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Effect of Foliar Spraying of Ascorbic Acid on Cell Membrane Stability, Lipid Peroxidation, Total Soluble Protein, Ascorbate Peroxidase and Leaf Ascorbic Acid Under Drought Stress in Grapes

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Abstract

Drought is the most common environmental stress in vineyard occurs in soils with low water content, even in irrigation condition. To evaluate foliar spraying of ascorbic acid under drought stress, a research during 2012 to 2013 years in the natural environment, and one year after the establishment of the cuttings in clay loam soil was conducted. Cultivars with two-level white seedless and khoshnav, two levels of water stress control (moisture content of 75% field capacity) and drought stress (moisture content of 25% field capacity or irrigation after 7 weeks) with three replications by factorial design in a randomized complete block design were studied.Drought was affected significance difference at 1% statistical level on total physiological characteristics. While the use of AsA had no effect on stability of cell membranes, lipid peroxidation, total soluble protein and leaf ascorbic acid, however was effective significantly different at 5% level ascorbate peroxidase. Cultivars in this study showed significant differences at 1% level in leaf ascorbic acid. AsA 300 mg per liter in Khoshnav under full irrigation caused increase leaf ascorbic acid and decrease ascorbate peroxidase activity as compared to control and this concentration in White seedless under full irrigation caused increase membrane stability and under drought stress decrease total soluble protein compared to the control. The results showed that ascorbic acid can affect some physiological responses of grapes.

Keywords: Grape, Drought, Antioxidants

Introduction

Important horticultural crops in temperate zones rainfed and in irrigation condition in semiarid regions are planted, subjected to frequent drought periods with little rainfall or deficit irrigation. Drought stress causes a detectable changes in plants and the destructive effects of drought stress vary depending on the investigation of the physiological responses (Schultz an Stoll, 2010). Unlike other fruit trees, grapes one of the oldest and and the most extensive crop in temperate rain fed and the most widely crop in irrigation areas of semiarid throughout the world which is more frequently subjected to periods of drought. In such

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a situation, understanding of stress responses in grapevine can be main role in the growth, performance and sustainability of viticulture (Bonda and Shutthanandan, 2012). Changes in the structure of cell membrane lipid induced by lipid changes is increased membrane permeability to ions and macromolecules (Popova et al., 2009). Ascorbate may play a role in regulate hydrogen peroxide produced to neutralize the hydrogen peroxide in order to stomatal closure (Chen et al., 2003). ABA induced production of hydrogen peroxide that acts as signaling to accelerate the closing of stomata. Hydrogen peroxide by ascorbate peroxidase becomes into water and oxygen. The increase in anti-oxidation enzyme activity, the concentration of superoxide and hydrogen peroxide reduces and the risk of hydroxyl radical decreases (Morello et al., 2005). Grapes are one of the most important fruits of the world in terms of production and area under cultivation. Among the various varieties that are grown in Iran, White seedless is the world's best cultivars and its importance in the production of raisins, molasses and fresh table extensive research areas in order to achieve maximum performance and quality of the fruit is has prepared extensive research to achieve maximum performance and quality of the fruit. In this context, apart from methods such as breeding programs, the use of chemicals such as ascorbic acid as well as a quick, easy and inexpensive method it can be used to increase the quantity and quality of this product. The positive effect of ascorbic acid on various aspects of the structure and its role in plant, encourages many researchers to use it to improved systemic resistance against hazardous risk factors that may exist in the surrounding environment of plants growth. According to Iran is located in arid and semi arid region, drought resistant plants with high performance is essential. To accomplish this, awareness of defensive situation against drought stress is important. Several greenhouse studies to investigate grape biochemical reactions under water stress have done, however, this type of research has performed in natural conditions that confidence in the results and information obtained will be higher. An important feature of these results is tested under natural conditions that increases ensure of present results compared to the greenhouse testing.

Material and Methodes

To evaluate the method of foliar spraying of ascorbic acid on lipid peroxidation, changes in stability of cell membranes, total soluble proteins and anti-oxidation compounds in grapes under drought stress a research during 2012 to 2013 in the research field of Horticultural Science, University of Kurdistan in the natural environment one year after the establishment of the cuttings in clay loam soil was carried out. In this study, cultivars with two-level white seedless and khoshnav, two levels of water stress control (moisture content of 75% field capacity) and drought stress (moisture content of 25% field capacity or irrigation after 7 weeks) with three replications by factorial design in a randomized complete block design were studied. Geographical characteristics of the study area with geographic coordinates 46 degrees 59 minutes east and was 35 degrees 16 minutes north of Sanandaj. Training was conducted in the first year with only two arms per vine and in the second year with two-branch and three buds on each branch was pruned. Stability of cell membranes (Ghoulam et al., 2002), lipid peroxidation (Heath and Packer, 1968), total soluble proteins (Bradford, 1979), ascorbate peroxidase (Nakano and Asada, 1981) and leaf ascorbic acid (Omaye et al., 1979) was measured. Information obtained from a factorial experiment in a randomized complete block design with three replications were analyzed using MSTATC and SAS software. Means using Duncan multiple range test with probability of 5% level were compared.

Results and Discussion

The results showed that the stability of the cell membrane is affected by drought and this respect has a significant difference at the 1% level in addition significant difference at the 5% level have between

interaction cultivar and drought stress and other treatments that have no effect on cell membrane stability. Under drought stress significantly decreased stability of the cell membrane in White seedless while a significant decrease was not observed in the loss of cell membrane stability in Khoshnav (Table 1). Based on the results reported by ghaderi et al (2011), in -1.5 MPa of soil water potential relative stability of the cell membrane (MSI) in Sahany, Farkhi and white seedless in the same treatment decreased relative to the control that these results are consistent with the present experiments. The results showed that lipid peroxidation was affected by drought. Significant difference in the level of 1% have between control and drought stress and other treatments had no effect (Table 1). Ionic leakage as an indicator of the stability of cell membranes and membrane integrity and causing drought tolerance in plants (Xu et al., 2008). MDA increased in leaves of plants tolerant and drought-sensitive crops like wheat and Kentucky have observed (Bian and Jiang, 2009) that this findings were consistent with this research and under stress conditions, no significant difference in the rate of cell damage in susceptible and tolerant to drought stress in grapes was observed (Table 1). Some metabolic activity may be reduced during dehydration. Maybe compounds that are the structure MDA levels in these plants is reduced (Jubany-Maria et al., 2010).

The results showed that the total soluble proteins affected by drought stress, interaction between cultivar and ascorbic acid and the interaction between cultivar and drought stress and other treatments had no effect on it (Table 1). AsA treated with either 150 or 300 mg per liter decreased in white seedless total soluble proteins while in Khoshnav 300 mg per liter of ascorbic acid increases the amount of protein. Drought affects the metabolism of amino acids. Amino acid content in leaves under drought stress often increases. This may is result in the de novo protein synthesis or protein breakdown. In investigation effect of drought stress on anti-oxidation enzyme activity in barley under water deficit, drought stress significantly increased the total protein content (Haddad and Salekjalali, 2000) that these results are consistent with the present experiment. Ascorbic acid with oxygen free radical scavenging is reduced damage to fatty acids, protein and thus reduce the destructive effects of drought stress (Lin and Wang, 2002).

The results showed that ascorbate peroxidase enzyme activity affected by drought stress, ascorbic acid, interaction between cultivar and ascorbic acid and the interaction between cultivars and drought stress and ascorbic acid and other treatments had no effect on it (Table 1). The use of ascorbate in non-stress conditions both the 150 and 300 mg per liter in white seedless the enzyme activity was increased in comparison with control. It seems that the response of cultivars in activating anti-oxidation enzymes different from each other in drought stress and non stress conditions. Different studies under stress conditions, changes in mitochondrial oxidation and reduction have shown. This phenomenon leads to the generation of reactive oxygen species that plays an important role in plant defense mechanisms (Huckelkoven and Kogel, 2007). Different levels of stress compared to control in barely had no effect on ascorbate peroxidase activity (Salekjalali et al., 2012). Effects of drought stress in wheat ascorbate peroxidase was not effectively (Gong et al., 2005). The results showed that leaves ascorbic acid were affected by cultivar and drought stress and there was significant difference in the level of 1% in addition there was significant difference in 5% level between interaction cultivar and drought stress, and the interaction between cultivar and ascorbic acid and drought stress and other treatments had no effect on it (Table 1). Ascorbic acid in White seedless under control further than Khoshnav, however it significantly decreased in drought stress. So in this situation was equal to the rate of Khoshnav. The amount of ascorbic acid in Khoshnav were not observed significantly different between control and drought conditions. The use of ascorbic acid in full irrigation in Khoshnav increased (Table 1). de novo synthesis of ascorbic acid and its recycling is important in maintain a high concentration. Among the non-enzymatic antioxidants ascorbic acid is one of the most important compounds that key role in plant cells. Ascorbic acid has ability to remove all kinds of reactive oxygen species (Foyer, 2001). Several correlations between water deficit and the amount of soluble intracellular antioxidants have been reported

(Jaleel et al., 2008e). It is believed that ascorbic acid plays a role not only in scavenging superoxide, but also in hydroxyl ions. With increasing severity of drought stress in cowpea, a decrease in ascorbic acid content in leaves was reported (Akhila et al., 2008). Drought stress in *Cochlearia atlantica* and *Armeria maritima*, resulting in a reduction amount of ascorbic acid in both cultivar (Buckland et al., 1991) that these results are consistent with the present experiment.

Table 1: Results meanes of MSI, MDA, Total soluble protein, APX and AsA combined drought stress and leaf spraying of ascorbic acid in two grape cultivars

AsA (mg/gFw)	APX (Changes in absorbance per minute/ mg protein)	Total soluble protein (mg/gFw)	MDA (nMol/gFw)	MSI (%)	AsA	Treatment	**Cultivar**
0.97	0.12	0.42	0.22	87.48	AsA0		
0.85	0.24	0.26	0.24	89.72	AsA150	**Control**	
0.81	0.23	0.23	0.22	90.58	AsA300		**White seedless**
0.55	0.13	0.64	0.48	84.41	AsA0		
0.70	0.07	0.51	0.5	84.67	AsA300	**Stress**	
0.63	0.11	0.31	0.46	85.70	AsA300		
0.48	0.26	0.22	0.26	87	AsA0		
0.36	0.22	0.24	0.23	86.04	AsA150	**Control**	
0.80	0.08	0.35	0.28	87.93	AsA300		**Khoshnav**
0.46	0.08	0.55	0.49	85.79	AsA0		
0.44	0.12	0.54	0.45	85.31	AsA150	**Stress**	
0.42	0.05	0.67	0.52	85.50	AsA300		

Conclusion

In this study two cultivars of grapes were subject to combined drought stress and ascorbic acid. Responses cultivars under drought stress (25% fc) and full irrigation (75%fc) were different from each other. Khoshnav in drought stress, higher levels of total soluble proteins was shown and White seedless in full irrigation, decreased total soluble protein.In khoshnav, no change in cell membrane stability, it seemes to suggest that this cultivar is more tolerant in water shortage conditions.

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