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THE EFFECTS OF DROUGHT ON THE LEVEL OF ISOFORMS OF AQUAPORIN IN CV. 'HOROZKARASI' GRAPEVINE

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Abstract

This study aimed to investigate the aquaporin expression of 'Horozkarası' grapevine. Therefore, own-rooted cv. 'Horozkarası' grapevines were exposed to two different irrigation treatments; well-irrigated and water-stress treatments under controlled environmental conditions in pots for six days. And then in the stressed plants lipid peroxidation (MDA), relative water content (RWC) and membrane permeability (MP) were measured in leaves. The expression patterns of different PIPs group of aquaporins (PIP2-1, PIP2-2) also were performed with root and leaf tissues. While significant decreases were observed in relative water content (RWC) and membrane permeability (MP) in stress treatment, increase was observed in lipid peroxidation (MDA) in leaves. Also, while significant decreases were observed in PIP2-1 and PIP2-2 in stress treatment in leaves, increases were observed in expressions of PIP2-1 and PIP2-2 genes in drought treatment in root.

***Key words**: Vitis, Aquaporins, water stress, MDA.*

INTRODUCTION

'Horozkarası' grapevine has significant economic importance, and is grown in Southeastern Anatolia, especially in Gaziantep and Kilis, for using in vine and dried grape production. Because of the increase in demand on fruits having high antioxidant content that is important for human health, the demand on 'Horozkarası' grapevine that is widely consumed in dried form started to increase day by day.

Its long, elliptic, and significantly large grapes make this variety to be consumed in dried form, as well as it is also consumed freshly. 'Horozkarası' grapevine grape, which is very common especially in Gaziantep and Kilis region, is grown in almost all of the cities in GAP region (Gürsöz, 1993).

This variety is also known as Kilis Karası and Antep Karası among the producers and, among 35-40 sorts of grapes grown in Gaziantep province, it comes to the forefront with its area and amount of production.

Grapevine is a cultivation plant that has high level of adaptability to inappropriate soil conditions. Except for a narrow line in eastern Black Sea region, all of the regions of Turkey are within the arid and semi-arid climate belt. Moreover, as in entire world, the reflections of global warming also increase in Turkey. Besides the global warming, especially the decrease in usable water sources, the increase in arid and semi-arid agricultural lands, and the significant increases in duration and severity of drought have significantly stressful effect on the cultivated plants being grown. As well as it decreases the yield and quality, it might also result in increase in product losses and even in death of plants. On the other hand, it is another important research topic if the actual agricultural lands, on which the agricultural activities are performed, will be suitable for agricultural production in future. Some of the studies on this subject indicate that the patterns in agricultural production might change in future due to the climate change. It is inevitable that such a climate change will directly affect the viniculture in future. Today's vineyards will be unsuitable for vinicultural activities under the effects of increasing temperature and limited water sources. Scientists make effort for developing solution suggestions for this problem; there are 2 solution suggestions. First of them is to obtain new hybrids by crossing actual varieties (*V. vinifera* L.) with those having high drought tolerance to be used in future. The second proposal is to evaluate the new microclimate regions to occur in the regions close to the poles due to the increasing temperatures. In order both to protect the actual limited water sources we have and to obtain high crop yield and quality by using relatively lower level of water use, it is necessary to determine the drought tolerance of existing grape varieties. Thus, in near future, it would be relatively easier to obtain varieties to be grown using less water and to have quality suitable for consumption and market requirements by utilizing varieties with high tolerance to drought. Even if it is attempted to increase the efficiency of water use through the technological advancements regarding the irrigation systems and to protect the water sources, these precautions are limited, short-dated, and very expensive. For this reason, it is very important to select the grapevine varieties having high drought-tolerance and to examine the effect of their interaction with rootstock on the drought tolerance.

The leading one among the most important abiotic factors affecting the quality and yield in agricultural production is the drought. The grape gives physiological, biochemical, and molecular responses to the drought. The chemical signals coming from the roots play important role in the adaptation especially in first phase of water stress (Schachman, 2008). The signals are conveyed through the xylem to the leaves, and play role in arranging the water losses. Under the conditions of drought, many chemical signals are transmitted from the roots to the leaves. Some of these chemical signals, abscisic acid (ABA), pH, cytokine, malate, and precursor compounds of ethylene play role in the use of water during the first phase of drought stress (Schachtman, 2008). ABA was reported to act as stress hormone under the environmental conditions such as drought and salt stress (Peleg, 2011; Fukaki 2009). But, regarding the importance of ABA for the root signals, there are debates among the studies due to the methodological differences (Schachman, 2008). It was reported that, during the drought stress, the pH changes in xylem core play role as chemical signal (Wilkinson, 1999). This change affects ABA metabolism or directly the water status of leaves. pH change in xylem causes the close of stoma by activating the ABA that is the cell protector (Wilkinson, 1999). In studies on the chlorophyll content of drought stress in grapes, when compared to irrigated plants, the increases were reported in the total chlorophyll content of unirrigated grapes by Maroco et al. (2000), while Flexas et al. (2000) reported remarkable decrease and Chaumont et al. (1994) found no change in total chlorophyll content.

In drought stress tolerance of various varieties, a close relationship was found between the antioxidant system and the decrease in oxidative damage (Zhang and Kirkham, 1996; Jiang and Zhang, 2002; Lima et al., 2002; Ramachandra et al., 2004; Sofo et al., 2005; Sanchez-Diaz et al., 2007; Aganchich et al., 2009; Ozkur et al., 2009; Wang et al., 2009). In their study on the antioxidative mechanism in drought adaptation of two varieties grown on their roots (Sabatiano and Mavrodafni), Alexandros and Angelos (2012) reported a rapid increase in hydrogen peroxide concentration of Mavrodafni variety under droughty conditions. The researchers emphasized that, while there was no significant change in CAT activity under drought stress, there was difference in APX and SOD activities.

The transportation of water in xylem in angiosperms is known to be affected from the anatomical characteristics such as vein size, distribution and intensity, core structure, vein permeability, and topology of xylem network. Besides them, the chemical signals (ABA) significantly affect the water transportation in plants in expression of aquaporin. Lovisolo et al. (2008) emphasized that ABA has important role in transpirational control in rehydrated grapes, and reported that the xylem embolism and abscisic acid hormone increased in stressed plants. In addition, they showed that aquaporin has important role in regulation of the leaf and root hydraulics. Aquaporin is a water channel protein that exists in various physiological processes among the organisms. In grapes, aquaporins play important role in drought adaptation of plants by maintaining their ion and water balance under varying environmental conditions. In studies on different plant varieties, among the plant that have not been exposed to drought stress, PIP1;1 aquaporin gene was expressed in roots at higher levels when compared to the expression in leaves (Galmes et al., 2007; Weig et al., 1997; Jang et al., 2004). It is known that, aquaporin plays important role in adaptation of grapes to drought due to varying environmental conditions. Aquaporins play important role in arranging the hydraulics in roots (Vandeleur et al., 2009) and leaves (Pou et al., 2013) of grapevines. Aquaporins play role in continuous root-to-leaf water transportation, and they might lead to rapid and inverse changes in cell's hydraulic conductivity by arranging the water permeability of membrane (Hayes et al., 2007; Surbanovski and Grand, 2014). Aquaporins play role in arranging the water movement throughout the plasma membranes in the metabolic path between the cells and in correcting the xylem embolization (Lovisolo and Schubert, 2006). Zarrouk et al. (2015) reported that, in their study on drought in cv. Toriga Nacional grape variety, the root-shoot signals responded by increasing the root hydraulic conductivity in mid-level water stress by being encouraged by the chemicals. In addition, they emphasized that the aquaporin isoforms played role as major-sub organizer and in sensing the water stress since the first phase of water stress.

MATERIALS AND METHODS

Growing the Plants, Implementation, and Taking the Samples

This study was carried out in greenhouses within the body of Kilis 7 Aralık University's Agricultural Implementation and Research Center (TUAM) and in Agricultural Engineering Faculty Laboratory of the university. The slips used in this study were collected from the vineyards in this region. The materials collected were cut into the suitable size for rooting, and then planted into rooting cases by sinking into 2% fungicide solution. The rooted healthy plants were planted into 5 L (1:1 v:v) peat/perlite mixture. In order to endure the homogeneity, they were grown on a single body with cut branches. As of the month of August, the plants were irrigated on regular basis, and then they were divided into control and drought groups. This study was carried out in experimental pattern of fully randomized coincidence parcels, in triplicated in accordance with factorial order (10 healthy saplings planted in pots in each repeat). Drought regime was implemented for 6 days. At the end of 6th day, the root and leaf samples were immediately treated with liquid nitrogen for molecular analyses, and kept at 80˚C until the analyses.

Relative Moisture Content (RMC)

The leaf samples collected a little while before the harvest were immediately weighed and the wet weight (WW) was determined. By keeping the samples in pure water for 4 hours, they were transformed into turgor, and then weighed again (TW). And, finally, the leaf samples were dried in drying cabinet at 60°C for 24 hours, and dry weight (DW) was determined (Dhanda and Sethi (1998). Using the formula below, the relative moisture content was calculated.

$$\mathrm{RMC}\ (\%) = [(\mathrm{WW}-\mathrm{DW})/(\mathrm{TW}-\mathrm{DW})]\mathrm{x}100$$

Membrane Permeability (MP)

The leaf samples taken before the harvest (0.1 g) were rinsed firstly with tap water and then with pure water. The plant samples were kept in 10 ml pure water at 40°C for 30 minutes, and EC (C1) of solution was measured (C1). EC was measured again for the sample that was kept in water bath at 100°C for 10 minutes (C2), and MSI was calculated using the formula below (Premchandra et al., 1990 and Sairam, 1994).

$$\mathrm{MP} = [1-(\mathrm{C1}/\mathrm{C2})] \mathrm{ x } 100$$

Determining the Lipid Peroxidation (MDA)

In order to show the stress effect on plant and to compare with the levels of gene expression, malondialdehyde (MDA) analysis was employed in determining the lipide peroxidation (Hodges et al., 1999). In calculating the values read on spectrophotometer and percentage MDA levels, the formulas below were used.

ABS=Absorbance

MDA=Malondialdehyde

1- ((ABS 532+TBA)-(ABS 600+TBA)-(ABS 532-TBA)-(ABS 600-TBA)= A

2- ((ABS 440+TBA)-(ABS 600+TBA)x0,0971=B

3- nmolMDA/ml= (A-B/157000)x106

RNA Isolation and qRT-PCR Analysis

All of the plant samples taken after the stress treatments were kept in refrigerator at -80°C until the RNA isolation procedure. For each of stress conditions, 3 biological replications were employed. For RNA isolation from the plant samples, the protocol of manufacturer company was followed (Total RNA Extraction Kit; Vivantis Malaysia). RNA concentration and amount were determined using the Spectrophotometer (Thermo Multiskan™ GO Microplate) with nano-drop feature. Moreover, RNA samples were swiped into 1% agarose gel. cDNA synthesis was determined using M-MuLV Reverse Transcriptase RNase H- (Vivantis, Malaysia) in accordance with the guidelines provided by the manufacturer. For real-time implementations, Thermo SYBR Green Master Mix and Real-Time PCR (LightCycler® Nano Roche; Mannheim, Germany) were used, and the reaction was performed in following order; at 95°C for 10 min. and then at 95°C for 15 s, 55°C (binding temperature varies depending on the primer) for 30 s and 72°C for 30 s for 40 cycles. Melting curve was obtained by heating the amplicon from 55°C to 95°C. Transcript abundance of PIP2.1 and PIP2.2 was analyzed using specific primers (Baiges et al. 2001). In order to determine the change in expression level, *VvActin* reference gene was used.

Statistical Analysis

The significance of differences was analyzed sing "Independent t-Test" by using JMP 13 software.

RESULTS AND DISCUSSIONS

Relative Moisture Content (RMC)

The effects on relative moisture content are seen in Figure 1 for 'Horozkarası' variety. Accordingly, it was determined that the treatments have statistically significant effect. When compared to control group, the relative moisture content of plants grown in drought treatment was found to significantly decrease (p:<0.05).

In Figure 1, the change in RMC by drought is presented.

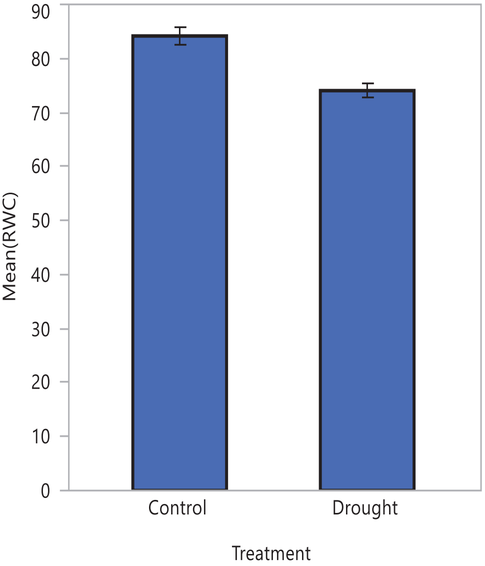

Figure 1. The effects on relative moisture content of 'Horozkarası' variety

Membrane Permeability (MP)

In Figure 2, the effects on membrane permeability for 'Horozkarası' grape variety can be observed.

Accordingly, the treatments were found to have significant effect.

When compared to control, 1% change in membrane permeability of plant grown in drought treatment was found to be statistically significant (p:<0.05).

In figure 2, the change in membrane permeability (%) by drought is presented.

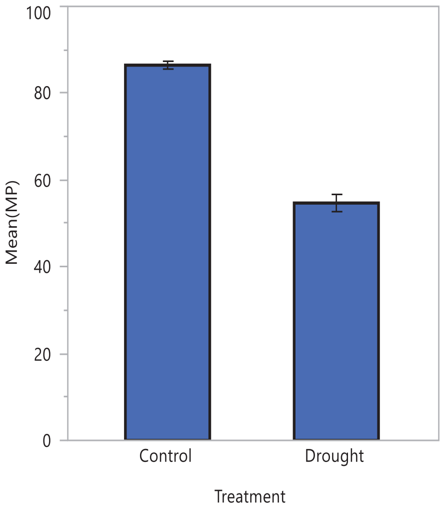

Figure 2. The effects on membrane permeability for ‘Horozkarası’ grape variety

Lipid Peroxidation (MDA) Level

In Figure 3, the effects on lipid peroxidation level for ‘Horozkarası’ grape variety can be seen. Accordingly, the treatments were found to have significant effect. It was observed that the increase in MDA level of plant grown in drought treatment was found to be statistically significant ($p:<0.05$). The change in MDA level (nmol ml^{-1}) by drought is presented.

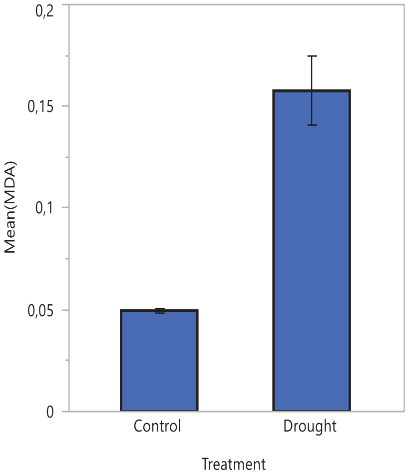

Figure 3. The effects on lipid peroxidation level for ‘Horozkarası’ grape variety

Aquaporin gene expression

PIP2-1 and PIP2-2 expression levels were examined in root and leaf samples taken from ‘Horozkarası’ grape variety exposed to stress. While significant decreases were observed in PIP2-1 and PIP2-2 in stress treatment in leaves, increases were observed in expressions of PIP2-1 and PIP2-2 genes in drought treatment in root ($p:<0.05$) (Figure 4). The role of AQPs in regulating plant’s water status under water stress is a complex issue, because the expression of different AQP genes may be stimulated, decreased, or unchanged under abiotic stress (Yamaguchi et al., 1992; Maurel 1997; Kirch et al., 2000; Kawasaki et al., 2001). In grapevine, highly vigorous rootstocks have higher fine-root hydraulic conductivity partly due to the higher aquaporin expression and activity (Gambetta et al., 2012). AQPs in plants often show a tissue/organ-specific expression (Tyerman, Niemietz & Bramley, 2002). Chemical signaling such as ABA and hydraulic signaling via aquaporins regulate the stomatal conductance. Relative abundance of transcripts in roots and leaves strongly depended on which AQP and the treatment given (Galmés et al., 2007). In non-stressed plants, PIP1.1 was more abundantly expressed in the roots compared to leaves, consistent with the observations described for other PIPs in the roots of other varieties (Weig et al., 1997; Jang et al., 2004). Plants exposed to short-term water stress showed an enhanced ratio of root-to-leaf AQP expression, particularly for the moderate stress treatment. In study of Aroca et al. (2012), it was emphasized that plasma membrane intrinsic protein (PIP) sub-group played important role in absorbing water from the soil. It was found that own-rooted grapevine cultivars that differ in their response to soil water deficits via differences in the regulation of the leaf water potential also vary in their root response to water soil deficits in terms of aquaporin expression (Vandeleur et al., 2009). In parallel with the results obtained in present study, it was observed that the expression of aquaporin genes in the leaves decreased to limit water loss via transpiration, whereas the expression of the same aquaporin genes increased in the roots to enhance water uptake to avoid plant water constraints when water deficits occurred.

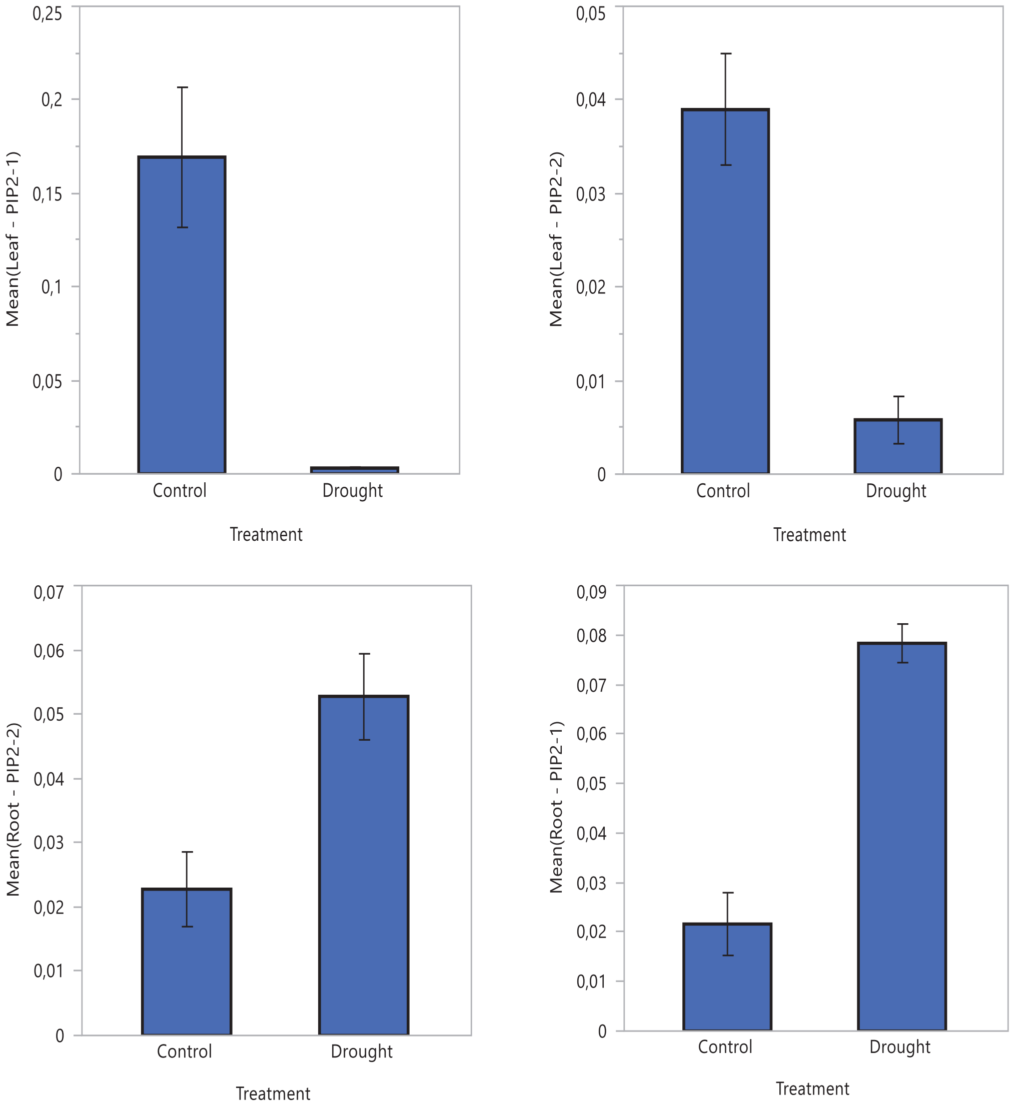

Figure 4. PIP2-1 and PIP2-2 expression levels in root and leaf samples taken from 'Horozkarası' grape variety

CONCLUSIONS

In this study, it was determined that the 'Horozkarası' grape variety, which has successfully adapted to low annual precipitation levels and arid summer conditions of Southeastern region of Turkey, gave physiological, biochemical and gene-level responses to drought and well-adapted to semi-arid regions. For this reason, it is believed to have potential to contribute to the breeding studies on developing varieties that have high tolerance to the drought.

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