicals [60, 61]. Campos *et al*. [62] reported that the high electrolyte leakage in coffee was mainly due to lipid degradation. Furthermore, MDA production after chilling indicated the occurrence of lipid peroxidation. Application of Spd in the present study reduced electrolyte leakage and lipid peroxidation (Figs. 1&2). Similar results were obtained by [63] who found that exogenous PAs could suppress effectively the electrolyte leakage and the increase of MDA content caused by chilling stress in both chilling sensitive and tolerant cucumber cultivars. They suggested that PAs might influence membrane oxidation system to enable plants to be adaptive to chilling stress. It has been well established that PAs play important roles in defence of plants against diverse environmental stresses [28, 36]. The effect of PAs in plant stress response could be exerted directly or via acting as scavengers of reactive oxygen species (ROS) [36, 64, 65], which is considered primarily responsible for lipid peroxidation of cellular membranes caused by environmental stresses. Moreover, radical scavenging ability of polyamines is due to their phenolic hydroxyl groups [66]. Polyamines being cationic in nature can associate with anionic components of membrane such as phospholipids thereby stabilizing the bilayer surface and retarding membrane deterioration. [67]. Moreover, Ammoaghaie and Moghym [68] showed that polyamines increased membrane stability and thermotolerance of seedling soybean

Because different forms of reactive oxygen, such as superoxide anion, singlet oxygen, hydroxyl radicle, or hydrogen peroxide (H_2O_2), are produced in specific sub cellular locations by distinct stimuli and then interact with distinct ROS sensors and signal transduction pathway components in each sub cellular compartment, changes in plant cellular redox state are well poised to modulate cellular responses to changing environmental conditions [69] as well as endogenous developmental processes [70, 61]. The results of the present study revealed that chilling stress induced a significant increase in H_2O_2 level of wheat seedlings. These data are in accordance with those obtained by [7, 59] who stated that chilling could cause H_2O_2 accumulation in *Zea mays* and mung bean plants. The increase in H_2O_2 levels could be attributed to decreased activity of key antioxidant enzymes. Also, H_2O_2 was shown to be produced through the degradation of polyamines by polyamine oxidase [71]. Chilling causes not only the changes of membrane structure of plants [72], but also the decreased activity of oxidant enzymes [14]. Antioxidant enzymes constitute the cellular defence against oxidative stress; therefore function importantly in protection of plants

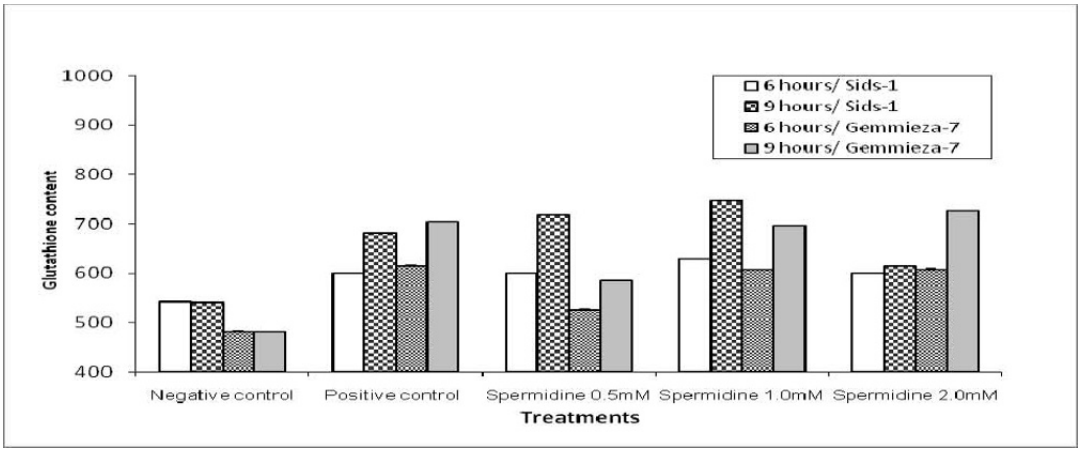

Fig.7. Glutathione content expressed as µg/g fresh wt in wheat seedlings. Fifteen-days-old seedlings pre-treated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pre-treated seedlings were chilled for 6 and 9 hours at 5ºC. Then transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

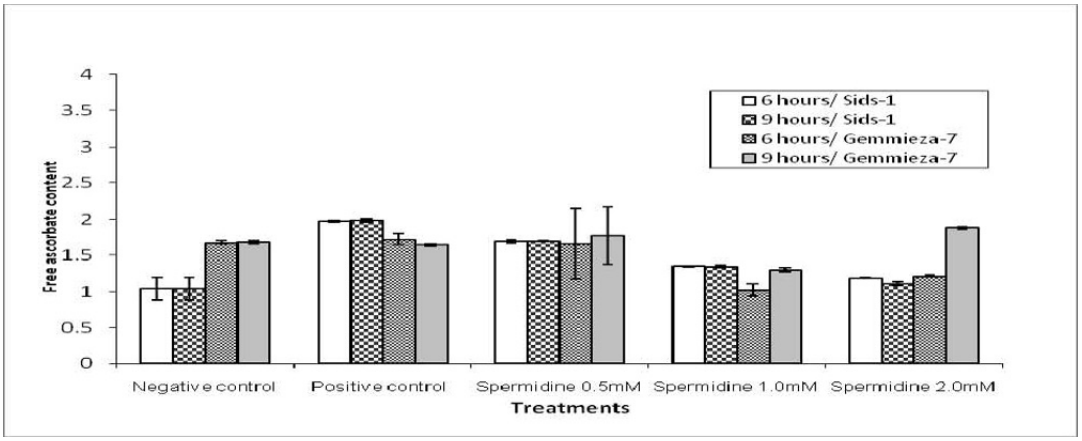

Fig. 8. Free ascorbate content expressed as mg/g fresh wt in wheat seedlings. Fifteen-days-old seedlings pre-treated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pre-treated seedlings were chilled for 6 and 9 hours at 5ºC. Then transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE

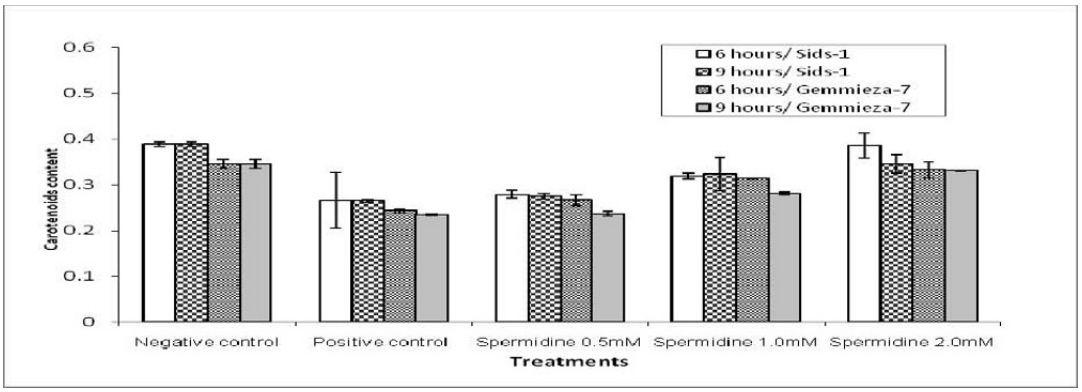

Fig. 9. Carotenoids content expressed as µg/g dry wt. in wheat seedlings. Fifteen-days-old seedlings pre-treated with 0.0, 0.5, 1.0 and 2.0mM Spd. The pre-treated seedlings were chilled for 6 and 9 hours at 5ºC. Then transferred to the pre-experimental conditions in the greenhouse, where they have recovered for 10 days. Data represented as mean of 3-replica ± SE.

Table1. Polyamines content expressed as ppm in wheat seedlings subjected to 5ºC for 6 and 9 hours. Fifteen-days-old seedlings pre-treated with 0.0, 0.5, 1.0 and 2.0mM Spermidine. The pre-treated seedlings were chilled for 6 and 9 hours at 5ºC.

Treatment	Polyamines content (ppm)			
	Sids-1		Gemmieza-7	
	6 hours	9 hours	6 hours	9 hours
Negative control	67.0	67.0	38.7	38.7
Positive control	83.5	94.0	49.0	51.7
Spermidine 0.5mM	104.6	102.6	63.1	59.0
Spermidine 1.0mM	121.5	133.3	92.0	101.9
Spermidine 2.0mM	180.8	195.2	137.8	162.1

from stress-induced damage [58, 33, 30, 36].

The present work showed that chilling stress induced a significant decrease in catalase, peroxidase and ascorbate peroxidase activities in wheat seedlings. However, spermidine treatments increased the activities of the mentioned enzymes when compared to non treated chilled plants. A large body of evidence has shown that the antioxidant enzyme systems are altered under abiotic stresses, including chilling [73]. Zhang *et al*. [63] found that exogenous application of Put and Spd could effectively prevent cucumber seedlings from chilling injury by inducing activities of several antioxidant enzymes other than NADPH oxidase, including SOD, POD, APX and CAT. These results strongly suggest that PAs, especially Put and Spd, act as antioxidant machinery to prevent chilling injury through counteracting the oxidative stresses imposed on chilled leaves of cucumber. High levels of PAs could confer plant tolerance to abiotic stress by acting as direct ROS scavengers or binding to antioxidant enzyme molecules to scavenge free radicals [58, 33, 30, 36].

The present work showed that chilling stress induced a significant increase in glutathione (GSH) and free ascorbate content in wheat seedlings. Accumulation of GSH during stress has been reported during chilling stress in zucchini [74], maize [16], Arabidopsis [75] and *Lupinus termis* [11]. Changes in glutathione pool size during chilling stress are the result of its altered synthesis or transport. Chilling increases the amount of cysteine, the end product of assimilatory sulphate reduction, in maize [76]. The amounts of the other precursors of GSH, glutamate and glycine, also increase in maize grown at 5°C [2] and γ-glutamylcysteine synthetase (γ-EC synthetase) [16], the key enzymes of GSH synthesis. Ascorbic acid/dehydroascorbic acid and glutathione (GSH)/glutathione disulfide act as redox buffers to regulate cellular responses to changes in environmental conditions [77]. The status of the GSH and ascorbic acid pools is generally regarded as a reliable proxy for the overall redox state of a particular tissue, cell, or cellular compartment.

Plants responded to the low temperature stress by reducing their chlorophyll content, increasing their ratio of total carotenoids to chlorophyll and by the accumulation of large amounts of zeaxanthin and antheraxanthin [78]. This leads to a reduction in the absorption of excitation energy on one hand and to reduce the redox state of plastids. Signaling from the plastids to other cellular compartments, especially the nucleus is likely achieved through multiple pathways, and the plastid redox state has been established as a central upstream signal in intracellular coordination of stress responses [77]. It was found that inhibition of photosynthesis during chilling stress is related to restriction of intercellular transport via plasmodesmata (PD), further connecting plastid physiology to regulation of PD [79].

Chilling stress, in the present study, increased the total polyamines content in both wheat cultivars under investigation when compared to negative control. Also, polyamine treatments induced a significant increase in PAs contents in both wheat cultivars (Table, 1). Similar results have been reported by [40] who provided evidence that PAs, Spd in particular, are involved in the chilling tolerance of cucumber cultivars. They showed that in a chilling-tolerant cultivar of cucumber, synthesis of Spd was increased in leaves during chilling treatment, while it was not in a chilling-sensitive cultivar. Moreover, [41] suggested that adding Spd to the growth medium, prior to cold exposure, significantly enhanced Spd content in all organs of cucumber and resulted in higher cold tolerance, even if their polyamine pools significantly decreased during chilling treatment. Kubiś [80] stated that some exogenous Spd are not degraded, but enters as a whole into the cell, enhancing the Spd content, while the other part is metabolized into spermine, and an interconversion pathway for putrescine biosynthesis was also confirmed.

The increase of the sum of Put, Spd, and Spm during chilling may well be ascribed to enhanced ornithine decarboxylase (ODC) activity. High level of endogenous PAs is a major factor responsible for tolerance of chilling stress. Zhang *et al*. [63] found that during exposure to chilling, the chilling-tolerant cv. *Changchun mici* exhibited high levels of free Put, Spd and Spm, whereas chilling-sensitive cv. *Beijing jietou* did not, except a slight increase of Put at late time point in leaves of the latter. Moreover, Çakmak and Atici [81] showed that putrescine may act as an agent inducing primary changes in the apoplastic antioxidant system of wheat leaves during reactive oxygen species-mediated damage caused by low temperature stress.

Conclusion

It could be concluded that wheat seedlings are sensitive to chilling stress. The chilling injuries were increased with increasing the chilling period. PAs, especially Spd, act as antioxidant machinery to prevent chilling injury through counteracting the oxidative stresses imposed on

chilled leaves of wheat cultivars. Also, PAs may modulate directly or indirectly plant defence response to chilling stress through regulation of H_2O_2 production. The high level of endogenous PAs could be considered as a major factor responsible for tolerance of the chilling-sensitive wheat cultivars. This fact alone implicates the possibility to modify the capacity of wheat tolerance and create wheat traits with improved chilling-tolerance to chilling through engineering high PA content species.

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