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# DEHYDRIN PROFILES OF SOME IRANIAN MELON VARIETIES (*Cucumis melo* L. MERR) UNDER DROUGHT STRESS CONDITIONS

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## ABSTRACT

Dehydrins are proteins that play a role in the mechanism of drought tolerance. This study aimed at establishing dehydrin profile and accumulation in four local melon varieties of Iran: Mino, Dargazi, Saveii, and Semsori, as well as in a commercial variety Honeydew. Plants were treated with drought stress by adjusting the soil water content to 75, 50, 40, 30 and 20% of field capacity (FC) by withholding water. Water status of plants was monitored based on the seedling fresh weight (FW) and relative water content of leaves (RWC). Total protein content was extracted, then heat-stable protein (HSP) fraction was isolated for each variety and water stress treatment. After SDS-PAGE of HSP, Western blotting analysis was carried out with Anti-dehydrin rabbit (primary) and Goat anti rabbit (secondary) antibodies. ANOVA results showed that with decreasing FC below 75%, FW and RWC decreased, but these changes significantly varied among genotypes. On the basis of FW and RWC data under different drought stress treatments, the following drought-tolerant ranking was established: Mino > Dargazi > Saveii and Honeydew > Semsori, from tolerant to sensitive order. Results of Western blot analysis showed that expression of some proteins with molecular weights of 19–52 kDa was induced in the studied varieties under drought stress (% FC). Expression level of the dehydrin proteins in different varieties was variable and also depending on the drought stress level applied. However, dehydrin proteins (45 and 50 kDa) showed strong expression levels in all varieties under severe drought stress (20% FC). The abundance of dehydrin proteins was higher in tolerant varieties (Mino and Dargazi) than in moderate and drought sensitive genotypes. Consequently, dehydrins represent a potential marker for selection of genotypes with enhanced drought tolerance.

**Keywords:** melon, drought stress, dehydrin, western blotting

## INTRODUCTION

Melon (*Cucumis melo* L.) is an important fruit crop widely cultivated in many regions of the world. The most prevalent environmental stress affecting melon is drought, which impacts considerably plant growth and fruit yield. Usually, melon fruits under drought stress become soft, wrinkled, and turn brown. Additionally, water deficiency hastens premature ripening, reducing fruit size [Shetty et al. 1997]. Plants have developed different strategies to face water deficit and, over the past few years, much attention has been focused on the identification of genes and proteins induced in response to environmental stresses [Kuşvuran et al. 2011].

Drought is one of the major abiotic factors limiting plant growth. Plants exhibit various responses at both the transcript and proteomic levels in adaptation

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to drought stress [Fathi and Tari 2016]. During the past two decades, several specific proteins have been characterized in drought-stressed plants that can be classified into several groups. LEA proteins are desiccation stress inducible, often responsive to ABA. Stress regulated enzymes are required for the biosynthesis of various osmoprotectants (e.g. sugars, Glycine-betaine). Some other polypeptides perform detoxification reactions (Superoxide Dismutase, Catalase, Ascorbate Peroxidase), while further protein factors are involved in the regulation of signal transduction and gene expression, such as protein kinases and transcription factors [Kyriakis et al. 1994]. Dehydrins are dehydration-induced polypeptides forming another group of drought-induced proteins. They share a consensus 15-amino acid sequence, the 'K' motif (EKKGIMDKI- KEKLPG), and exhibit a wide range of total molecular masses from 9 to 200 kDa. The 'K' motif is involved in hydrophobic interactions probably leading to macromolecule or cellular structure stabilization. This way it helps in maintaining the integrity of cell membranes [Close et al. 1996, Beck et al. 2007] in response to adverse environmental conditions, such as drought, cold, and salinity. Dehydrins may act synergistically with compatible solutes [Olave-Concha et al. 2005, Beck et al. 2007]. The known physical properties of dehydrins also suggest roles as stabilizers of nuclear or cytoplasmic macromolecules under water stress conditions [Campbell and Close 1997].

Dehydrin proteins have been most extensively studied in relation to drought stress [Jiang and Huang 2002]. The accumulation of dehydrins was observed in various tissues, such as roots, leaves, coleoptiles, crowns and seeds [Houde et al. 1992]. Dehydrin accumulation has been reported to be positively correlated with dehydration tolerance in annual crops [Close et al. 1996, Mohammadkhani and Heidari 2008].

The molecular response of commercially important crops, especially melon, to drought stress has not been extensively studied. In this paper, we report the results of expression analysis of melon dehydrins induced by drought stress.

## MATERIALS AND METHODS

**Plant material.** Seeds of four Iranian melon landraces (Dargazi, Mino, Saveii and Semsori) as well as Honydew (commercial variety) (Fig. 1) were sown into peat (Kekkila, Vantaa, Finland), watered daily at field capacity (FC) and incubated under 150 $\mu Em^{-2}s^{-1}$ PAR at 24–25°C and with a 16-h photoperiod. After two weeks of growth in well-watered conditions (FC), drought stress was initiated by withholding watering. Control plants for each variety were watered daily at FC condition. Fresh weight (RFW) of seedling at control (FW1) and also under drought stress conditions (FW2) were measured after withholding water when water content reached 75, 50, 40, 30 and 20% of FC:

$$RFW = (FW2) \cdot 100\%/FW1$$

RWC was calculated in control and drought stressed melon plants with the same method, according to the formula of Barrs and Weatherley [1968]:

$$RWC\ (\%) = [(FW - DW)/(TW - DW)] \cdot 100$$

Fresh weight (FW) was immediately recorded after leaf excision (with three replications). Turgid weight (TW) was measured 24 h after soaking leaves in distilled water at 4°C. Dry weight (DW) was measured after drying for 24 h at 100°C. Results were expressed as RWC at 75–20% FC in relation to the control according to the formula: (RWC of plants after drought treatment) × 100%/( RWC of control plants) and calculated for each variety.

Leaves from control and at 75, 50, 40, 30, and 20% FC values were sampled, frozen in liquid nitrogen, and stored at –80°C until used for protein isolation.

**Isolation of leaf proteins from melon.** 250 mg leaf sample was ground in liquid nitrogen and crude protein extracts were solubilized in 1.5 ml ice-cold extraction buffer (100 mM Tris–HCl (pH = 7.6), 2 mM EDTA (pH = 8) and 2 mM phenylmethyl sulphonyl fluoride (PMSF). The protein concentration of the supernatant was determined by the Bradford method [1976] at 595 nm, with bovine serum albumin as standard. The samples were equalised to 3 mg in 1 ml with Tris–HCl extraction buffer. These equalized protein samples were then subjected to heat treatment (100°C) for 10 min, kept on ice for 5 min, and centrifuged at 15000 rpm at 4°C for 20 min. Heat-stable proteins in the supernatant were precipitated with 100% trichloroacetic acid and centrifuged at 15 000 rpm at 4°C for 15 min. The concentrated pellet was washed twice with ice-cold 80%


**Fig. 1.** Representation of the three axes for the walnut (*Juglans regia*) compression test

acetone with incubation for 45 min at 4°C. After centrifugation (15,000 g at 4°C for 10 min), the obtained pellet was dissolved in 30 µl 500 mM Tris-HCl pH 8.0 buffer, the samples were dissolved thoroughly by vortexing.

**Western blot analysis.** 15 µl of heat stable protein per sample, as well as control sample (well-watered seedling) and +control sample (extracted from *Arabidopsis thaliana* that overexpressing dehydrin), were separated by one-dimensional SDS polyacrylamide gel (1D 12% SDS-PAGE) and electroblotted onto nitrocellulose membrane (Schleicher and Schuell) using the semi-dry system (Bio-Rad). Nitrocellulose filters were blocked with 0.5% Tween-20 and 10% milk powder in BST (Tris Buffered Saline). The blots were probed with a primary anti-dehydrin antibody (Rabbit antibody) (1/5000 dilution) raised against the heat-stable protein [Vaseva et al. 2011] and shaken for 30 min. This antibody was developed in rabbit against the conserved consensus TGEKKGIMDKIKEKLPGQH (Stressgen Ltd, USA). The blots were washed with TBST (Tris Buffered Saline with Tween 20) buffer with 0.1% Tween-20 three times (5 min/wash). Then placed in solution with alkaline phosphatase conjugated goat anti-rabbit IgG (Jackson Immuno Research Laboratories Inc.) in 1 : 5000 dilution for 30 min. Then the membrane was placed in NBT (Nitro Blue Tetrazolium) and BCIP (5-Bromo-4-Chloro-3-Indolyl-Phosphate) substrates for 1 h in dark condition [Vaseva et al. 2011].

## RESULTS AND DISCUSSION

**Fresh weight of seedling (FW).** FW remained unchanged at 75% FC in relation to control, and then sharply decreased to reach an average of 43–58% at the end of the drought period (20% FC, severe drought stress). Comparisons showed that mean FW of plants subjected to drought stress (50 and 40% FC) and of control plants differed significantly for four varieties (Semsori, Saveii, Dargazi, and Honeydew) ($p < 0.001$), but for Mino variety the differences were statistically insignificant ($p > 0.05$). Means of FW were significantly different at 30 and 20% FC for all varieties when compared with control plants ($p < 0.01$). Among those five varieties, 'Mino' had the lowest decrease of FW in relation to control after drought stress (30 and 20% FC). Therefore, this variety seems to be more tolerant to drought stress than other genotypes. Dargazi was another variety showing more tolerance to drought stress.

According to ANOVA, the factors 'varieties' and 'stress treatments' (50–20% FC) significantly affected FW in this experiment ($p < 0.001$). However, FW was not influenced by 75% FC treatment, with decreasing FC, FW significantly ($p < 0.05$) decreased in all varieties, but Mino had the lowest decrease of FW when its seedlings were exposed to any drought stress treatments. Therefore, it seems that Mino was more tolerant to drought stress than any other varieties. Moderate tolerance was observed in Dargazi, Saveii, and Honeydew to 50–30% FC, but the first had the similar response to Mino at 20% FC and it can be considered as a drought tolerant. Among the varieties studied Semsori was the most sensitive and had the highest FW decrease in water stress (Fig. 2).

**Relative water content (RWC).** In the present study, we found non-significant decrease of RWC of five melon varieties when seedlings were exposed to 75% FC in relation to control plants ($p > 0.05$). However, RWC of three varieties (Semsori, Saveii, and Honeydew) ranged from 75 to 55% under drought stress (50–20% FC) significantly decreased in relation to control plants ($p < 0.05$). In 50% FC, Dargazi, and Mino had

RWC of about 84–89%, which was not statistically different to that of the control. Therefore, cv. Dargazi and Mino had higher RWC than other varieties at 50% FC. However, with increasing drought stress (40–20% FC), differences between stressed plants and controls significantly increased in Dargazi and Mino, but this happened at slower speed than in other varieties. The lowest RWC in relation to control was in the variety 'Semsori'. Therefore, this was the most sensitive genotype towards drought stress among those studied.

RWC of stressed plants significantly decreased with decreasing FC below 75% FC in all varieties, but these decreases were different among varieties. Analysis of variance for 50–20% FC treatments shows significant differences among RWC values of varieties. Mino variety had the highest RWC in all drought stress treatments and can be considered as a tolerant genotype. In second ranking, Dargazi could save a considerable amount of water upon drought stress treatment. Saveii and Honeydew were statistically different from Semsori in terms of RWC. Therefore, it seems that Semsori was the most sensitive variety because it had the lowest RWC in all drought stress treatments (Fig. 3). In bread wheat varieties, RWC in drought stress decreased in all bread wheat varieties tested [Keyvan 2010]. Water stress however made a significantly different effect on leaf RWC, depending on varieties reported in bean [Korir et al. 2006]. Schonfeld et al. [1988] found that with increasing level of drought stress in wheat, RWC usually but not always decreased. They found that the varieties that are more tolerant to drought are more likely to retain high RWC. These results are similar to those reported by Sinclair and Ludlow [1985] and in general agreement with results of this study. In our experiments the correlations between RWC and FW were statistically significant in all drought stress treatments: $r = 0.81^{**}$ for 50% FC, $r = 0.71^{**}$ for 40% FC, $r = 0.89^{**}$ for 30% FC and $r = 0.92^{**}$ for 20%.

**SDS-PAGE of total and heat stable proteins.** The SDS–PAGE profiles of total and heat stable proteins show sharp and detectable bands for different varieties and drought stresses. This testified that our total and heat stable protein extraction protocols yielded intact proteins. Sample concentrations were equalized and loaded on SDS-PAGE gels as shown in Figure 4. The total and heat stable protein profiles of five varieties of melon, showed differences between the plant grown in normal (whiteout stress) and drought stress conditions. In drought stress condition new protein bands were found (Fig. 4).

**Immunoblotting result.** Testing for the presence of dehydrin proteins by Western blotting method showed that no expression of dehydrin was found in any of the varieties at 75% FC samples and also in control plants (Fig. 5). Results regarding FWs and RWCs of seedlings showed that 75% FC could not affect plant responses and did not trigger production of dehydrin proteins. Western blotting confirmed that 50% FC tended to induce minor expression of dehydrins showed by the appearance of a 50 kDa protein band only in stressed seedlings of Mino and Dargazi varieties. Therefore, Mino and Dargazi (drought tolerant varieties) accumulated dehydrins under this condition and also conserved higher percentage of RWC than other varieties. Dehydrins weren't observed in any of the well-watered plants or other varieties (moderate or sensitive) subjected to 50% FC (Fig. 6). Expression of dehydrins in drought tolerant plants is a common response to water stress condition [Close 1997, Hara 2009]. The dehydrin in drought-tolerant and drought-sensitive soybean varieties were also found different based on molecular weight [Arumingtyas et al. 2013]. Dehydrins with the relatively high molecular weight of 40 kDa were also detected in other investigations in response to water stress condition [Volaire et al. 2001].

The moderately expressed 45 and 50 kDa dehydrins and faint bands at 19 kDa were present in Mino variety when the seedlings were exposed to 40% FC. These three bands were observed on Saveii variety but at a lower level. At this stress level Dargazi cultivar already revealed moderate expression of dehydrins with apparent molecular masses of 22–25 kDa. Volaire et al. [2001] reported that different sized dehydrin proteins were expressed in drought stressed tissues in *Poa bulbosa*. Under this low stress level Mino and Dargazi also had higher percentage of RWC and FW than other varieties. Therefore, positive correlation between dehydrin expression/intensity and FW and RWC in tolerant varieties could be observed [Lopez et al. 2002]. Differential accumulation of a 24-kDa dehydrin protein in wheat seedlings correlated with drought stress tolerance at grain filling [Lopez et al. 2001]. In our experiments the other drought-sensitive melon varieties

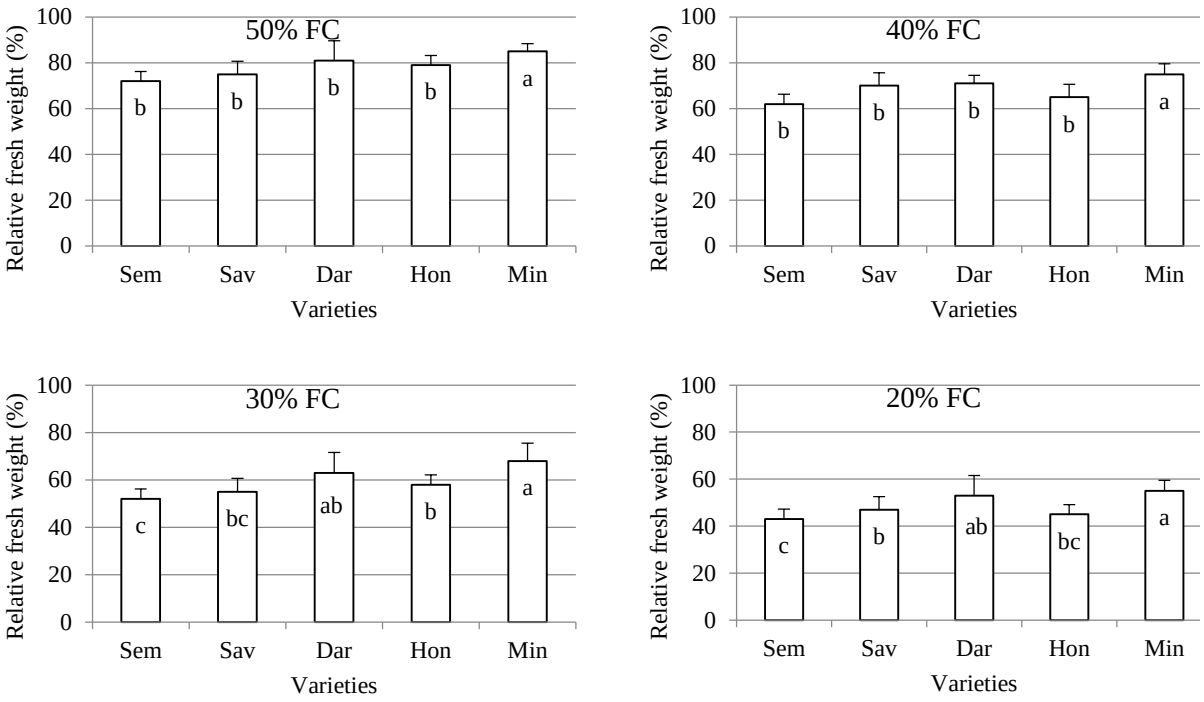


**Fig. 2**. Seedling fresh weight after drought stress (50, 40, 30, 20% FC) in melon varieties. Sem – Semsori, Sav – Saveii, Dar – Dargazi, Hon – Honeydew, Min – Mino. Means in each column followed by different letters are significantly different at $P \leq 0.05$ by Duncan's new multiple range test

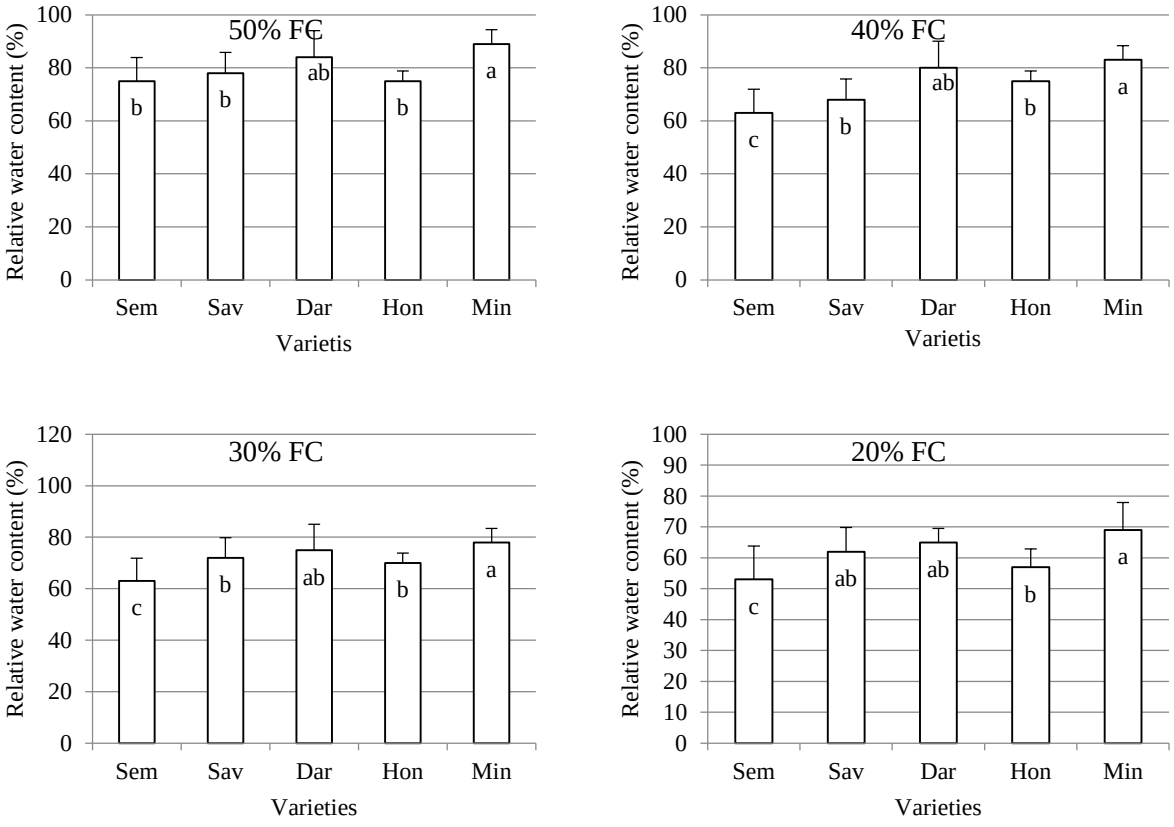


**Fig. 3.** RWC of leaves after drought stress (50, 40, 30 and 20% FC) in melon varieties. Sem – Semsori, Sav – Saveii, Dar – Dargazi, Hon – Honeydew, Min – Mino. Means in each column followed by different letters are significantly different at $P \leq 0.05$ by Duncan's new multiple range test

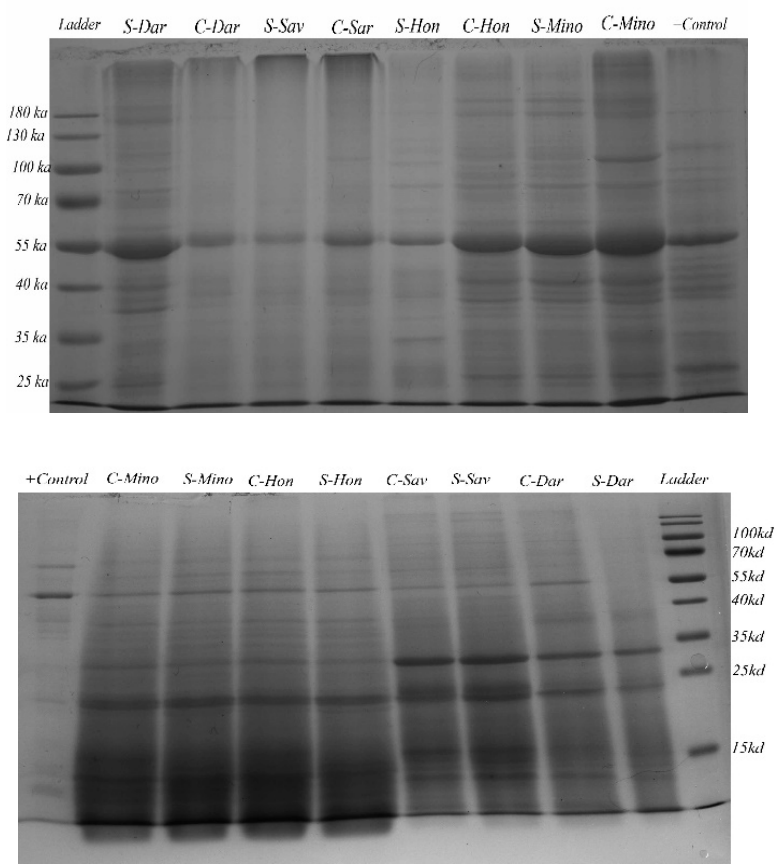


**Fig. 4.** SDS-PAGE of total protein (left) and heat-stable protein (right) on polyacrylamide gel (1D 12%). Hon – Honeydew, Sav – Saveii, Dar – Dargazi, S – stressed plant, Control – well-watered plant, +Control (extracted from *Arabidopsis thaliana* that overexpressing dehydrin)

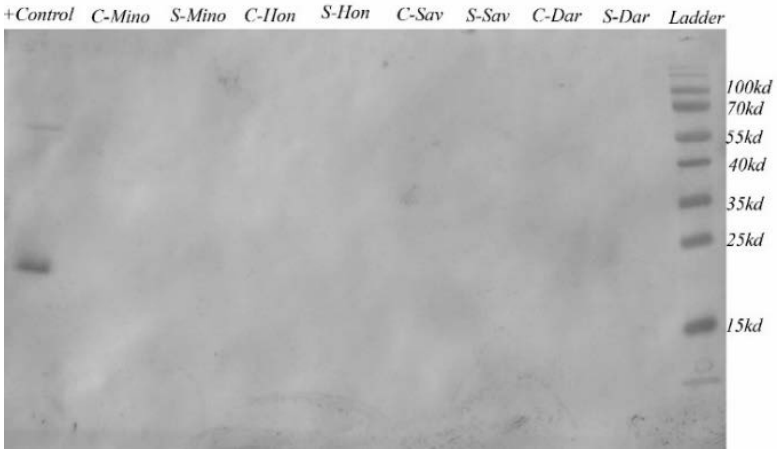


**Fig. 5.** Western blot analysis of leaf samples from five melon varieties with anti-dehydrin antibody at 75% FC. S – stressed plant, C – well watered plant, Hon – Honeydew, Sav – Saveii, Dar – Dargazi, +Control (extracted from *Arabidopsis thaliana* that overexpressing dehydrin)


**Fig. 6.** Western blot analysis of leaf samples from five melon varieties with anti-dehydrin antibody at 50% FC. S – stressed plant, C – well watered, +Control (extracted from *Arabidopsis thaliana* that overexpressing dehydrin)

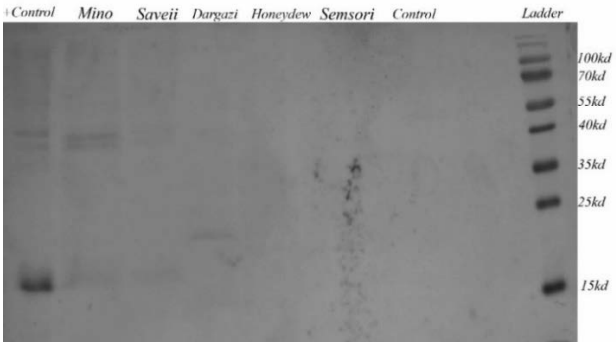


**Fig. 7.** Western blot analysis of leaf samples from five melon varieties with anti-dehydrin antibody at 40% FC. C – well watered plant, +Control (extracted from *Arabidopsis thaliana* that overexpressing dehydrin

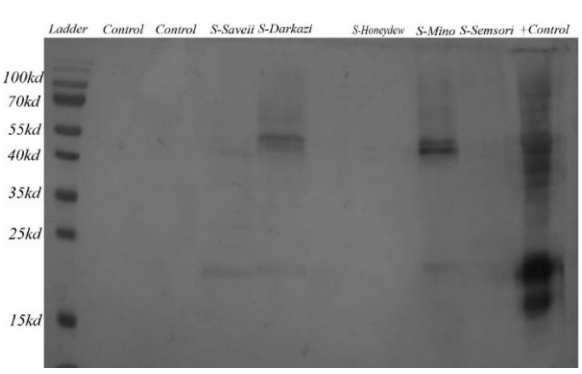


**Fig. 8.** Western blot analysis of leaf samples from five melon varieties with anti-dehydrin at 30% FC. S – stressed plant, C – well watered plant in two varieties

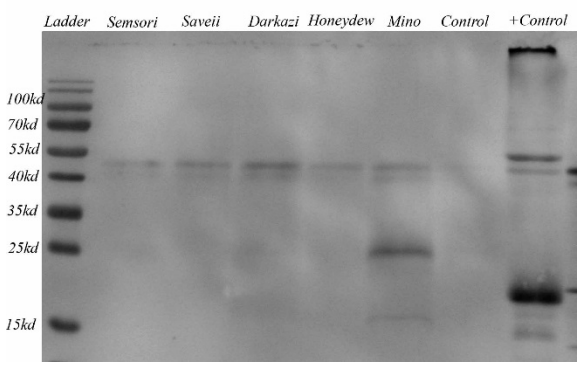


**Fig. 9.** Western blot analysis of leaf samples from five melon varieties with anti-dehidrin antibody at 20% FC. Control – well watered plant, +Control (extracted from *Arabidopsis thaliana* that overexpressing dehydrin)

did not accumulate any dehydrin bands under drought stress at 40% FC and appeared to have the lower values of FW and RWC. As controls, well-watered plants did not contain any dehydrin specific bands in Western blots (Fig. 7).

The dehydrin profiles in leaves of drought-sensitive varieties did not change at 30% FC, whereas changes were seen in the drought-tolerant genotypes. Severe drought stress caused increasing dehydrin protein accumulation in the drought-tolerant varieties. With progressive stress (at and under 30% FC), dehydrin expression was observed at Mino, Darkazi and Saveii varieties. Moderate expression of a 19 kDa dehydrin was revealed in these three varieties. High expression of the 45 and 50 kDa dehydrins appeared in Mino and Dargazi. However, no dehydrins were observed in Honeydew and Semsori varieties subjected to drought stress or any of the well-watered plants (Fig. 8). Mino and Dargazi were drought tolerant varieties based on FW and leaf RWC data of seedlings, which correlated with high expression of different dehydrin proteins. This indicates that tolerance to drought stress coincided with high amount of dehydrins accumulating during the stress. Our data confirm similar observations reported from other plant species [Lopez et al. 2002].

Several transgenic plants are overexpressing genes coding for dehydrin proteins [Ochoa-Alfaro et al. 2012, Kumar et al. 2014]. Four dehydrin genes, PmLEA10, PmLEA19, PmLEA20 and PmLEA29 were chosen for individual over-expression in tobacco plants. The transgenic tobacco plants showed lower relative content of malondialdehyde, relative electrolyte leakage and higher relative content of water than control plants when exposed to cold and drought stress. These results demonstrated that PmLEAs were involved in plant responses to cold and drought [Bao et al. 2017].

According to Cellier et al. [1998] dehydrin expression is associated with a tolerance mechanism leading to maintenance of turgor pressure, suggesting that dehydrins might also be involved in preventing cellular dehydration. Dehydrins may possibly be oxidized by radicals like hydroxyl and peroxyl radicals, so they could eliminate radicals and in turn lessen oxidative damages induced by water stress [Hara et al. 2004].

After seedlings had been subject to high level drought stress (20% FC), the 45 and 50 kDa dehydrins were observed at strong level in all studied varieties. Some researches were confirmed by observing that drought stress caused an increasing accumulation of dehydrins [Close 1996, Hara et al. 2004]. The 45 and 50 kDa immune-detected dehydrin bands probably corresponded to high molecular weight proteins, expressed at high drought stress level. Comparing the expression intensity among the varieties showed that each variety demonstrated different expression intensity of the dehydrin proteins (Fig. 9). Arumingtyas et al. [2013] showed that a soybean drought tolerant variety had the highest expression intensity of dehydrin proteins among the varieties studied.

Generally, the expression intensity of dehydrin proteins in Mino and Dargazi in all stress conditions was higher than other varieties based on 45 and 50 kDa (Darkazi > Mino and Saveii > Semsori > Honeydew). The expression intensity of the 50 kDa dehydrin was higher than that of 45 kDa protein in all varieties. Tolerant genotypes seem to be capable of increasing defense proteins against drought stress compared to sensitive varieties. In this study drought stress caused an increased production or accumulation of dehydrin proteins in both groups of varieties (tolerant and sensitive) as it has been reported in other research [Close 1996]. The strongest dehydrin expression of a 25 kDa protein was observed in Mino at 20% FC. This band probably corresponded to a specific low molecular weight dehydrin protein which was expressed in Mino under severe drought stress conditions. However, a 25 kDa dehydrin band with weak intensity was also present in Dargazi but was not present in any other variety. On the other hand, a 19 kDa dehydrin was also moderately expressed only in Mino (Fig. 9). A dehydrin protein with similar molecular weight was reported previously [Close et al. 2000]. Dehydrin-like proteins of 28, 32, and 34 kDa MW were detected in mature seeds by Samarah et al. [2006]. Vaseva et al. [2011] reported that clover plants submitted to drought accumulated high amounts of 30 and 22 kDa dehydrins as well as those between 40 and 50 kDa. The differences in the plant responses to drought stress indicate that there are many mechanisms used by plants to survive drought stress [Vaseva et al. 2011]. Different genetic determination of tolerance against drought stress has also been suggested, as this trait is certainly controlled by many factors in nature [Arumingtyas et al. 2012].

## CONCLUSION

Our results indicate that accumulation of 45 and 50 kDa as well as 25 kDa dehydrin proteins was associated with the relative drought tolerance which was characterized by a lower reduction in FW and leaf RWC of seedlings in Mino and Dargazi melon varieties. Consequently, these dehydrins represent potential markers for selection of genotypes with enhanced drought tolerance [Lopez et al. 2001]. Using such markers, screening techniques might be developed with the advantage of sensitive detection of drought tolerance in early developmental stages.

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