The Role of Cosmotic Adjustment On Drought Stress of Some Olive Cultivars

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Abstract

Generally native genotypes of Olives have different reaction against drought stress. The current research was conducted in order to determine the metabolic index under drought stress conditions in olive cultivars (Nabali, Gordal, Arbequine, Zard, Roghani and Feshomi). It was designed on a RCBD experiment based on factorial with full irrigation and drought stress treatments in three replicates. The evaluated parameters in this research were several metabolic solutions, including, sucrose, starch, proline, and betaine. There were significant differences between control treatment and drought stress in different cultivars for the evaluated parameters. The amount of sucrose in three continual experiments showed significant differences between two control treatments and drought stress. Highest Sucrose was measured in Gordal (40.83 mg/g\(^{-1}\)) and lowest amount was measured in Roghani cultivar (18.88 mg/g\(^{-1}\)) in the fresh weight samples. Sucrose content increased under drought stress intensity and starch content decreases under water stress condition (20.83 and 37.75 mg/g\(^{-1}\) respectively). Proline content increased under drought stress conditions in the cultivars which is Nabal; 12.33 and Arbequine; 9.64 Micro mol/g\(^{-1}\) in the fresh weight samples respectively), however, it’s the reason for differences of physiological and bio-chemical behaviors in these cultivars. Betaine content increased under water stress conditions (101.342 Micro mol/g\(^{-1}\) in fresh weight samples), so far that contrary to the metabolic solutions betaine is not quickly metabolized after drought stress and it may be considered as an indices of previous stress inside the plant, and it could be concluded that Gordal and Nabali were the best cultivars compared to Arbequine, Zard, Roghani and Feshomi due to drought resistance levels.

Keywords: Olive –Drought Stress –Proline–Trehalose –Betaine

Abbreviation: MPa; Megapascal, nm; Nanometer, µl/g; Milligram per litter, FW; Fresh weight. DW; dry weight,

1. Introduction

From economic points of view water is considered as a very important resource in most parts of the world, particularly in arid and semi-arid areas and nations have always been suffering from the risks of water shortages. Recently the need to water in all industrial sectors is being increased and horticultural industry is one of the important economics activities endangered by this situation (Paschal and Zengerke, 1997). Plants compatibilities to control water situation and their resistance against drought are different. Generally native genotypes, with regards to their ecological conditions in different seasons are able to adapt themselves with environmental changes and maintain their efficiency for the same environment (Chaves et al., 2003). Researchers believe that olive is an ever green plant and its cultivation in the Mediterranean region is a common practice. Cultivation of this plant is endangered by several unfavorable environmental stresses. For example, water shortage, cold, salinity and etc. are stress causing factors which limit the plant growth. Therefore physiological and biochemical processes of these plants are capable to endure stress mechanism (Wang and Stutte, 1992). Temperature is another important environmental factor for enzyme synthesis (Balaji and Ebenezer, 2008). However drought stress decreased photosynthesis process in olive trees, because water acts as electron release (Angelopoulos et al., 1996). Furthermore in metabolic and physiologic cycles, affecting cellular growth, proline synthesis, opening of stomas, photosynthesis and transfer of nutrients are effective. Most of plants have different mechanisms, when responding to drought stress. Accumulation of metabolic materials is one of the compatibility mechanisms. These compounds have low molecular weight and high solvability rate which are non-toxic in high rate of concentration. Accumulated compatible solutions are different in cultivars and they include betaines, polyoils, carbohydrates, like mannitol, sorbitol, trehalose, aminoacids, like proline (Smirnoff,1998) and these solutions are accumulated in sytocells, where avoid inactivation of metabolites processes (Stoop et al., 1996). Accumulations of compatible osmolitos to regulate osmosis reduce the osmotic potential and allow the cell to absorb more water from environment. Therefore it will have quick compensation effect on water deficit in living creatures (Ramanjula and Sudhakar, 2000). Olive tree is one of plants, which endure drought stress in very low potentials (~6 to-8MPa) (Xiloyannis et al., 1999). Decrease of osmotic potentials up to ~6 MPa is reported in two cultivars of Koroneiki and...
Mastoidis. Significant decrease of osmotic potential in this plant is due to accumulation of dissolved materials in leaf which is an indication of osmotic adjustment in olive (Chartzoulakis et al., 1999). When the plants are exposed to drought stress, the level of metabolism cell must be such to tolerate the stressing factors. The first solution is to stimulate the structure of molecules, which did not exist before and along with this action the new characteristics required for cell’s metabolism under stress conditions are also stimulated. Secondly the inner environment of the cell is optimized in order to provide the best enzymic system operation by accumulating organic compounds with low molecular weight, protective properties or osmotic adjustment (Kuznetsov and Shevyakova, 1999). It seems that osmotic adjustment phenomena is one of the most important compatibility mechanism of trees against water deficit, which has been in connection with olive, pistachio, almond and peach trees. These phenomena caused plants resistance under drought stress (Ramanjula and Sudhakar, 2000). Some scientists believe that molecular structure, resisting drought stress in plants lead to better or more complete recognition of plant metabolism. This study includes the expression of genes in control plants and those under treatment. Expositions of several of these genes cause synthesis of osmotic preservatives, like aminoacids, proteins, sugars and betaines (Iturriaga et al., 2000). Finally these substances may play an important role on osmotic regulation, protection of large molecular structures (protein compounds), collection of free radicals, ionic adjustment, cellular embrane strength, CO₂ content stabilization and photosynthesis capability protection by environmental stresses (Smirnoff, 1998). The main objective of this study was determination of young olive plants capability towards compatibility under drought conditions by applying different water deficit stress treatments in order to achieve detailed identification of metabolic and bio-chemical mechanisms affecting this phenomenon.

2. Materials and Methods

In order to study the effect of drought stress in six olive cultivars, including Nabali, Feshomi, Arbequine, Zard, Gordal and Roghani and experiment was designed based on factorial and RCBD in three replicates, with two treatments (full irrigation) and drought stress (-1.5MPa), with three alternate course (drought stress and recovery). The 36 olive of the cultivars with two years old were selected in this study. It was conducted in a green house condition with humidity between 22 to 31 percent. A soil media of sand and agronomy soil was used (1:1:1v/v/v). Gypsum blocks were used to evaluate the moisture of the pots soil and start irrigation treatments. The apparatus was adjusted under -15 bar pressure and the amount of moisture in Gypsum block was noted. This moisture has been the basis for determination of irrigation time during the course of growth (Alizadeh, 2002). This study was conducted in summer to studying the effects of drought stress on the metabolic and physiologic characteristics under various treatments.

To measure the amount of soluble carbohydrate (sucrose and trehalose) amount of fresh olive leaves were distributed and then smashed in mortar. Total of 0.5g of smashed olive leaf and 5ml 96% ethanol were added into each test tube. Then they were centrifuged for 15 minutes at 4500 rpm. Carbon active was added to remove green color of solution and it was heated for 10 to 15 minutes and then the solution was filtered.

Amount of 1one milliliter of the above solution were taken and 6 ml anthrone was added after water-bath process on a hot water bath and cooling, then sulfuric acid was added up to 10 ml.

The absorption was measured using spectrophotometer in 626 nm. A method for measuring insoluble carbohydrate (starch) is nearly similar to the method for measuring soluble carbohydrate, with only difference that centrifuge process has been repeated for several times in order to eliminate sugar. The remaining sediment was added 5 ml distilled water and 52% perchloric acid, and then it was centrifuged for 15 minutes with 4500 rpm (Beckman model Allegra 64 R, USA) then after adding sulfuoric acid and anthrone it was left in boiling water bath and when the samples were cooled, their absorption in 626 nm wave long nanometer with spectrophotometer were read (Khochert, 1978).

To determine the amount of proline, 0.5g smashed olive leave along with 3% sulfuric acid were centrifuged with 15000 rpm for 10 to 15 minutes and under 4 centigrade. Then it was added 2 ml ninhydrine acid and 2 ml classical acetic acid. The samples were heated in a water-bath for one hour, when they were cooled, 4 ml toluen was added and vortex for 20 minutes. Proline concentration of the samples was calculated on the standard curve and by using formula. The absorption rate was read by spectrophotometer in 520 nm (Bates et al., 1973).

The betaine content of olive leaves was measured by Grieve and Grattan (1983) method. First of all the plant samples were dried fewer than 55 to 65 °C, then 0.5 g leave mixed with 20ml distilled water and it was put on shaker for 48 hours. One ml of plant concentrate was mixed with 1ml sulfuric acid 2M and placed in ice bath, and after preparing iodine potassium (15.7g pure iodine plus 20g Iodine potassium), 0.2 ml of iodine potassium and iodine were poured into laboratory tube and vortex. The sample was centrifuged for 15 minutes at 10,000 rpm. In each tube the upper phase was taken away and only the crystal part at the bottom of tube was kept. Then the existing crystals were solved with 9 ml. Amount of I & 2 Dichloromethane solvent after vortex process and 2 to 2.5 hours the amount of absorption was measured in 365 nm. A statistical software (SAS) was used to analyze the data and means were comprised by Duncan test.

3. Result

Data analysis of the amount of sucrose, under control treatments, drought stress at 1% level showed significant differences. The results of study indicate that the amount of sucrose in dif-
Different olive cultivars has increased in the first and second stages but in the third stage, when stress increases, the amount of sucrose shows decrease (Figure 2). Different cultivars such as Gordal, Nabali, Arbequine, Zard, Feshomi and Roghani, had the highest amount of sucrose contents respectively and it became significant in the level of 1% (Figure 1). The amount of starch decreased under drought stress and there were significant differences in different treatments. Significant differences results were showed in control treatment, drought stress and olives cultivars in Table 1 ($P \leq 1$). So there is least amount of starch in stressed cultivars of Roghani, Arbequine, Zard, Feshomi, Gordal and Nabali respectively. There was significant difference in the amount of trehalose in control treatment and cultivars under drought stress and the amount of trehalose showed significant difference in 1% level in different cultivars. In this study, the changes in the amount of trehalose indicate that under drought stress conditions, Gordal, Roghani, Arbequine, Zard, Nabali and Feshomi had the highest rates of trehalose respectively (Figure 4). There were significant different levels of proline indicate in the treatments ($P \leq 1$). Nabali, Gordal, Feshomi, Roghani, Zard and Arbequine showed highest amount of proline respectively (Figure 6). Different level of proline was observed and specified in this study. Amount of proline increased in the first and second stages, but it was decreased in the 3rd stage stress. Different levels of betaine were indicated among of control plant, drought stress and olive’s cultivars. The highest of betaine were measured among the Gordal, Zard, Nabali, Arbequine, Feshomi and Roghani respectively under drought stress condition (Figure 6).

**Fig. 1.** Comparison of sucrose content in different olive cultivars under drought stress conditions and control plant in the course of three continual experiments. (F.W: Fresh weight)

**Fig. 2.** Comparison of changes of sucrose content on different time. (F.W: Fresh weight)

**Fig. 3.** Changes of the starch content in different olive cultivars under drought stress conditions and control plant in the course of three continual experiments.

**Fig. 4.** Changes of Trehalose content different olive cultivars under drought stress conditions and control plant in the course of three continual experiments.
Fig.5. Changes of Proline content on different olive cultivars under drought stress conditions and control plant in the course of three continual experiments.

Fig.6. Changes of Betaine content different olive cultivars under drought stress conditions and control plant in the course of three continual experiments.

Table 1. Mean comparison of the parameters in different olive cultivars under drought stress conditions and control plant.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Bataine (Micro mol g⁻¹ DW)</th>
<th>Proline (Micro mol g⁻¹ FW)</th>
<th>Trehalose (mg g⁻¹ FW)</th>
<th>Starch (mg g⁻¹ FW)</th>
<th>Sucrose (mg g⁻¹ FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arbequina</td>
<td>77.07b</td>
<td>9.64 c</td>
<td>94.61ab</td>
<td>1.56c</td>
<td>31.72c</td>
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<tr>
<td>Feshomi</td>
<td>64.12c</td>
<td>10.29 b</td>
<td>76.64 c</td>
<td>2.00a</td>
<td>22.61c</td>
</tr>
<tr>
<td>Gordal</td>
<td>99.25a</td>
<td>10.99ab</td>
<td>111.61 a</td>
<td>2.53a</td>
<td>40.83a</td>
</tr>
<tr>
<td>Nabali</td>
<td>82.96b</td>
<td>12.32 a</td>
<td>93.24 b</td>
<td>2.98a</td>
<td>35.72b</td>
</tr>
<tr>
<td>Roghani</td>
<td>62.33c</td>
<td>10.37 b</td>
<td>96.22ab</td>
<td>1.35c</td>
<td>18.88f</td>
</tr>
<tr>
<td>Zard</td>
<td>85.05b</td>
<td>9.65 c</td>
<td>93.92 b</td>
<td>1.92b</td>
<td>26.29d</td>
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</tbody>
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Times

<table>
<thead>
<tr>
<th>Times</th>
<th>Proline (Micro mol g⁻¹ FW)</th>
<th>Proline (Micro mol g⁻¹ FW)</th>
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<tbody>
<tr>
<td>Time 1</td>
<td>77.57a</td>
<td>8.81 b</td>
</tr>
<tr>
<td>Time 2</td>
<td>80.98a</td>
<td>9.99 a</td>
</tr>
<tr>
<td>Time 3</td>
<td>76.85a</td>
<td>6.93 c</td>
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Treatments

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<tr>
<th></th>
<th>Control</th>
<th>Stress</th>
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<tr>
<td></td>
<td>55.59b</td>
<td>101.34a</td>
</tr>
<tr>
<td></td>
<td>5.78 b</td>
<td>11.38a</td>
</tr>
<tr>
<td>Sucrose</td>
<td>79.99b</td>
<td>92.77a</td>
</tr>
<tr>
<td></td>
<td>79.99b</td>
<td>92.77a</td>
</tr>
<tr>
<td></td>
<td>2.92a</td>
<td>1.20b</td>
</tr>
<tr>
<td></td>
<td>20.83b</td>
<td>37.75a</td>
</tr>
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</table>

4. Discussion and Conclusion

In this study it was specified that under drought stress conditions the amount of sucrose has been increased and it has been significant from statistical points of view. Increase of sucrose in Gordal, Nabali and Arbequina cultivars has been more than other cultivars respectively. Scientists believe that concentration of soluble carbohydrates in olive leave increase under drought stress, it could show that a soluble carbohydrate in this plant has a considerable role in adjustment of osmotic pressure (Morgan, J. M. 1984). The results indicate that when drought stress increases (in the stage 3 of this experiment) not only the amount of sucrose increased but also there was a decreasing process. To justify this subject, Vasseyand et al., (1991) believe that under drought stress conditions stomas close it has been happened because of propagation of Co₂ into intercellular space and this prevent biochemical processes and sucrose synthesis. Tombesi et al., (1986) declare that the amount of soluble carbohydrate in the leaves of Leccino olive cultivar has been increased when the rate of soil water was decreased from 7 to 21 percent. The soluble of carbohydrate was decreased when the soil water content reduced to 4 percent. In the same connection, Pedro et al., (2004) report that severs water shortage decreases soluble sugar concentration in the oak plant. The result of this study is nearly similar to the above studies.

The amount of insoluble carbohydrate (starch) decreases under the effect of water stress condition. In this connection, Fernandez and Moreno (1999) report that olive leaves, under drought stress conditions, try to overcome drought by increasing soluble carbohydrates through analyzing starch. Thus, under drought stress conditions, photosynthesis, in compare with the materials produced in the course of growth, are less affected. Therefore metabolic produces (carbohydrates) except starch are accumulated under stress conditions (Hsiao, 1993). An experiment performed on Lichino olive cultivar confirms this subject (Tombesi et al., (1986). Trehalose was increased under drought stress. Researchers reported the same case of soluble carbohydrates in olive leaves, like, trehalose, sucrose, glucose, reffinose under drought stress conditions (Moing et al., 1999). Scientists believe that the main mechanism of the decrease of osmotic potential is by accumulation of materials, which are not toxic in high concentration in plants. This mechanism leads to the maintenance of cell tourgance and keeps the stomas open Maintenance of cell tourgance by lowering the osmotic potential, can keep the stomas open for photosynthesis process (Bosabalidis and Kofidis 2002).

Proline content of olive considerable increased under drought stress compared with control plants. Ennajeh et al., (2004) in a
Similar study reported that proline content in Chemlali-olive cultivar has been increased under water stress conditions.

Different theories are presented in connection with the reason of accumulation of proline in drought stress condition. Sanchez et al., (1998) reported that proline accumulation helps the plant to survive in a short course after drought stress process and regain its growth when the stress conditions are over. This theory almost corresponds with the present research, because there was drought stress in the third stage and when drought stress was increased, the proline content decreased.

The betaine content increased under the effect of water stress and the amount of this substance in olive cultivars was nearly 78-125 µl/g of dry weight leave and the limit of betaine synthesis has been reported 40-400 µl/g of dry weight leave under drought stress by Rhodes et al., (1993). Several researches believe that most of the plants respond to the drought stress conditions by accumulation of adaptable substances in the cell, including betaine, which adjusts osmotic pressure (Wang et al., 1992). So far that existence of osmotic adjustment in olive, apple, peach, cherry and grape, against drought stress is confirmed (Xiloyannis et al., 1999). In this experiment, betaine accumulation under drought stress conditions in Gordal, Zard and Nabali were measured more than other cultivars. It could be conclude that accumulation of betaine content in Gordal and Zard cultivars could be an adaptable cytoplasm solution for osmotic control. So far those betaines, contradictory to proline, are not metabolized quickly after drought stress and these could be considered as an index of former stresses in the plant (Chartzoulakis et al., 1999). This has to be mentioned that the betaine content is identified in several plants (Rhodes et al., 1993), but the activity of betaine is not reported in olive plant so far.

5. References


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