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# Investigation of hormonal treatments in physiological alterations of Sistan ruby grapes

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## Introduction

Bunch density is an undesirable trait in ruby grapes, and besides reducing its marketability, it causes the bursting of pressed pods and the spread of fungal infections in grape bunches. On the other hand, the most valuable agronomic characteristic of Ruby grape is its resistance to heat stress in Sistan region. Another valuable feature of the ruby grape that multiplies its commercial value is early ripening. Ruby grape is one of the earliest grape varieties in the world, which ripens in the middle of May or the end of May. At this time, other grape varieties are finally in the stage of cluster formation. Extensive studies have been done on the growth and development processes of Ruby grape grapes. Studies have shown that the reason of high resistance of ruby grape to heat stress is the high expression of HSP family genes. Also, studies have shown that the flowering stage is the most important growth period in the ruby grape in the field of bunch density control, heat resistance and early maturity of the product. Foliar spraying of growth regulators with the effect they have on their internal counterparts in the plant, antioxidant system and source-reservoir relationships, as a solution to reduce the effects of environmental stress at the molecular, cellular, biochemical levels. They are considered physiological and productive. Especially plant hormones, by interfering in signal transduction pathways, leave behind an immediate action. The studies have shown that gibberellin hormone foliar application reduces cluster density in ruby grapes. Also, foliar application of abscisic acid hormone increases the plant's resistance to heat stress.

## Materials and methods

Lichtenthaler's method was used to measure photosynthetic pigments. First, 0.1 gr of plant tissue was ground in 10 ml of 80% acetone and the resulting solution was passed through Whatman No. 1 paper. Finally, the absorption value at wavelengths of 663 nm for chlorophyll a, 646 nm for chlorophyll b, and 470 nm for carotenoids was read by a spectrophotometer. To measure protein by Bradford method, 5 cc of Bradford solution was added to 0.1 cc of protein extract from each sample, and then it was vortexed for 20 minutes and then absorbance was recorded at 595 nm wavelength. To measure the amount of carbohydrates, 0.1 gr of the green tissue of the plant was placed in sealed test tubes with 10 cc of distilled water and heated for 15 minutes in a bain-marie bath at a temperature of $100^{oC}$ after cooling. 1 cc of the samples was taken and 1 cc of 5% phenol and 4 cc of 98% sulfuric acid were added to it. Finally, it was read using a spectrophotometer at a wavelength of 488 nm. To measure proline, their absorbance was read by a spectrophotometer at a wavelength of 520 nm after processing. The activity of catalase enzyme was calculated by the method of Daizi. The activity of guaiacol peroxidase enzyme was calculated according to the method of Daizi. The reaction mixture included 25 mM potassium phosphate buffer, 40 mM hydrogen peroxide, and 20 mM guaiacol. The reaction was started by adding 100 microliters of enzyme extract in a final volume of 3 milliliters. The absorption increase due to the formation of tetragaiacol was recorded at the wavelength of 470 nm for 3 minutes. The activity of ascorbate peroxidase enzyme was calculated by the method of Nakano and Asada. The amount of ascorbate peroxidation was investigated by increasing the absorbance at the wavelength of 290 nm. The reaction mixture included 50 mM phosphate buffer, 0.5 mM ascorbic acid, 1.2 mM hydrogen peroxide, and 0.1 mM EDTA. Enzyme activity started by adding hydrogen peroxide. Measurement of polyphenol oxidase enzyme activity was calculated by the method of Raymond et al. The reaction mixture included 50 mM phosphate buffer and 0.5 mM pyrogallel. The reaction was started by adding 200 µM of enzyme extract in a final volume of 3 ml and at 40 degrees. Polyphenol-oxidase absorbance changes were recorded in 4 minutes at 430 nm wavelength.

## Results and discussion

In general, the flowering stage had the lowest level of photosynthetic pigments compared to the pre-flowering stage and the post-flowering stage. The comparison of three graphs of chlorophyll a, chlorophyll b and total chlorophyll shows that in the stage after flowering, the decrease in the level of chlorophyll a is compensated by the increase in the level of chlorophyll b and vice versa. Under the influence of all three hormonal treatments, the protein level decreases drastically. Gibberellin treatment increased the carbohydrate level with the growth and development of grapes, while abscisic acid treatment increased the carbohydrate level to the highest level in the pre-flowering stage, but the carbohydrate level gradually decreased in the flowering and post-flowering

stages. The comparison of the four factors of photosynthetic pigments, the amount of protein, proline and carbohydrate shows that under hormonal treatment, especially the abscisic acid hormone, plant resistance to heat stress increases. In fact, proline can be used as a source of carbon and nitrogen storage for tissues that are being repaired, an effective compound in regulating and modulating osmotic pressure, a buffer to stabilize pH, a cleaner for reactive oxygen species in the cell, and also act as a protective molecule and play an important role in plant cells in adaptive and protective responses against stresses. The amount of proline under the influence of gibberellin treatment in the post-flowering stage and abscisic acid treatment in the flowering and post-flowering stage clearly shows that the hormonal treatment induces stress conditions in ruby grapes. Despite the increase in the amount of proline, the breakdown of proteins is such that we see a decrease in the protein level. However, gibberellin and indole acetic acid treatments have less effect than abscisic acid in inducing heat resistance. Hormonal treatments of indole acetic acid and abscisic acid showed the most positive role in increasing the activity level of polyphenol oxidase enzyme. It seems that ascorbate peroxidase is the main antioxidant enzyme in ruby grapes under the influence of hormonal treatments. While all three hormone treatments almost decreased the activity level of catalase enzyme in all three growth stages studied. The ruby grape has adapted to the heat stress conditions of its region. The reason for the short growth period of ruby grapes is also rooted in this compatibility. Because the short growth period has the advantage that the ruby grape can protect its clusters from the heat of $50^{oC}$ in June.

## Conclusion

Hormonal treatment, especially the abscisic acid hormone, plant resistance to heat stress increases. However, gibberellin and indole acetic acid treatments have less effect than abscisic acid in inducing heat resistance. Hormonal treatments of indole acetic acid and abscisic acid showed the most positive role in increasing the activity level of polyphenol oxidase enzyme. It seems that ascorbate peroxidase is the main antioxidant enzyme in ruby grapes under the influence of hormonal treatments. While all three hormone treatments almost decreased the activity level of catalase enzyme in all three growth stages studied.

***Keywords**: Antioxidants, chlorophyll, grapes, plant physiology, phytohormones.*

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***Conflict of Interest***

There is no conflict of interest.