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Molecular mechanisms of ambient temperature perception in peppermint plants

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Abstract

Unlike animals that can move when living conditions are no longer favorable to them, plants are mostly fixed. They have therefore developed adaptation strategies to respond to environmental changes by constantly modulating and adjusting their metabolic systems.

Plants must face different types of abiotic aggression or stress and adapt to them: lack or excess of water, high or low light, air pollution, soil salinity, extreme temperatures and wind, The plants produce the major energy products they contain, which cause the light or energy sources, and also the sources of more chemicals and medicines, fibers and other sources. These economically important products are used for the production of bio-renouvelable products. The plants are equipped with an important ecological and environmental value. Thanks to the photosynthesizers, the plants produce oxygen that is an essential element for sustaining all living organisms, animals, and vegetables. All these reasons also include the gardens in the garden, including the development steps at the croissance course, and the regulatory voices that allow the plants to grow and adapt to all changes in the environment. These changes may affect stress biotiques or abiotics. These plants are resistant to stress and can allow them to survive and ensure their growth and recovery.

Vegetable agriculture is directly influenced by biotech manufacturers such as the temperature, photoperiod, availability of water and nutrients, etc. Temperature variations affect the sense of biochemical reactions that improve or stimulate the sleep level of grains and axillary pores.

Keywords: physiological traits, peppermint plants, salinity, heat stress.

Introduction:

Abiotic stresses are environmental conditions (physical or chemical) that adversely affect plant development. They include drought, extreme temperatures (cold and heat), salinity, and oxidative stress. Abiotic stress conditions are detrimental to plant growth and crop yield. Unlike animals, plants cannot move and must therefore endure adverse growth conditions. Many plants have therefore developed more or less extensive capacities to tolerate extreme climatic episodes, such as large temperature variations. The optimum temperature for the growth of many plants is around 20°C, but some can tolerate exposures of 0°C to 45°C. Plants can slightly control their temperature through anatomical structures, such as hairs on the leaves, and through mechanical adjustments, by changing the orientation of leaves, but their temperature is largely dependent on the ambient temperature. Some plant species can continue to grow and reproduce in physical conditions far from the optimal growing conditions. Other plant species restrict their life cycle to a less extensive range of parameters; they are said to be adapted to a particular environment. [1]

For many cultivated plants, the thermal optimum at the beginning of their development is significantly higher than later. The temperature in the period

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immediately following planting affects the subsequent development of the plant. When the soil temperature remains below 6°C at the beginning of vegetation, bolting is favoured. In other cases, the responses to the cold are quite different. For example, winter cereals shown in spring do not produce ears; they are said not to bolt. If the temperature at germination is above 5°C, only vegetative development is observed. On the other hand, if during growth the temperature drops to 0°C for a sufficient time, for example in autumn, heading occurs the following spring. This is called the process of vernalization. The so-called alternative varieties can be sown in spring, but their earing is earlier if germination takes place at low temperatures. As for spring varieties, they are not influenced by the germination temperature. [2]

The completion of each phase of the vegetative cycle of a given plant has its special requirements, especially regarding temperature. One thing is certain that only plants that can develop frost tolerance will be able to survive the winter. Frost can cause traumatic damage.

The formation of ice crystals at the expense of free intra or extracellular water is accompanied by mechanical damage that can lead to tissue death. Many tropical plants do not survive in low temperatures, but higher temperatures than the freezing point. Cereals, on the other hand, can have a high resistance to winter frosts. In addition to atmospheric conditions, the plant itself must be taken into consideration. Depending on the case, tissue freezing occurs at an external temperature above or below the freezing point. The increase in frost resistance occurs through acclimatization of the plant during a period of falling temperature to around 0°C, which ensures a progressive dehydration of the tissues. [3] In general, the resistance of the plant to cold, as well as to drought, is a function of the water retention energy in the tissues. It is greatest during the period of growth arrest in mature tissues. On the contrary, young tissues, rich in water and floral organs, are particularly sensitive, hence the danger of spring frosts for fruit crops.

Plant growth at temperatures above 32°C inhibits chloroplast biogenesis, and temperatures between 30 and 32°C reduce the photoreductive activity of chloroplasts. There is a disruption of lipid-protein interaction, causing disorientation of the thylakoid membrane structure, and a change in the distribution of excitation energy between photosystem I and II. Several enzymes have limited capacity at temperatures above 40°C, while at this temperature leaves decrease their photosynthetic capacity and inhibit starch formation in leaves and fruits. [4]

The study of responses to abiotic stress is therefore of fundamental interest and agronomic interest. Indeed, abiotic stress constitutes a major cause of yield loss for all types of crops. Improving the adaptation of plants to their growing spaces is therefore a major challenge for 21st-century agriculture. Molecular control mechanisms of stress tolerance

Abiotic stress is based on the activation and regulation of stress-related genes. These genes are involved in various events of stress response, such as signaling, transcriptional control, protection of membranes and proteins, and toxic free radical compounds. The response of plants to abiotic stresses is complex, involving many genes and biochemical molecular mechanisms. The scientific techniques used to improve plants and plant products are known as plant biotechnology. They include genetic engineering, or methods of improving plants using modified genes and transferring them from plant to plant. Genetically modified plants are sources of nutrition and vaccines, for example, and some are resistant to diseases, toxic ions, and herbicides. Plant biotechnology can lead to the development of tools to more easily select varieties best suited to particular growing conditions. [5]

1_ Concept of thermal stress:

nutrients and water, and use them at the rate imposed by heat stress, a reduction in the yield of late sowings, linked to a decrease in the number of ears and the average weight of the grain, caused by the effects of high temperatures. They also note that the penalizing effect of heat stress materialized by an acceleration of the development and a reduction in the dimensions of the constituent organs of the plant. The result is a negative effect on the overall productivity of the plant; the decrease in yield due to terminal stress is positively correlated with the reduction in the average weight of the grain and the variation in the number of grains/m². The rise in temperature, late in the plant development cycle, and particularly after anthesis, is a constraint on the increase in yields in semi-arid zones. [6]

The effect of high temperatures is manifested by an acceleration of leaf senescence and the cessation of grain growth, showing that the optimum temperature for grain development and filling ranged from 12 to 15 C° for many straw cereal genotypes. They observed a 3 to 5% decrease in grain weight for each degree increase in temperature from the base of 12 to 15 C°. In the temperature range of 12 to 15 C°, a reduction in filling time was compensated by an increase in filling rate, with little effect on average grain weight.

1_1_Low temperatures

The altitude and a Mediterranean climate impose a very cold and rainy winter; the winter cold limits growth when water is available and lengthens the plant cycle to expose it to the drought of early summer. Late frost damage is very common on cereals, making the adoption of early varieties too risky. The adoption of the strategy of dodging as a means to escape thermal stress at the end of the cycle is not very effective in the case where the selected early genotypes are not genetically resistant to cold. [7]

The sudden drop in temperature, below 0 °C, causes many disturbances within the plant. When the temperature drops sharply, ice crystals form in the intercellular spaces, dehydrating the cells whose water is drawn to these spaces. The plasma membrane loses its specific permeability, and there is a disruption in cellular functioning.

The reversibility of the phenomenon only occurs if the cellular structure is not seriously damaged. During thawing, intact cells rehydrate and become functional again. The dehydration of cells is accompanied by an increase in the concentration of organic substances and mineral salts. If the cold persists, there is leaf drying. Low temperatures reduce growth during the winter, while plants can use the water stored in the soil following the low climatic demand that characterizes this period. [8]

Heat stress is often defined as when temperatures are high or low enough for a sufficient time to irreversibly damage plant function or development. Plants can be damaged in a variety of ways, including low or high day or night temperatures, warm or cold air, or high soil temperatures. When temperatures approach these limits, growth declines, and beyond these limits, growth stops. Heat stress is a complex function that varies with the intensity (degree of temperature), duration, and rates of temperature increase or decrease.

1_2_ Impact of heat stress in plants:

In arid and semi-arid high-altitude areas, heat stress can occur early in the cycle, resulting in a strong reduction in the number of plants emerging per unit area, following the effects of high autumn temperatures. These effects diminish as sowing is done late [9]

The effect of high temperatures on sowing is manifested by a reduction in the length of the coleoptile.

2-method

accuracy of one millimeter. A digital scale (Sartorius BP211D, Germany) was used to measure the fresh and dry weight of the shoots and roots of peppermint plants with an accuracy of one ten thousandth of a gram. In order to measure the dry weight, the plant tissue samples were first dried in air for two days and then placed inside aluminum foil for 48 hours in an oven at a temperature of 65 degrees Celsius. The content of total phenol was measured by Folin-Ciocaltio method [10] the amount of proline was measured by the method of Bates et al. and the amounts of sodium and potassium were measured using the Flame Photometer method [11] The amount of sugar in leaf solution was measured by the method of Kochert the amount of chlorophyll and carotenoids by the method of Lichtenthaler and the amount of calcium was measured by titration with EDTA [12]

All the experiments were done in the form of a completely randomized design (CRD) based on a factorial design in three replications. Data variance analysis was done using a three-way ANOVA test, and data mean comparison was done using Duncan's test in SPSS version 26 software.

3_Results and discussion:

The results of analysis of variance of physiological evaluations (Table 1) showed that the factors of temperature, salinity and time and their interaction had a significant effect on changes in the level of calcium, carotenoid, potassium to sodium ratio, phenolic compounds, soluble sugar, proline and total chlorophyll at the levels of one and has five percent, only the salinity treatment interaction with time did not show a significant effect on proline changes.

Table 1- Variance analysis of physiological traits in peppermint plants under salinity and heat stress.

Sources of Change	Degree of Freedom	Aerial Body Fresh Weight	Root Fresh Weight	Dry Weight of Aerial Parts	Root Dry Weight
Temperature	1	0.759**	42.22**	0.032**	0.193**
Salinity	2	31.8**	6.176**	0.148**	0.154**
Time	2	01.2**	29.55**	0.029**	0.540**
Temperature × Time	2	0.417**	6.11**	0.009**	0.009**
Temperature × Salinity	2	0.171**	6.75**	0.068**	0.017**
Temperature × Time × Salinity	4	0.099**	8.69**	0.054**	0.003**
Time × Salinity	4	0.294**	5.1**	0.018**	0.004**
Error	36	0.005	0.024	0.001	0.002
Coefficient of Variation (CV%)		02/1	1.3	0.98	0.95

Activity of plant extracts, and it may be recommended as a suitable method to increase the antioxidant capacity of medicinal plants [13]

The total phenol in peppermint seedlings at a temperature of 25 degrees Celsius at a salinity level of 60 mM showed a significant decrease in the first 24 hours compared to the control, and over time, its amount increased, so that in 72 hours after the treatment, with an increase of 1.88 times the witness accompanied the ratio. At the salinity level of 120 mM, a significant increase in the phenol level was observed up to 48 hours, but at 72 hours, it was reduced by 1.39 times compared to the control. At a temperature of 35 degrees Celsius, in the first 24 hours, a significant decrease in the level of phenol was observed compared to the same sample at a temperature of 25 degrees Celsius, which had an increasing trend over time. Comparison of the corresponding treatments between two temperatures of 25 and 35 °C showed that the simultaneous effect of salinity and temperature resulted in a greater increase in phenol levels than salt stress alone (Figure 1-D). When faced with an adverse environmental condition, plants stimulate some physiological responses and increase the biosynthesis capacity of various phenolic acids, which ultimately leads to improved plant performance in difficult conditions [14] In the present study, the changes in phenol level in peppermint seedlings under salt and heat treatment, after decreasing in the first stages of stress, showed an increasing trend with time. This indicates that the formation of the biosynthesis processes of phenolic compounds is delayed in mint plants, which makes this plant sensitive to stress conditions. The significant changes in the phenol level at the heat stress of 35 degrees Celsius also confirm this mechanism. Excessive production of phenolic compounds and their intermediates has been reported in tomato plants under different abiotic stresses [15]. The increase of phenolic compounds in different stressful conditions not only protects the plant against oxidative damage but also improves the nutritional values of edible tissues through the accumulation of different antioxidant substances. Therefore, damage concluded that the effects of stress are more pronounced for some reactions, and this affects the allocation of substances in the pathway. Under stressful conditions, plants must decide how to allocate their resources for growth or defense metabolite production.

The amount of calcium under the treatment of different levels of salinity and temperature of 35 degrees Celsius had a significant decrease compared to the same samples at 25 degrees Celsius. The comparison of the corresponding treatments between two temperatures of 25 and 35 °C showed that the simultaneous effect of salinity and temperature caused a greater decrease in calcium level than salt stress alone, so that the calcium level in 72 hours after application of salinity was 120 mM at 35 °C Celsius, reached its lowest level (Figure 1-E). In the present study, the amount of calcium under the treatment of different levels of salinity and temperature of 35 degrees Celsius had a significant decrease compared to the same samples at 25 degrees Celsius. Calcium maintains water content and regulates plant growth by affecting mineral transport and metabolite accumulation.

Salt stress led to a decrease in growth in terms of height and biomass accumulation in soybean plants, which was more obvious in plants with calcium deficiency. In this study, it was found that the availability of calcium under normal growth conditions and salt stress affects the synthesis of proline, glycine betaine, and soluble sugars. Optimal calcium supplements regulate the activity of antioxidant enzymes and the content of non-enzymatic antioxidants (ascorbate, glutathione, and tocopherol) and are reflected in the improvement of oxidative damage caused by salt stress [16]

The results showed that the changes in the amount of total chlorophyll under the treatment of different levels of salinity and temperature of 35 degrees Celsius had a significant decrease compared to the same samples at 25 degrees Celsius. The comparison of the corresponding treatments between two temperatures of 25 and 35 °C showed that the simultaneous effect of salinity and temperature caused a greater decrease in the level of chlorophyll than the salt stress alone, so that the total chlorophyll level in 72 hours after the application of salinity of 120 mM at 35 °C, reached its lowest level (Figure 1-F).

The results showed that at the temperature of 25 degrees Celsius, increasing the level of salinity causes a significant decrease in the number of carotenoids, so that at a salinity of 120 mM, the number of carotenoids was 1.34, 1.37, and 1.67, respectively, compared to the control in 24, 48, and 72 hours. decreased by At 35°C, carotenoids decreased with increasing salinity level (Figure 1-G).

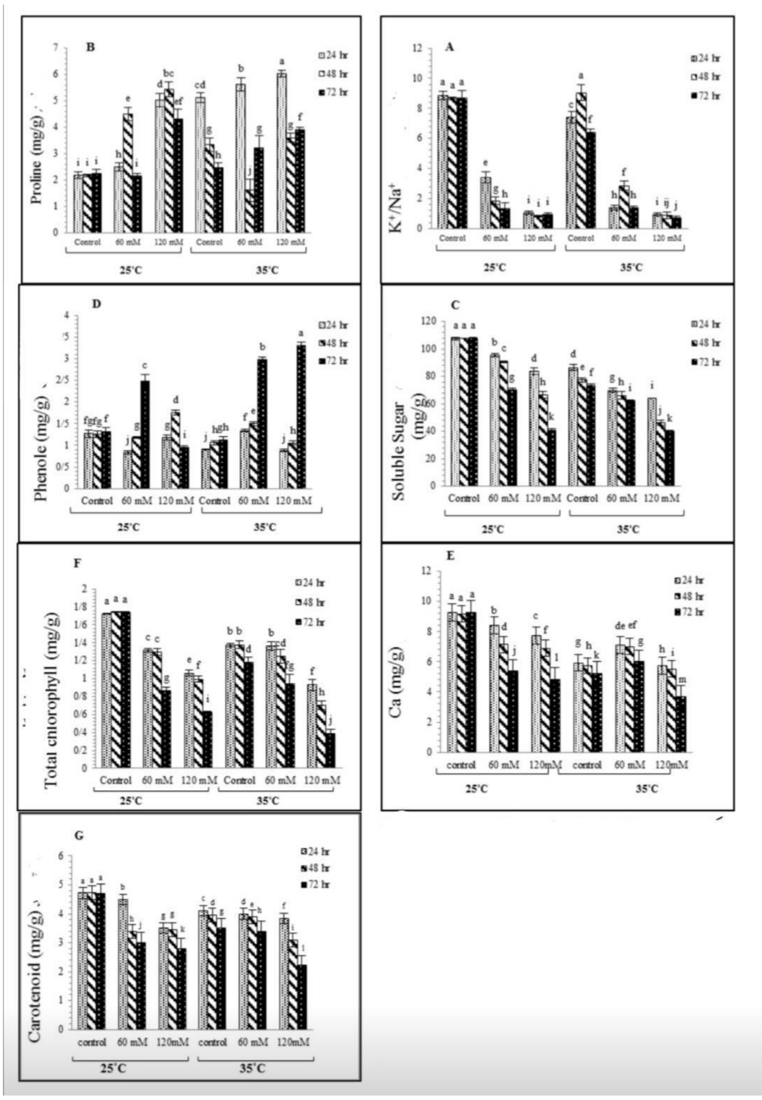

Figure 1: Variance analysis of chemical traits in peppermint plants under salinity

The results of comparing the averages showed that by applying salt stress at 25 degrees Celsius, the number of soluble sugars showed a significant decrease. This decreasing process increased with the increase of salinity level and the passage of time after the application of the treatment, so that in 72 hours after the application of 120 mM salinity, it was associated with a 2.62-fold decrease in soluble sugar compared to the control. At the temperature of 35 degrees Celsius, this decreasing trend was also observed, so that increasing the level of salinity stress, the number of soluble sugars in peppermint plants decreased significantly (Figure 1-C).

Evaluation of chlorophyll and carotenoid content is a tool to interpret stress tolerance in plants. Plants under salinity stress usually produce large amounts of free radicals that lead to chlorophyll degradation. When chlorophyll decreases, photosynthesis is expected to decrease, and so does sugar. The inhibition of photosynthesis by heat stress is interpreted as the inability to maintain Rubisco in an active form [17] In the present study, the effect of salinity treatment at both levels of 60 and 120 mM and temperature treatment of 35 °C decreased the content of chlorophyll and carotenoid, and of course, reduced the level of photosynthesis and reduced the amount of soluble sugar in peppermint seedlings.

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This decreasing process of chlorophyll and carotenoid content under the influence of abiotic stressors has been reported in various studies.

Chrysargyris showed that the simultaneous application of salinity treatments and copper metal toxicity in Mentha spicata reduced the content of chlorophyll and soluble sugar, in the simultaneous evaluation of salinity and heat stress in Jatropha curcas plants that high temperature increases the negative effects of salt stress on the main physiological processes and causes a decrease in the level of photosynthesis and chlorophyll destruction.

4_Evaluation of vegetative traits

The results of the analysis of variance for the evaluation of vegetative traits showed that heat, salinity, and time treatments and their interaction had a significant effect on the changes in fresh and dry weight of roots, fresh and dry weight of aerial parts at the level of 1% (Table 2).

The comparison results of average vegetative traits (Figure 2) showed that with the increase in salinity and temperature, the wet and dry weight of aerial parts decreases. On the other hand, with the increase of salinity and heat level, the fresh weight and dry weight of the root showed a significant increase. So that the fresh weight of the roots at 35 degrees and 120 mM salinity increased by 1.15, 1.17, and 1.45 times, respectively, in 24, 48, and 72 hours. Also, the root dry weight increased by 1.54, 1.33, and 1.34 times.

Figure 2- The mutual effect of salinity and heat stress on root dry weight (A), root wet weight (B), stem dry weight (C), and stem fresh weight (D) in peppermint seedlings. Data are means of 3 replicates ± SE. The same letters indicate no significant difference at the $p<0.05$ level.

Table 2- Variance analysis of vegetative traits in peppermint plants under salinity and heat stresses.

Source of change	df	Total chlorophyll	Proline	Soluble sugar compounds	Phenolic compounds	The ratio of potassium to sodium	Carotenoid (mg/ml)	Calcium
Temperature	1	0.568**	271.3*	363.5711*	0.472**	834.2**	1.33*	56.43**
Salinity	2	450.2**	439.16**	225.5974*	922.1**	120.269**	5.72**	39.13**
Time	2	0.576**	9.035*	354.1598*	062.4*	944.2**	2.13**	12.88*
Temperature × time	2	0.224*	927.3*	230.254*	0.899*	0.863*	1.12*	69.12*
Temperature × salinity	4	0.014*	338.14*	595.130*	1.185*	946.3*	0.079*	0.629
Temperature × salinity × time	4	0.012*	831.2*	565.128*	514.1**	523.1**	0.441*	0.672*
Error	-	0.003	0.092	274.38	0.029	0.116*	0.001	0.004
Coefficient of variation (%)	-	3/4	3/7	9.8	2/3	2/2	95/3	36/2

Table 2- Analysis of variance for morphological characteristics in peppermint under salinity and heat stresses, and ns respectively significant at one percent and five percent level, and non-significant. *and ns: significant at $p\leq0.01$, significant at $p\leq0.05$, non-significant respectively.

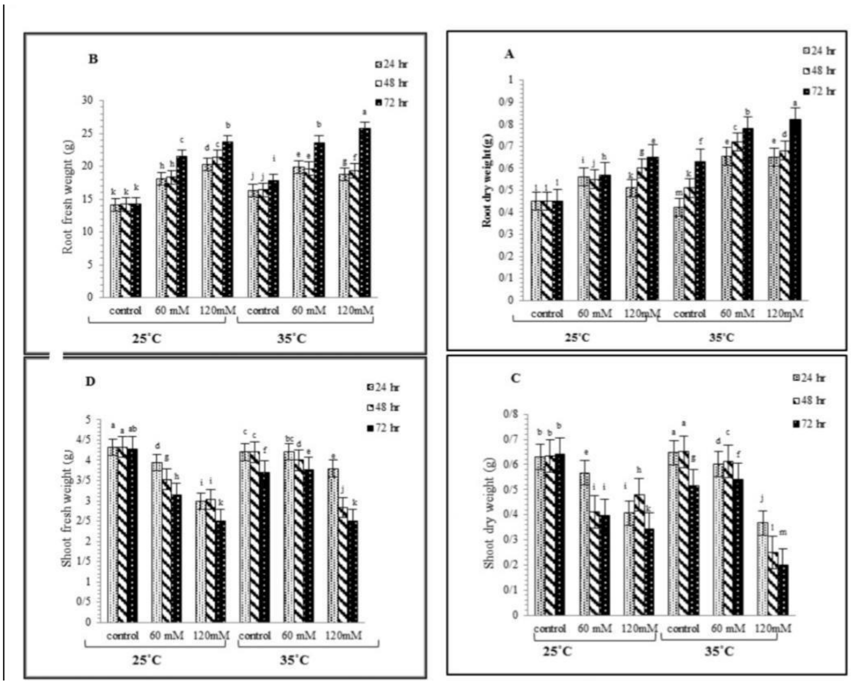

Figure 2- Interaction effects of salinity and heat stresses on root dry weight (A), root fresh weight (B), shoot dry weight (C), and shoot fresh weight (D) in peppermint seedlings. Data averaged 3 replicates ± SE. The same letters indicate no significant difference at the level of $p<0.05$.

The mechanism of plant adaptation to salinity conditions is very complex, one of the causes of plant growth reduction in saline conditions is the accumulation of toxic sodium ions in plant tissues, which causes a decrease in enzyme activity and a change in the distribution pattern of carbohydrates [18] It has been shown in several studies that the root/shoot ratio of many plants increases under salt stress [19] Root development is important for plant survival in dry and desert areas. In this sense, salinity has a positive effect on root growth per hectare. On the other hand, growth reduction in the presence of salt stress is generally explained by two effects. First, water deficit begins immediately after the osmotic potential decreases to a threshold level, and then in the second stage, when salt accumulation in leaves reaches a toxic concentration, leaves die. In some plants, shedding old leaves is a way to prevent the toxic effects of excess sodium salts that accumulate in the leaves. Salt can be secreted in old leaves and the leaves die, which is very important for the survival of the plant [20] In the present study, with the increase in salinity and temperature, the fresh and dry weight of shoot decreased and compared to the fresh weight and dry weight of root, it showed a significant increase.

The significant reduction of vegetative growth indices under salinity and heat stress has been evaluated in various studies. [21] reported reduced growth of canola under salt stress. Similar to these results in barley [22], tomato [23,24], orange [25], and rice [26] have also been reported. [27] Evaluating the results of more than 120 case studies published on the response of different plants to drought stress and heat stress showed that the combination of drought and heat stress significantly reduces the harvest index, shortens the life cycle, and changes the number. The size and composition of the seed affect the yield of the plant. Furthermore, these effects are more severe when the stress combination is applied during the reproductive stage of plants.

Conclusion:

The results of this research showed that salinity and heat stress are limitations for peppermint cultivation. These factors affect plant physiology and reduce its growth and performance. The simultaneous salinity and heat stress decreased the absorption of potassium and calcium, increased the absorption of sodium, caused the breakdown of chlorophyll and carotenoids, caused a decrease in photosynthesis and a decrease in soluble sugars, and ultimately decreased plant growth. Instead, the simultaneous stress of salinity and heat increased the amount of phenol, and this can affect the quality of peppermint. One of the plant's tolerance mechanisms against these two stresses was the production of proline. Agricultural methods, use of biological fertilizers, and cultivation of resistant cultivars can be effective in reducing the adverse effects of salinity and heat stress on peppermint plants.

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