

## Comparison of Cold-hardiness Levels of Wheat Cultivars Based on Alterations in Alternative Oxidase Protein Level

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### Abstract

In plant mitochondrion, electron transport chain has a second terminal oxidases named as alternative oxidase (AOX) in addition to cytochrome oxidase (COX). AOX pathway is known to be alternative respiration or cyanide-resistant respiration and linked to stress tolerance of plants. This study aimed to assess cold-hardening levels of four different cultivars of wheat by comparing alterations in protein levels of AOX. The AOX protein levels of Yıldırım and Dogu-88 cultivars were markedly higher than those of Ayyıldız and Alpaslan cultivars in control conditions, which indicate that alternative respirations of Yıldırım and Dogu-88 cultivars are more active than those of the other cultivars. Cold stress resulted significant increases AOX protein levels of all cultivars in comparison to their controls. The increases rates were 47, 34, 31, and 70% in Yıldırım, Doğu-88, Ayyıldız, and Alpaslan cultivars, respectively. These findings revealed that although all cultivars tried to resist to cold stress by improving their alternative respirations, Yıldırım and Dogu-88 cultivars are more tolerant to cold stress than the other cultivars.

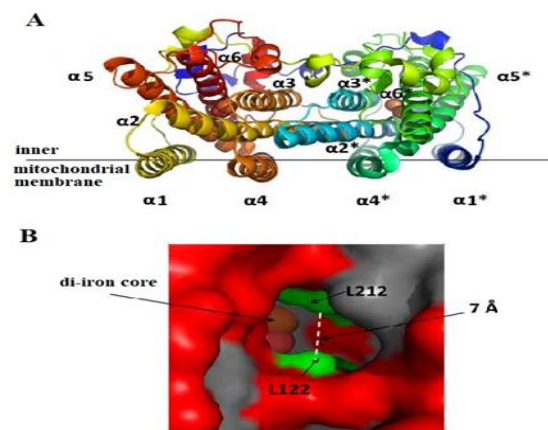
**Keywords:** Alternative oxidase, cold stress, wheat, immuno-blot analysis

### 1. Introduction

The cyanide-resistant respiration, known as alternative respiration, in plant mitochondria was first

discovered by van Herk in 1937 (van Herk 1937). This pathway is common to all plants besides some fungi and protists (Schonbaum et al. 1971). Electron transfer through alternative respiration is not coupled to ATP synthesis because it prevents formation of membrane potential, and eventually occurs heat instead of ATP (Bendall and Bonner 1971; Siedow and Moore 1993). This heat produced by AOX activity is used to make primary amines volatile in thermogenic plants and thus pollinating insects and animals are attracted (Meeuse 1975), (Watling et al. 2006).

AOX is found in the structure of dimer or monomer depending on its oxidized or reduced state in inner mitochondrial membrane (Lambowitz et al. 1989), (Elthon et al. 1989), (Erdal et al. 2015). AOX protein was firstly proposed to be consist of two transmembrane helices (Siedow and Umbach 1995). In the following years, Andersson and Nordlund (1999) suggested a new model of AOX as an interfacial protein rather than a transmembrane protein (Figure 1).



**Figure 1.** The structure of alternative oxidase (AOX) (MAY et al. 2017) (A) In the dimeric structure of AOX, helices are labelled  $\alpha 1$ -  $\alpha 6$  and  $\alpha 1^*$ -  $\alpha 6^*$  on the neighbouring monomer. (B) Surface model of AOX hydrophobic cavity.

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Low temperature is a crucial factor restricting the crop production (Erdal 2012), (Turk and Erdal 2015), (Turk and Genisel 2019), (Turk et al. 2019a). In addition to normal metabolic processes, low-temperatures enhances alternative pathway capacity in plants (Vanlerberghe and McIntosh 1992), (Purvis and Shewfelt 1993), (Erdal and Genisel 2016). Today, one of the major troubles for plant breeders is to augment plant tolerance to low temperatures and to select cold-resistant varieties of plants (Fowler et al. 1995).

It is well-documented that under the cold conditions, the transcription of some cold-responsive genes (COR/LEA) are significantly activated, and the high level of these proteins confers to freezing tolerance to plants (Thomashow 1999). Kobayashi et al. (2004) reported that differences in the protein levels of COR/LEA are linked to tolerance of wheat cultivars to low temperature stress. Similarly, Nobuyuki et al. (2008) informed that alternative respiration might be partly linked to the cold/freezing tolerance in wheat. However, there is limited information about AOX protein levels of different cultivars of plants to reveal the relationship between AOX protein level and cold hardiness.

The aim of study was to determine and to compare differences among protein expression levels of AOX in four different wheat varieties. The comparison of alternative respiration pathways in laboratory conditions would be a very useful method for determination of cold hardiness levels in both between cultivars of one species and many species.

## 2. Material and methods

### 2.1. Plant material, growth conditions, and applications

The seeds of wheat (*Triticum aestivum* cv. Yıldırım, Doğu 88, Ayyıldız, and Alpaslan) were subjected to surface-sterilization for 10 minutes with 5% NaOCl solution, and then the seeds were rinsed a few times with pure water. All of the sterilized seeds were sown in pots containing sand and grown for 14 days in a plant growth cabinet set at a constant temperature regime of  $20 \pm 1$  °C (day/night) for a 14 h photoperiod at  $40 \pm 5\%$  relative humidity. The plants were divided into two groups (control and cold group) and cold stress ( $5/2$  °C for 48 h for wheat cultivars)

were applied to the seedlings in the second groups. The control group was grown on same cycle of  $20 \pm 1$  °C (day/night). After 48 h, the seedlings were harvested for biochemical analyses.

### 2.2. Mitochondria isolation

The leaves of wheat cultivars studied were extracted in grinding based on the method of Chien et al. (2011). After differential centrifugation steps and Percoll gradient, the purified mitochondrial pellet was obtained (Grabel'nykh et al. 2011). The protein concentration of the pellet was determined according to the method of coomassie brilliant blue (Bradford 1976).

### 2.3. Immuno-blot analysis

The mitochondrial proteins separated SDS-polyacrylamide gel were transferred to PDVF western blotting membrane in semi-dry Western blotting. While the monoclonal antibody (*Sauromatum guttatum*) and the anti-mouse secondary antibody were used (Elthon et al. 1989). The density of the occurring protein bands was calculated by comparing band intensities of their controls.

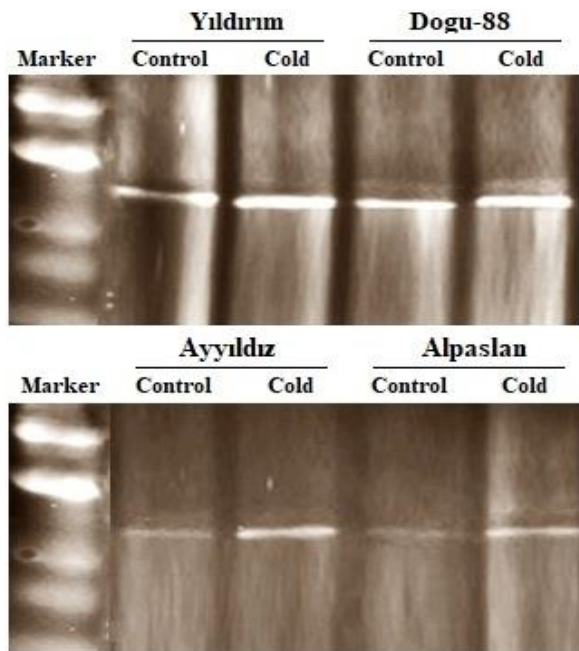
### 2.4. Statistical analysis

All experiments were an entirely random design with three replications. The data was analysed by ANOVA with Duncan's multiple range test with SPSS 20.0. The significant differences were evaluated at  $P < 0.05$ .

## 3. Results and Discussion

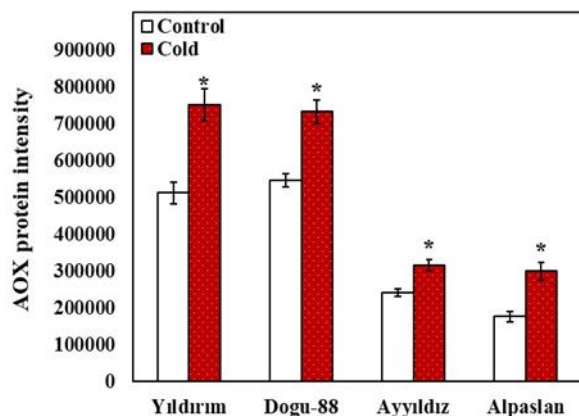
In the present study, it was assessed the alterations in level and intensity of AOX protein in the leaves of four different wheat cultivars grown normal and cold conditions, and they were compared with among themselves. Immuno-blot analysis showed that AOX protein bands of Yıldırım and Dogu-88 cultivars were obviously more intensify than those of Ayyıldız and Alpaslan cultivars grown under control conditions (Figure 2).

Cold stress gave rise to significant increases in intensity of AOX protein bands of all varieties studied when compared with their controls.



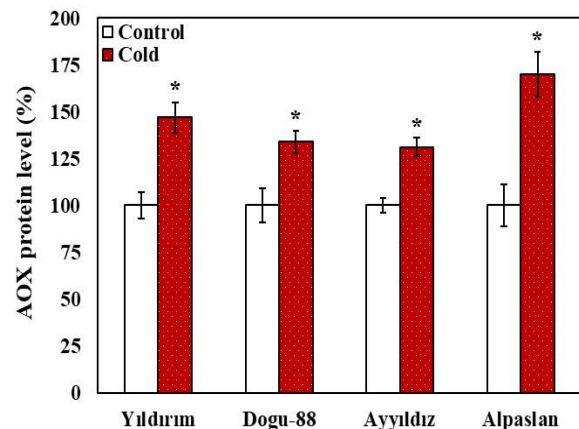
**Figure 2.** The AOX protein bands in the leaves of wheat cultivars grown under control and cold stress. (A) The AOX protein bands of Yildirim and Dogu-88 varieties. (B) The AOX protein bands of Ayyıldız and Alpaslan cultivars.

As seen from Figure 3, Yildirim and Dogu-88 cultivars exhibited more two-fold intensity of AOX protein than Ayyıldız and Alpaslan cultivars. Similarly, under cold stress, AOX protein intensity of these cultivars was higher than in the other cultivars. However, cold-induced increase rates of AOX proteins were significantly different among cultivars.



**Figure 3.** AOX protein intensity in the leaves of four wheat cultivars grown under control and cold stress.

Compared to their controls, the increase ratios in AOX protein intensities were 47% for Yildirim, 34% for Dogu-88, 31% for Ayyıldız, and 70% for Alpaslan, respectively (Figure 4). These data means that even if their AOX protein levels are different from each other, all cultivars attempted to improve their alternative respiration pathways in response to cold stress.



**Figure 4.** The cold-induced increase rates of AOX protein in the leaves of four wheat cultivars in comparison to their controls.

It is well-documented that plants tend to enhance activity of their alternative respiration pathways in response to environmental stresses, such as low temperatures and salinity (Turk 2019), (Turk and Genisel 2019). Number of studies have reported that low temperatures increase velocity and activity of mitochondrial respiration, in particular alternative respiration, in plants. It was informed that cold stress augmented alternative respiration rate and AOX protein level in chickpea (Erdal et al. 2015). Similarly, in cold-stressed maize seedlings, the activity of alternative respiration and AOX protein level was higher than control plants (Erdal and Genisel 2016). The enhanced mitochondrial respiration rate is considered as a common phenomenon in response to cold stress. Although alternative respiration pathway constitutes a small part of the total mitochondrial respiration, its stimulation contributes to increasing plant's resistance to cold stress (Wang et al. 2011). The stress alleviating effect of alternative respiration is linked to its multifarious properties. Firstly, it prevents

the blockage of mitochondrial electron transport chain in plants exposed to cold stress or the other abiotic stress conditions. Since cytochrome pathway is very sensitive to environmental stress conditions, it might be easily affected and thus mitochondrial electron transport might be disturbed. In similar cases, electrons are transferred through AOX and the possible congestion in electron transport chain is prevented. The other property of AOX is to produce heat. Because AOX activity produces heat instead of ATP energy, enhanced activity of AOX results in an elevation in inner heat level, and thus confer to tolerance to plants against cold stress.

On the other side, cold stress leads to excessive production of reactive oxygen species (ROS) in plants by disturbing mitochondrial respiration pathway and the other main processes. The high level of ROS causes drastic damages to biological macromolecules, cellular compartments, and genetic material (Dumlupinar et al. 2007), (Cakmak et al. 2010), (Erdal and Demirtas 2010), (Erdal 2012), (Genisel et al. 2013), (Turk and Erdal 2015), (Turk et al. 2019b), (Turk and Genisel 2019). The mitigating role of AOX on reactive oxygen species has been well documented in plants in response to cold stress (Cvetkovska and Vanlerberghe 2012). AOX minimizes the production of reactive oxygen species by preventing the over-reduction of ubiquinone via direct transportation of electrons from ubiquinone to oxygen. Eventually, cold-induced damages are reduced to some extent by activation of alternative respiration. It has been reported that when the expression levels of antioxidant enzymes' genes are low levels, activity, gene expression, and protein level of AOX augments significantly and finally reduce the level of reactive oxygen species (Maxwell et al. 1999), (Cvetkovska and Vanlerberghe 2012), (Wang et al. 2011), (Erdal and Genisel 2016).

In this study, high level of AOX protein in Yildirim and Dogu-88 indicates that these cultivars had more effective alternative respiration and thus more tolerant to cold stress than Ayyildiz and Alpaslan cultivars. Cold stress-induced elevations in AOX protein level of all cultivars studied suggest that plants improve their alternative respiration capacities to resist to cold stress and to maintain their survival.

## 2. Conclusion

When taking account into the properties of alternative respiration on plant resistance to environmental stress factors, it is possible to say that determining alternative respiration capacities, including activity, gene expression, and protein level of AOX in laboratory conditions could be useful in the selection of more tolerant varieties or cultivars of plants depending on environmental conditions, and this method could be used in practice to save time and to prevent product loss.

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