Membrane fatty acid compositions and cold-induced responses in tetraploid and hexaploid wheats

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Abstract Plant cells often increase cold tolerance by reprogramming their genes expression which results in adjusted metabolic alternations, a process enhanced under cold acclimation. In present study, we assessed the changes of membrane fatty acid compositions along with physio-biochemical indices like H_2O_2 and malondialdehyde (MDA) contents and lipoxygenase (LOX) activity during cold stress (CS) phases in acclimated and non-acclimated durum (SRN and Gerdish) and bread (Norstar) wheat genotypes. During thermal treatments, MDA was an end product of lipid peroxidation via oxidative stress (H_2O_2 content) rather than LOX activity. LOX activity plays a double role in mechanism of cold tolerance in wheat, particularly at severe stress. With increase in severity of CS especially in non-acclimated plants, LOX activity decreased along with an increase in MDA and other responses helped increase or maintaine unsaturated fatty acids (FAs) whereas in acclimated plants (moderate CS), increasing of LOX activity along with a decrease in MDA indicates probably its role in secondary metabolites like jasmonic acid signaling pathway. Significant increase of total FAs and particularly unsaturated FAs showed distinct cell endeavor to protect against CS in Norstar and Gerdish compared to SRN genotype. Results showed that an increase in double bond index and LOX activity and low MDA under CS could be reasons for plant cold tolerance.

Keywords Damage index · Durum · Cold acclimation · Cold responses · Fatty acids · Wheat

Abbreviations
DBI	Double bond index
CA	Cold acclimation
CS	Cold stress
FA	Fatty acid
FAMEs	Fatty acid methyl esters
FM	Fresh mass
GC	Gas chromatography
JA	Jasmonic acid
LOX	Lipoxygenase
MDA	Malondialdehyde
NBT	Nitro blue tetrazolium
ROS	Reactive oxygen species
TCA	Trichloroacetic acid
UFAs	Unsaturated fatty acids

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Introduction

Cold stress (CS), like other types of abiotic and biotic stresses, induces oxidative processes in plant cells. These processes are initiated by reactive oxygen species (ROS), which interact nonspecifically with many cellular components, triggering peroxidative reactions and causing

significant damage to essential macromolecules, such as photosynthetic pigments, proteins, nucleic acids, and lipids, and especially damaging the membranes [1]. Although the high level of ROS is potentially harmful to plant cells, its production during CS could have a role in stress perception and protection [2]. Plasma membrane is the primary cell site that plays a crucial role in the perception, transduction and transport of environmental signals [3]. Environmental changes affect the functions of cell membranes and as a result either the cell adapts to these changes or it would kill it [4]. Toxic hydrogen peroxide (H_2O_2), commonly taken as an indicator of oxidative stress, is the most stable form of the ROS due to capability of rapid diffusion across cell membrane and also influencing the level of lipid peroxidation [5, 6]. Malondialdehyde (MDA), one of the final products of lipid peroxidation of unsaturated fatty acids (UFAs) can also be considered as an evaluation factor of membrane damage. On the other hand, lipoxygenases (LOXs) are responsible for membrane degradation because they catalyze the dioxygenation of UFAs producing hydroperoxy fatty acids which are toxic to the cell. For adapting to these changes, organisms use reprogramming of gene expression which results in changing lipid and fatty acid (FA) membrane compositions and generally in the adjusted metabolic alterations [3, 7]. Such processes were enhanced by a phenomenon known as cold acclimation (CA) and could be different in tetraploid (durum) wheat with A and B genomes and in hexaploid (bread) wheat with A, B and D genomes. Increasing evidences have suggested that CA and conditioning nearly in every aspect of plant structure prior to CS reduce induced damages [8–11]. It is possible that different genomic structures can be potentially participated in cellular redox reactions. It can be interesting to plant physiologists to study the effects of CS in durum and bread wheat genotypes following CA. These differences probably afford plants with cold tolerance; however the degree of tolerance is a genotype-based trait.

In Iran, the area under durum wheat cultivation is about 400–500 thousands hectares with an annual production of 400–500 thousand tons, which covers about 60 % of country demands. In spite of the importance of durum wheat for Iranian rural economies, the country has not been successful in its research and development efforts to substantially improve durum wheat productivity. The combinations of increasing demand for durum and durum products, as a result of demographic pressure, and relatively low durum productivity partly due to abiotic stresses like CS made the country to be an importer of durum [12, 13]. The extent of damages due to winterkill in durum wheat vary greatly from region to region and from year to year. Because of the inherent sensitivity of durum wheat to CS, there has been a great interest in new varieties that are

tolerant to CS. Most often, CS affect autumn-sown wheat during winter. Less often, CS can occur during late frosts in spring, causing leaf or spike injury. Due to the limited genetic variation for CS, conventional breeding efforts have been directed mostly to escape frost by selecting for later flowering. Cold-resistant durum varieties can be used as a promising gene pool for extending the growing season and geographical range of durum appropriate for planting in high (cold) regions, where other durum genotypes do not have successful performance. Gerdish plants as a durum wheat is cultivated in some regions of West of Iran as an alternative wheat plant. However, available information on physiological, biochemical and molecular studies of its cold tolerance is still limited. These studies could help in identifying differences associated with the capability of each genotype to cope with CS because understanding the mechanisms underlying a specific stress is also essential for the later strategies to be applied. In order to evaluate durum genotypes (selected from previous studies) more precisely, SRN and Norstar genotypes were used as a sensitive durum wheat and tolerant bread wheat, respectively. The objectives of this study were to compare the changes in cellular responses induced by CS after CA among these genotypes.

Materials and methods

Plant material and growth conditions

Seeds of Norstar (bread wheat), and two genotypes Gerdish and SRN (durum wheat) provided by Dryland Agriculture Research Institute (DARI) of Iran were sterilized with 10 % sodium hypochlorite for 5 min, soaked in distilled water, and then germinated in Petri dishes on filter paper for 72 h at 25 °C in a thermostat. Subsequently the seedlings were planted in pots containing soil, sand, and farmyard manure. Plants were grown in a growth chamber at 25 °C, an irradiance of 200 μmol m^{-2} s^{-1} from white light luminescent lamps, a 16 h photoperiod, and 75 % relative humidity for 14 days. Plants were irrigated with distilled water as needed. Although there is an overlap among species, genotypes of bread wheat (*Triticum aestivum* L.) have the higher cold tolerance compared to durum wheat [14] especially when CS followed by long-term CA. We used CA in order to assess cold tolerance of genotypes profitably in a short time [15] because, few studies have been reported on their physiological properties under CS after short-term CA (briefly, CA). The genotypes, showing the capacity of cold tolerance after short-term CA, can make plants ready to encounter lower temperature in environmental conditions. The cooling regime adopted in our experiments allowed us to differentiate the examined genotypes in terms of their tolerance to CS. In our

experiment, plants are moved from control conditions into acclimated temperature 4–5 °C for 14 days with the same photoperiod and irradiance. Leaf samples of genotypes were harvested and analyzed after 7 and 14 days under these conditions. After 14 days CA, plants were placed into a climatic chamber chilled preliminary to 0 °C. During further treatment, the temperature was lowered gradually to −5 °C (at the rate of 0.5 °C min⁻¹), and the plants were incubated at this temperature for 1 day. Non-acclimated plants were transferred from control conditions to CS at −5 °C. Plants under control conditions remained at 25 °C. The cooling regime (i.e., the combination of temperature and incubation period) was chosen in preliminary experiments. In various treatments, leaves were harvested immediately after removing the plants from the cold exposure room for physiological analysis. All measurements were made on 2 cm of the middle parts of the first leaves of wheat seedlings. The seedlings were considered to be of similar physiological age [16].

Estimation of the H_2O_2 content

H_2O_2 content was determined according to Loreto and Velikova method [17]. 0.35 g of the leaf fragments were ground under liquid nitrogen using a mortar and pestle and then were homogenized in an ice bath with 5 ml of 0.1 % trichloroacetic acid (TCA). The homogenate was centrifuged at $12,000 \times g$ for 15 min and 0.5 ml of the supernatant was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M Potassium iodide. The absorbance of the supernatant was measured at 390 nm in a spectrophotometer (Shimadzu UV-160, Shimadzu Corporation, Kyoto, Japan). The content of H_2O_2 was calculated by comparison with a standard calibration curve previously made by using different concentration of H_2O_2 and expressed in μmol g⁻¹ fresh mass (FM).

Lipid peroxidation (MDA) analysis

The measurement of lipid peroxidation in leaves, the thiobarbituric acid test, which determines MDA as an end product of lipid peroxidation, was assessed according to Heath and Packer method [18]. 250 mg FM leaflets were hemogenated in 2 ml extraction buffer TCA 1 % (w/v), centrifuged at $13,000 \times g$ for 15 min. One ml of the supernatant was added to 2 ml of 5 % (w/v) thiobarbituric acid in 20 % (w/v) TCA. The mixture was incubated in boiling water for 30 min and the reaction was stopped by placing the samples in an ice bath. Then the samples were centrifuged at $10,000 \times g$ for 10 min and the absorbance of the supernatants was measured at 532 nm with a spectrophotometer. The amount of MDA was calculated using: C = D/EL, where C is the concentration of MDA, D is the optical density, E is the coefficient of molar extinction (1.56×10^5 cm⁻¹ M⁻¹); and L is the thickness of the layer of solution in the vessel (1 cm). Content of MDA was expressed in μmol g⁻¹ FM.

Assay of LOX activity (EC-number: 1.13.11.12)

0.1 g FM leaflets was suspended in 2 ml extraction buffer (0.1 M Tris–HCl pH 8.5 consisting of polyvinylpyrrolidone (PVP; w/v) 1 %, CaCl₂ 1 mM, DTT 5 mM and 15 % glycerol), homogenized and centrifuged at $11,000 \times g$ at 4 °C for 20 min and the supernatant was removed for activity assay. LOX was assayed according to Axelroad et al. [19]. Linoleic acid (C18:2) was used as substrate. Substrate solution was prepared by mixing 50 mg Tween 20 (w/v), 10 ml Na₂HPO₄ buffer (pH 7.8) to 50 mg C18:2, by stirring and ultrasonic dispersion and being cleared by adding 20 μl NaOH and diluted to 25 ml with the buffer. The reaction was started by mixing 0.95 ml Na₂HPO₄ buffer, 50 μl supernatant and 5 μl substrate. The increase in absorbance of the solution was measured at 234 nm for 7 min using spectrophotometer. LOX specific activity was reported as ΔA_{234} min⁻¹ mg⁻¹ protein for each sample.

Extraction and analysis of fatty acids (FAs)

Total lipids were extracted according to the modified method of Bligh and Dyer [20]. One g leaf sample was homogenized in glass tubes and extracted with 15 ml chloroform:methanol 2:1 (v/v) and vortexed. 5 ml distilled water was added before final centrifugation. The lower phase was collected and dried under a stream of N₂. The lipid samples were processed for FA analysis following the methods described by Metcalf et al. [21]. The FAs of isolated lipids were methylated into reaction vials by refluxing with sodium methoxide (2 %) for 10 min at 100 °C and then were transmethylated by refluxing with 2.175 ml boron trifluoride methanol 14 % for 3 min at 100 °C. The fatty acid methyl esters (FAMEs) were extracted from the reaction vials three times with hexane, and then concentrated. The FAMEs were analyzed on gas chromatography using a Unicam 4600 series (Unicam Limited, Cambridge, UK) equipped with a BPX70 capillary column (0.25 mm × 30 m, 0.2 mm film thickness), a flame ionization detector. The column oven temperature was set at 160 °C for 5 min, then increased to 180 °C at 20 °C min⁻¹ and was held for 9 min, then increased to 200 °C at 20 °C min⁻¹. Components were identified by comparisons of retention times and peak curves with authentic standards. Double bond index (DBI) was calculated according to the formula of Orlova et al. [22]:
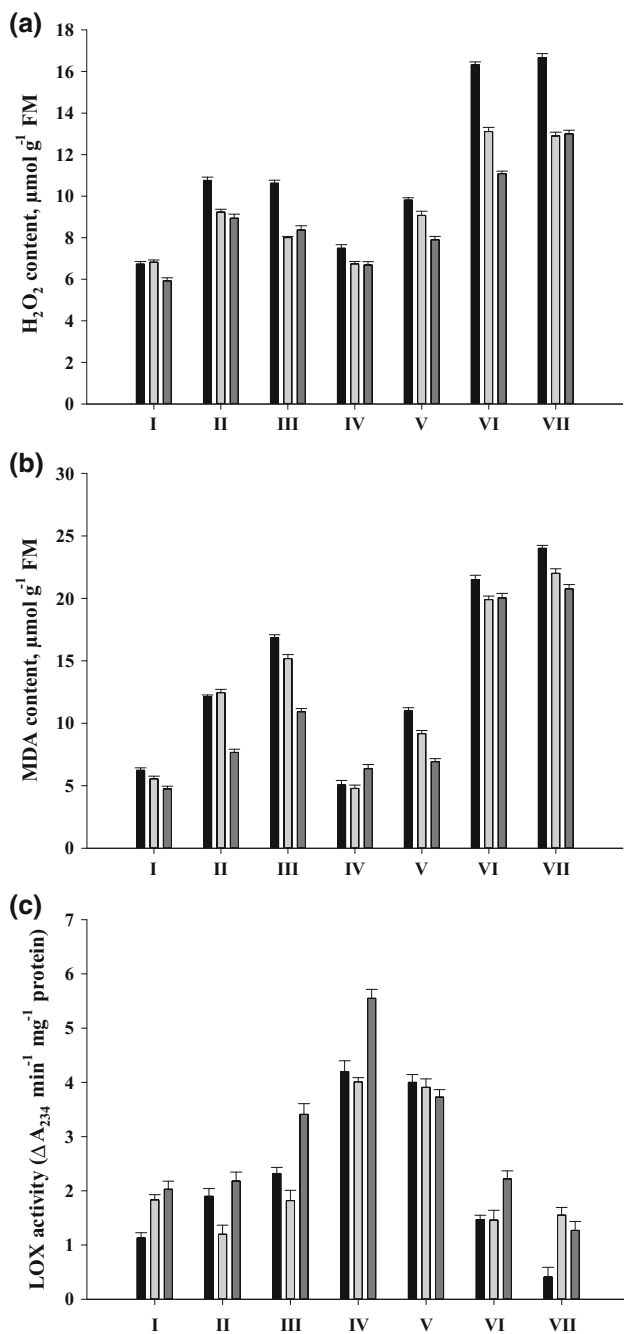
Fig. 1 Effects of thermal treatments on H_2O_2 (**a**) and malondialdehyde (MDA) (**b**) contents and lipoxygenase (LOX) activity (**c**) in the leaves of wheat incubated during control (25 °C) (I), acclimation phase (4 °C) after 7 days (II) and 14 days (III), cold phase (-5 °C) after 12 h (IV) and 24 h (V) in acclimated plants and cold phase (−5 °C) after 12 h (VI) and 24 h (VII) in non-acclimated plants conditions. The error bars represent the standard deviation (± SD) for replicates. *Black*, *light grey*, and *dark grey bars* indicate SRN and Gerdish genotypes (durum wheat) and Norstar (bread wheat genotype), respectively

$$DBI = [(1 \times C16:1) + (2 \times C16:2) + (1 \times C18:1) \\ + (2 \times C18:2) + (3 \times C18:3)]/[100],$$

where 16:0 = palmitic acid, 16:1 = palmitoleic acid, 16:2 = hexadecadienoic acid, 18:0 = stearic acid, 18:1 = oleic acid, 18:2 = linoleic acid, and 18:3 = linolenic acid.

Statistical analysis

Experimental data of physiological and biochemical sections of this research were analyzed by SPSS.19.0 computer software on the basis of randomized complete design (RCD) with three replicates. The treatment means were compared using Duncan's multiple range tests.

Results

H_2O_2 content

The H_2O_2 content has shown significant differences among treatments. Under control conditions, genotypes showed a significant difference with respect to H_2O_2 content, so that its content in Norstar was lower. Transferring seedlings from control conditions to CA phase caused a significant increase in H_2O_2 content in all genotypes especially in SRN. CS in acclimated plants induced a decreasing (after 12 h) and an increasing trend (after 24 h) in H_2O_2 content. CS in non-acclimated plants increased H_2O_2 content particularly in SRN genotype (Fig. 1a).

MDA

Comparing experimental data means of MDA content has shown significant differences in thermal treatments. Under control conditions, genotypes showed a significant difference with regard to MDA content, so that its content in Norstar was lower. In CA phase, MDA content significantly increased although this increase in Norstar and SRN genotypes were minimal and maximal, respectively. CS in acclimated plants caused a reduction in MDA accumulation whereas in non-acclimated plants, CS increased lipid peroxidation in all genotypes especially in SRN (Fig. 1b).

LOX activity

LOX activity in each of the thermal treatments has shown significant differences among genotypes. Under control

Table 1 Fatty acids (FAs) composition in leaf lipids of SRN and Gerdish genotypes (durum wheat) and Norstar genotype (bread wheat) subjected to the thermal treatments: control (25 °C), acclimation phase (4 °C) after 7 days and 14 days, cold phase (−5 °C) after 12 h and 24 h in acclimated plants and cold phase (−5 °C) after 12 h and 24 h in non-acclimated plants conditions

Fatty acids	Fatty acid content (µmol g⁻¹ FM)																				
	Control (25 °C)			Acclimation phase (4 °C)						Cold phase in acclimated plants (−5 °C)						Cold phase in non-acclimated plants (−5 °C)					
	Day 0			Day 7			Day 14			12 h			24 h			12 h			24 h		
	SRN	Gerdish	Norstar	SRN	Gerdish	Norstar	SRN	Gerdish	Norstar	SRN	Gerdish	Norstar	SRN	Gerdish	Norstar	SRN	Gerdish	Norstar	SRN	Gerdish	Norstar
C14:0	0.46	0.478	0.692	0.932	0.32	0.641	0.1	0.289	0.43	0.254	0.551	0.509	0.101	0.294	1.001	0.254	0.301	0.481	0.104	0.901	0.831
C16:0	1.604	1.099	1.139	0.734	1.009	1.01	0.497	0.199	0.425	0.664	0.86	0.537	0.233	0.709	0.907	0.9	0.65	0.523	0.121	0.708	0.974
C16:1	1.03	0.957	0.947	0.642	1.171	1.135	0.54	0.619	1.779	0.406	1.263	0.939	0.177	1.453	1.172	1.51	1.084	1.377	0.402	1.179	1.004
C16:2	0.281	0.25	0.928	0.907	1.301	1.437	1.614	0.416	0.956	1.168	0.593	1.722	0.344	1.169	0.528	0.568	0.315	1.721	0.925	1.393	1.153
C18:0	0.775	1.087	1.277	0.533	0.396	0.848	0.438	0.126	0.33	0.802	0.694	1.068	0.284	0.551	0.459	0.27	0.574	0.593	0.393	0.401	0.94
C18:1	1.031	1.586	1.712	1.016	2.596	1.929	0.927	2.228	3.861	3.429	1.504	4.098	2.674	2.636	2.966	1.382	2.37	3.113	1.476	1.994	2.047
C18:2	0.465	1.601	1.438	1.201	1.459	1.799	1.234	1.987	2.52	1.629	2.573	1.326	1.515	2.039	3.041	1.014	1.102	1.262	0.641	2.008	2.533
C18:3	3.727	4.576	6.798	7.488	7.794	9.566	9.642	9.596	12.718	13.182	16.042	21.125	9.903	11.82	15.52	9.685	10.896	13.175	6.731	6.859	9.193
C20:0	0.209	0.31	0.529	0.38	0.138	0.998	0.622	0.479	1.14	0.95	0.389	1.39	1.656	1.051	1.463	1.061	0.693	1.549	1.31	1.808	1.202

conditions, genotypes showed a significant difference with respect to LOX activity, so that its content in Norstar was higher. Assessing the effect of CS in acclimated and non-acclimated plants, LOX activity has shown increasing and decreasing trends, respectively (Fig. 1c).

Fatty acids

Along with the absolute content of lipids in wheat seedlings under thermal treatments, we determined the composition of their FAs. Nine FAs were identified in the leaves of wheat genotypes with the chain length from 14 to 20 carbon atoms (Table 1). The main parts of them were C16:0, C16:1, C16:2, C18:0, C18:1, C18:2 and C18:3. By transferring plants to 4 °C temperature, we have observed a decreasing trend in C16:0 in all genotypes compared to control plants, which were gradual in Gerdish plants and gently in SRN and Norstar genotypes. CS phase in acclimated plants induced an increase in Gerdish and Norstar and a decreasing trend in SRN. CS in non-acclimated plants caused a decrease in C16:0 in all genotypes especially in SRN. Acclimated plants compared to control plants, showed an increase in C16:1 content in Norstar and a significant decrease in SRN and Gerdish. CS in acclimated plants increased C16:1 in Gerdish and decreased it in SRN and Norstar. In non-acclimated plants, 12 h CS treatment induced an increasing trend in all genotypes but after 24 h, a decreasing trend especially in SRN was observed. In CA phase, C16:2 content compared to control plants increased in all genotypes. CS in acclimated plants caused a decrease in C16:2 in SRN and Norstar and an increase in Gerdish but, in non-acclimated plants, C16:2 increased in genotypes especially in Norstar. During CA phase, C18:0 significantly decreased especially in Gerdish whereas in acclimated plants, C18:0 showed an increasing and then decreasing trends. CS in non-acclimated plants decreased C18:0 in all genotypes compared to control plants. CA phase increased C18:1 content in Gerdish and Norstar while decreased it in SRN. CS in acclimated and non-acclimated plants increased C18:1 content in all genotypes. C18:2, C18:3 and C20:0 contents in thermal treatments in all genotypes showed an increasing trend.

Discussion

Nowadays, it is denoted that the modification of membrane lipid components has a crucial role in cell activities during CS [23, 24]. This capacity of plants to withstand CS is not constant but increases noticeably upon exposure to progressively lower temperatures known as CA. The results indicated that Norstar and then Gerdish showed a more satble physio-biochemical responses to CS than did SRN

genotype especially after CA phase. Durum and bread wheats are two distinct species with different genomes (tetraploid AABB, and hexaploid AABBDD, respectively [25]. It was supposed that such responses are distinct in these species due to the reprogramming of gene expression which results in adjusted metabolic alterations such as cellular osmotic stabilization, changing lipid and fatty acid membrane compositions and increasing antioxidants activities. [3, 7]. Like other stresses, CS induces oxidative processes in plant cells that are initiated by ROS production. Under CS conditions, the H_2O_2 level is very important, because it can promote the formation of hydroxyl radical, another and more toxic species of reactive oxygen [26]. The different levels of H_2O_2 in control conditions may be a sign of significant differences between two species wheat (durum and bread) so that more tolerance to CS in bread wheat may be predictable. Under CA phase, H_2O_2 content significantly increased in all genotypes which was a sign for perception of temperature change signal in genotypes. However, increased level of H_2O_2 in SRN plants was more than Gerdish and Norstar plants. This may refer to faster responses to 4 °C in SRN genotype which probably is one of the characteristics of sensitive plants under environmental conditions [27]. This point was also confirmed by the observed increase in H_2O_2 content during CS in acclimated and non-acclimated SRN plants. Results showed that CA in SRN plants occurred with less effect than that Gerdish and Norstar plants. Induction of freezing temperature for 12 h in acclimated plants caused significant decrease in H_2O_2 content and in that regards the lowest and the highest H_2O_2 contents belonged to Norstar and SRN, respectively. It seemed that a new equilibrium established by the cells in CA phase when ROS products (H_2O_2 content) served in other metabolism pathways as LOX, which will be explained in detail here. It was supposed that genotypes showed relative tolerance to freezing temperature in 12 h compared to 24 h CS treatment, which may be due to the activation of primary and secondary metabolites produced in CA phase. However, after 24 h CS in acclimated plants, an increase in H_2O_2 content was observed particularly in SRN genotype. Comparing acclimated and non-acclimated plants during freezing temperature revealed interesting points. Although H_2O_2 content in acclimated plants was lower than non-acclimated plants, its content in SRN and Norstar were the highest and the lowest, respectively that confirm previous data. These findings indicated that CA phase could trigger cellular pathways which was led to lower damage in Norstar and Gerdish plants compared to SRN ones. Also applied thermal treatments could screen sensitive and tolerant wheat genotypes. In order to verify this assumption, we measured the MDA content. Previous studies showed that higher susceptibility of some wheat genotypes to CS was

evidenced by MDA production and concentrations which commonly indicates the occurrence of lipid peroxidation [28–30]. MDA data analysis showed significant differences under control conditions among genotypes which were similar to H_2O_2 results. During CA phase, MDA content significantly increased probably due to an increase in ROS. Recent studies have shown that ROS increase as a signal can be involve in perception and alteration of cold tolerance gene expression in plants [3, 31]. The pattern of changes in MDA content in acclimated plants after freezing temperature was similar to H_2O_2 levels so that for 12 h it declined and then after 24 h it increased, but MDA contents in this treatment was lower than plants in CA phase. The decline in MDA level could possibly be associated with higher activity of defensive mechanisms such as decreasing of 16:0 and 18:0 and dramatic rise in 16:1, 16:2, 18:1, 18:2 and 18:3 in genotypes especially in Norstar, Gerdish. Our studies have shown that the decreased MDA content under CS was related to the enhanced UFAs in plants [7, 31]. And plants with higher levels of 18:3 showed less membrane injury. Thus increase in H_2O_2 content could not be due to oxidative stress in CA phase and may play as cellular defensive signal. Such results were observed in MDA data under CS in acclimated and non-acclimated plants so that increasing in MDA content during CS was higher in SRN compared to other genotypes after 12 and 24 h CS treatments.

During control and CA phases, LOX activity was higher in Norstar genotype unlike H_2O_2 and MDA contents and its minimum was observed in SRN genotype. It seemed that LOX activity plays more important role in Norstar and Gerdish for making plants tolerance in control and CA phases. As known, MDA may be an end product of lipid peroxidation via enzymatic (LOX) and non-enzymatic (mediated by ROS) reactions [32]. Comparing data of MDA and LOX activity after CS in acclimated and non-acclimated plants showed interesting point. It seemed that in thermal treatments, important factor in MDA content were environmental stresses (ROS changes) rather than LOX activity because during CS, changing of MDA showed a close relationship with H_2O_2 content. Such mutual relationship between MDA content and LOX activity also was observed in tomato and chickpea [31, 33, 34]. CS in acclimated plants increased LOX activity especially in Norstar genotype after 12 h, whereas 24 h CS did not significantly change the LOX activity. Relative increase in MDA content after 24 CS in acclimated plants confirmed low activity of LOX. Inducing of CS in non-acclimated plants did not cause forced changes in LOX activity compared to control ones so that just in SRN genotype, LOX activity decreased after 24 h. Maximum activity of LOX after 14 d CA, occurred in Norstar plants that showed more activity of LOX in defense pathways. Under such conditions more decrease in MDA content may

be observed in Norstar compared to other genotypes, which confirmed these results. It was supposed that higher LOX activity could not be directly related to the CS-induced cellular damage.

In stressed plants, the total content of FAs exceeded than that of control plants. Results showed that the status of CA was associated with more total FAs in acclimated plants compared to non-acclimated ones after CS. Thus, thermal treatments could enhance the biosynthesis of membrane lipids than their degradation [7]. This suggested that cell homeostasis of Norstar and then Gerdish could be maintained partly due to membrane FA composition to withstand CS compared to SRN genotype. The efficiency of FA contents was confirmed by comparing the SFAs and UFAs in the leaves of genotypes under thermal treatments (Figs. 2a, b, c). Significant increases of SFAs and UFAs under thermal treatments in Norstar compared to durum genotypes showed distinct cell endeavor to protect against CS. Interestingly, at 25 °C also such situations in FAs was observed, which may be considered as an index for cold tolerance even under control conditions. It was noted that under thermal treatments in durum genotypes, Gerdish showed higher UFA contents than SFA compared to SRN, which may be a reason for higher tolerance to CS. We presumed that the higher biosynthesis of membrane lipids and higher activity of desaturase enzymes in Norstar and then Gerdish would affect cellular responses during thermal treatments compared to SRN genotype. Regarding to values for C18 in cells and their absolute content alterations, it seems that C18 was the major determinants for cold tolerance in wheat and FAs elongation happens in adaptation to new environmental condition as well as desaturation. CS markedly affected DBI. A significant increase of DBI was observed during thermal treatments when values for H_2O_2 and MDA contents were the lowest. Saturated FAs showed a sharper decline than UFAs at this time in point. Our results indicated that during CS in acclimated and non-acclimated plants, UFAs contents reached a peak. It is clear that the increasing of UFAs content in cell membrane structure causes the stability of cellular membranes in plants and acts as a factor for cold tolerance in plants [33]. Comparing DBI and other physiological responses revealed interesting points. In control plants, DBI content was like LOX activity and was in contrary to H_2O_2 and MDA contents, so that the highest DBI belonged to Norstar, Gerdish and SRN, respectively. During CA phase, DBI increased to defend cell membranes and after 7 days, maximum increase occurred in SRN. This result confirmed significant changes in H_2O_2, MDA contents and LOX activity in this study which showed sensitive genotypes had faster metabolic changes. In acclimated and non-acclimated plants after 12 h CS, DBI was higher in Norstar than that of SRN and Gerdish plants which
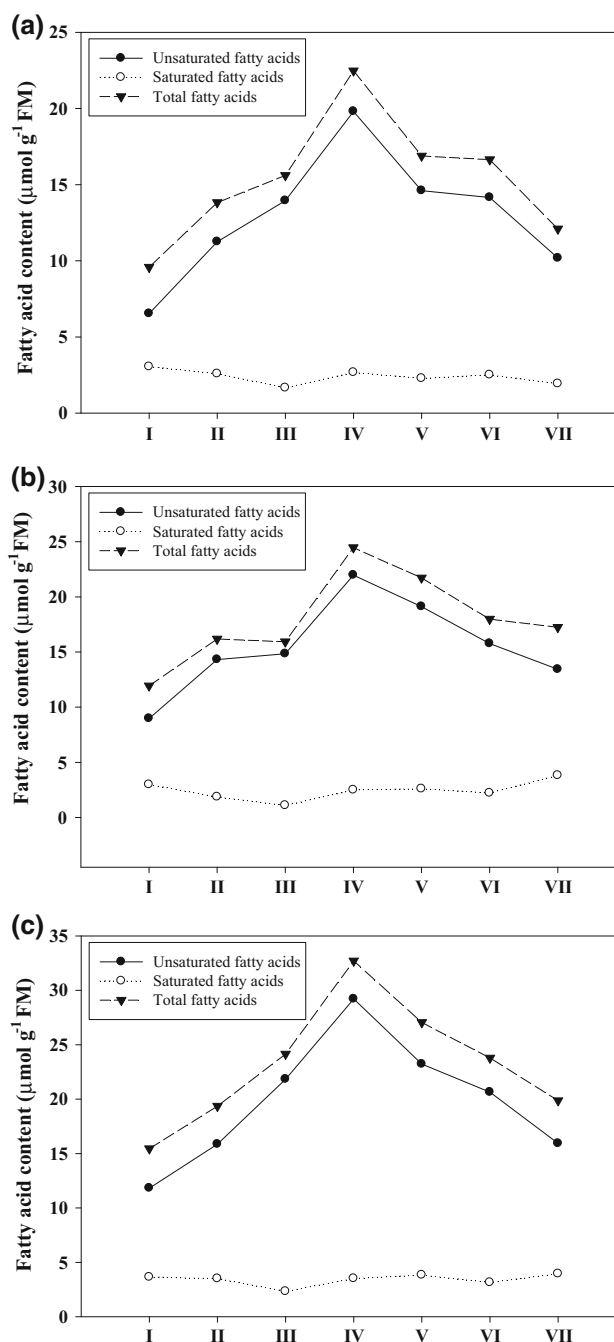

Fig. 2 Comparison among the changes of saturated, unsaturated and total fatty acids in leaf lipids of SRN (a) and Gerdish (b) genotypes (durum wheat) and Norstar (c) genotype (bread wheat) subjected to the thermal treatments: control (25 °C) (I), acclimation phase (4 °C) after 7 days (II) and 14 days (III), cold phase (-5 °C) after 12 h (IV) and 24 h (V) in acclimated plants and cold phase (-5 °C) after 12 h (VI) and 24 h (VII) in non-acclimated plants

revealed positive relationship between LOX activity and DBI (Figs. 3a, b, c). Under such conditions, Norstar with the highest LOX activity showed the highest DBI that to be associated with their defensive responses. Different studies
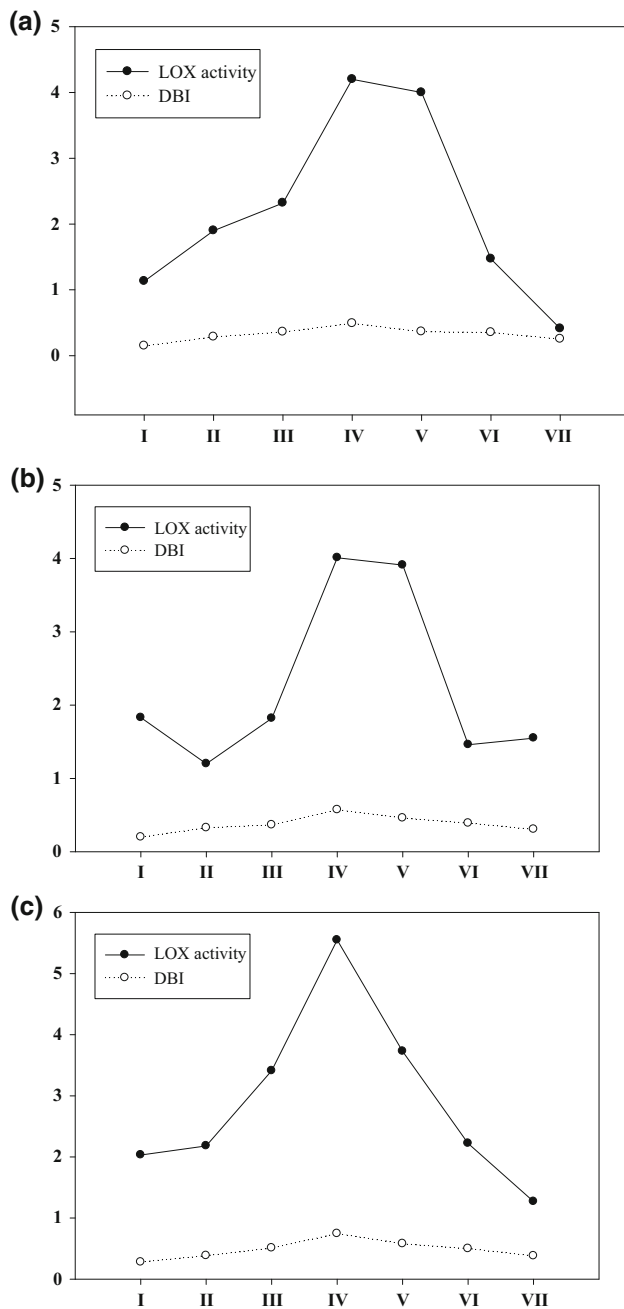
Fig. 3 Comparison between the changes of lipoxygenase (LOX) activity and double bond index (DBI) in leaf lipids of SRN (a) and Gerdish (b) genotypes (durum wheat) and Norstar (c) genotype (bread wheat) subjected to the thermal treatments: control (25 °C) (I), acclimation phase (4 °C) after 7 days (II) and 14 days (III), cold phase (−5 °C) after 12 h (IV) and 24 h (V) in acclimated plants and cold phase (−5 °C) after 12 h (VI) and 24 h (VII) in non-acclimated plants

have reported the cold induction of LOXs isozymes in transcript and proteome levels [35, 36] and it was suggested to be involved in the biosynthesis of jasmonic acid (JA) and several secondary metabolites and antioxidants that have specific roles in development and in responses to stress [37, 38].

Comparing the results of DBI and MDA, the lower levels of lipid peroxidation and membrane damage, which is conferred to the maximum DBI and lower MDA, is matching with the maximum level of LOX activity at 12 h CS in acclimated and non-acclimated plants. These data indicated that higher LOX activity could not be directly related to the CS-induced cellular damage. It proved the effective cold-induced activity of LOX on membrane fluidity [39] and a limited relation between LOX activity and MDA levels in wheat [34].

In plants, both 18:2 and 18:3 are oxygenated by 9-LOX and 13-LOX, respectively and further metabolized via seven different pathways into multiple biologically active oxylipins such as JA biosynthesis [38, 40, 41]. Our data suggest that LOX activity induced by CS could utilize 18:2 as a primary substrate like 18:3 [31]. It is likely that JA-dependent signaling in response to CS is subject to LOX substrate specificity behavior [42]. Consequently, LOX activity may play an important role in the mechanism of cold tolerance in durum and bread wheats. Interestingly, the LOX activity particularly at 24 h CS in acclimated and non-acclimated plants declined when lipid biosynthesis and DBI index due to direct and indirect effects of CS [33] reduced. It seemed that under such situations with increasing the severity of CS, some especial mechanisms were activated along with LOX activity, on one hand for protection against ROS and with increasing or maintaining of unsaturated FAs (DBI) on the other hand keep cell membrane activity against CS. Therefore under thermal treatments relationships of LOX activity and DBI can be changed which is an indication of defensive reprogramming in plants during CS. However it should be emphasized that the role of LOX in response to CS is complex and still remains to be investigated further.

Conclusion

In this research, hexaploid (bread wheat) and tetraploid (durum wheat) genotypes showed different physio-biochemical responses to CS under acclimation and non-acclimation conditions, that confirmed the existence of wide range genetic capacities in genotypes to increase cold tolerance when environmental conditions change. This could be as a result of a significant proportion of changes in cellular indices like H_2O_2, MDA, LOX activity and DBI that provided tolerance partly against cold-induced oxidative stress in genotypes. However, it will be valuable to find out what specific molecular responses happen along with thermal treatments. That is the area that needs to be examined in detail because our findings could be due to genomic factors or genes activities in A and B genomes in tetraploid and A, B and D genomes in hexaploid wheat. It

is possible that different genomic structures affect on tolerance to CS. LOX activity plays a double role in mechanism of cold tolerance in wheat, particularly at severe degrees of stress that could not be related directly to defensive responses. Thus, comparing the responses could be useful in understanding differences associated with the relative capacity of durum to cope with CS. Considering the studied protecting mechanisms, it can be concluded that Gerdish plants like Norstar showed better responses to CS compared to SRN, probably due to their more activities and/or synthesis of metabolites. Thus, it could be noted that these general responses are conserved in tetraploids and hexaploids but the degree of their adaptation and tolerance could be different in tetraploid and hexaploid wheat. This could be due to the genomic structures and other characters that some of them induce physiological characters, increase genetic capacity to cold tolerance.

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