Increasing temperatures as a result of global warming cause climate change with precipitation regime. The drought, which is felt by the increasing effects of these factors, is among the most important threats limiting the development of plants. In recent years, research on the impact of global warming has been intensified. In vitro studies are one of the most effective methods used in the world in response to stress applications in a short time. In vitro studies should first develop the appropriate method depending on the plant type. In the literature review, no standard method of high temperature stress was found in vitro. Therefore, in this study, temperature grades and exposure times to stresses in plants belonging to some vine varieties grown in vitro conditions (Çalkarası, Öküzgözü and Narince) were determined. As a result of the study, 36 hours at 35°C and 12 hours at 40°C did not lose the vitality of the plants but seriously entered the stress. Therefore, in vitro selection technique provides new opportunities for improving stress tolerance in grapevine for environmental sustainability.

**Keywords**: Grape, Abiotic Stress, Proline, In Vitro, Global Warming

**INTRODUCTION**

All living organisms constantly interact with their external environment by nature [1] and stress is observed if they fail to adapt to an extreme condition they encountered. Stress is investigated under two categories as biotic (pathogens such as viruses, bacteria, and fungi, insects, and herbivores) and abiotic (environmental conditions such as low and high temperatures, drought, salinity, excess water, radiation, various chemicals, oxidative stress, wind, nutritionally-deficient soils) [2,3]. As one of the sources of abiotic stress, the effects of high temperatures on all organisms is observed widely as a result of the global warming-induced climate change and its effects will reportedly continue in the following years.

Viticulture is an important branch of agriculture and may possibly be affected by global warming and the resultant drought in the future. Therefore, considerable changes both in the physiological activities of grapevines and the yield and quality of grapes are expected [4]. Countries that generate high income from grape growing and have developed growing techniques have carried out planning and intensive studies to take precautions against the stress caused by drought and high temperatures effects of which are already present and will be aggravated by global warming in days to come [5].

Turkey is listed among the five regions at an elevated C in each decade [2,3]. While the eastern regions of the country are expected to increase by about 3-4°C, the western regions of the line will be affected by temperatures that are projected to increase by about 3-4°C, while the eastern regions of the line will be affected by temperatures that are projected to increase by 4-5°C. According to the projections, majority of Turkey will be under the effect of a hot and dry climate by 2030 and temperatures will increase by 2 to 3°C [6].

Viticulture and winemaking are intertwined practices. In grapes, the quality changes brought about by soil structure, topographic properties, exposure to sunlight, and water-soil relationship are well-reported. To obtain high-quality wines, grapes should be cultured under conditions suitable for desired outcomes through cultivating in suitable soils and under appropriate climatic conditions and receiving sunlight at an angle suitable for the characteristics of the grapes and water as much as they need [7,8]. All these conditions are described by the ’Terroir’ concept [9]. The basic elements of ’Terroir’ include temperature and humidity regime and the temperature during the growing season of a grapevine is among the factors limiting grape growing. The number of the studies focusing on how the local wine grape varieties grown in Turkey will be affected by the estimated temperature changes is not sufficient.

The South Australian Regional Office of the Bureau of Meteorology (Bureau) defines a heatwave as either 5 consecutive days with maximum daily temperatures above 35°C, or 3 consecutive days with maximum daily temperatures above 40°C. Many viticulturists in southern Australia make vine management decisions based on this definition [10].

The studies until now have mostly focused on the drought stress in grapevines and were carried out under in vitro [11,12,13,14] and in vitro [15,16,17] conditions. In vitro studies enable a more effective observation of the plant behaviors in smaller areas, in shorter periods, and under controlled conditions and thus, are complementary to the studies carried out under field conditions [18,19,20].

No studies were found in the viticulture literature from Turkey or other countries examining the heat stress in the explants grown under in vitro conditions. Although information is available on at what temperature levels the vines grown outdoors are stressed, there is no information on the effects of duration of high temperature levels on the plants grown under in vitro conditions. Therefore, the study aims to determine the temperature levels and durations that are suitable for the plants grown under in vitro conditions in studies on high temperature levels.
MATERIALS AND METHODS

The Procurement of the Varieties and Explants

In the study, Narince, Çalkarası and Öküzgözü, which is the major wine varieties in Turkey, it is used. Çalkarası and Öküzgözü varieties procured from the Manisa Viticulture Research Station and single-node cuttings derived from the Narince variety obtained from the vineyard of a grower in Çarıkız Village of Turhal District, Tokat, Turkey that was monitored and grown in advance were used as the explant sources. The cuttings were kept at + 4°C until planting in a sterilized perlite medium. Then, after surface sterilization with 10% sodium hypochlorite, the single-node cuttings were planted in the perlite medium kept in plant growth chambers at 24-25°C for shooting. Prior to planting, perlite was saturated with pure water and sterilized in an autoclave. When needed, irrigation was continued with ½ Hoagland solution.

In Vitro Propagation

The active buds collected from the growing shoots were brought to the laboratory in sterile pure water. For the surface sterilization of the active buds, first, the buds were kept under running water for 20 min; then, the micro-cuttings each carrying an active bud were kept in a solution containing 10% sodium hypochlorite and 0.01% Tween 20 for 20 min and rinsed three times with sterile pure water. The single-node micro-cuttings were dissected into 0.4-0.5 mm pieces with a scalpel in a vertical air-flow cabinet under aseptic conditions. The micro-cutting samples were cultured in 105 cc glass jars containing basic MS (Murashige and Skoog, 1962) medium with a pH adjusted to 5.7-5.8. The media were sterilized in an autoclave at 121°C and 1.05 atm for 15 min. The micro-cuttings were then transferred to a plant growth chamber at 24-25°C with a photoperiod of 16-h light and 8-h darkness. After a 4-week growth period, prior to re-sterilization, the shoots were cut into pieces that each contain a single node and planted in 300 cc glass jars with 40-45 ml MS medium so that each jar contained a total of 5 plants and stress treatments were not imposed until the plants reached appropriate sizes.

Stress Application

The durations of the stress conditions were determined with the preliminary study carried out with the Çalkarası variety and, then, the three varieties used in the study were exposed to stress for the same durations. As it was the case for the growth chamber, plants with appropriate sizes (5-6 leaves) were placed in a plant growth cabinet (Nüve TK 600 model) at 25°C with a photoperiod of 16 h. Taking the heat stress levels determined by the South Australian Bureau of Meteorology into account, temperatures of 35°C and 40°C were applied.

For the stress treatment at 35°C, the ambient temperature was gradually increased from 25 ºC to 35°C in 5 hours. The time at which the temperature reached 35°C was accepted to be the initial hour and the explants placed in the plant growth cabinet were monitored every 3 hours (Figure 1). The plants were observed to show paleness, yellowing, and intense necrosis. The durations of the stress conditions were determined with reference to these observations. During the period between the first and ninth hours of the stress treatment at 40°C, slight paleness was observed in 5 of 7 plants. Since yellowing was observed at the end of the 12th hour, 12-h stress treatment was deemed appropriate for the stress treatment at 40°C.
Since no method was found for the evaluation of the high temperature-induced morphological changes in plants, a scale was developed in the study. For this purpose, after the heat stress treatments, the plants were transferred to the plant growth chamber at 25°C with a photoperiod of 16-h light and 8-hour darkness and rested in the chamber for 6 weeks. Then, based on the damage on the leaves and shoots of the plants, a 0-4 scale was developed.

According to this
0- no damage
1- 25% of plant leaves and shoots rewind and dry
2-50% of plant leaves and shoots rewind and dry
3- 75% of plant leaves and shoots rewind and dry
4- death

In our study Narince, Çalkarası and Öküzgözü, which is the major wine varieties in Turkey, it is used. 30 plants were used for each stress application.

RESULT AND DISCUSSION

When defining the mechanisms of stress, the type, severity, and duration of stress have been reported to be the most important factors that shape the consequences of these mechanisms [21,22]. Correspondingly, the results obtained in this study revealed that the first stress signs observed in the plants were more affected by the duration of high temperature treatment rather than its level. The results showed that all varieties used in the study were affected by the stress factors and the in vitro plants from the Çalkarası, Öküzgözü, and Narince grape varieties that were exposed to predetermined stress levels and durations showed significant signs of stress (Figure 4,5).

Table 1. Number of damage effects of varieties at 35°C high temperature stress

<table>
<thead>
<tr>
<th>0-4 Scale Damage Scale</th>
<th>Narince</th>
<th>Çalkarası</th>
<th>Öküzgözü</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
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<td>10</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>6</td>
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</tr>
</tbody>
</table>

Table 2. Number of damage effects of varieties at 40°C high temperature stress

<table>
<thead>
<tr>
<th>0-4 Scale Damage Scale</th>
<th>Narince</th>
<th>Çalkarası</th>
<th>Öküzgözü</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<tr>
<td>1</td>
<td>4</td>
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<tr>
<td>4</td>
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</table>

After the stress application, the plant was taken in the growth chamber and after 6 weeks the degree of damage of the varieties was determined. All varieties were affected by high temperature stress. At 35°C there were plants that were not damage (Scale 0 ) in all varieties, but no damage plants were observed at 40°C. The number of deaths plants increased as the temperature increased (Table 1,2; Figure 6,7).
CONCLUSION
The study investigates the effects of the duration of high temperature stress on three different grape varieties that are important for Turkish agriculture and were grown under in vitro conditions. We are of the opinion that the method and scale employed in the study have room for improvement and can serve as models for future studies examining other vine varieties and fruits.

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