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Research article

Interactive effect of thiourea application on morphological and physiological characteristics in *Cicer arietinum* L. grown at different temperatures

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Farklı sıcaklıklarda yetiştirilen *Cicer arietinum* L.'de tiyüre uygulamasının morfolojik ve fizyolojik özellikler üzerine interaktif etkisi

Abstract: Global warming affects many metabolic events in plants and significantly reduces yield and product quality. One of the physiological events most affected by heat stress is nitrogen metabolism. In this study, 5 and 10 mM thiourea was applied to chickpea plants grown at 15, 25, and 35 °C and it was aimed to determine how the plant can cope with heat stress with nitrogen supplementation. It was determined that the root length decreased significantly at all three temperatures depending on the increasing thiourea concentration, while the shoot length increased at 15 and 35 °C compared to the control. There was a decrease in root fresh weight in all three experimental groups due to increasing thiourea concentrations. Only at 5 mM at 15 °C was a highly significant increase seen over the control. When the experimental groups at all temperatures were compared, the highest chlorophyll a, b, and total chlorophyll values were found at 35 °C. It was determined that SOD activity decreased at all three temperatures compared to the control, while CAT and APX activity increased. A significant increase in NR and GS activity was determined in both thiourea treatments at 25 and 35 °C compared to the control.

Key words: Antioxidant enzymes, chickpeas, morphological parameters, heat stress, nitrogen metabolism

Özet: Küresel ısınma bitkilerde birçok metabolik olayı etkilemekte, verimi ve ürün kalitesini önemli ölçüde düşürmektedir. Sıcaklık stresinden en çok etkilenen fizyolojik olaylardan biri de azot metabolizmasıdır. Bu çalışmada, 15, 25 ve 35 °C'de yetiştirilen nohut bitkisine 5 ve 10 mM tiyoüre uygulanmış ve azot takviyesi ile bitkinin sıcaklık stresi ile nasıl başa çıkabileceğinin belirlenmesi amaçlanmıştır. Artan tiyoüre konsantrasyonuna bağlı olarak her üç sıcaklıkta da kök uzunluğunun önemli ölçüde azaldığı, sürgün uzunluğunun ise kontrole göre 15 ve 35 °C'de arttığı belirlendi. Artan tiyoüre konsantrasyonlarına bağlı olarak her üç deney grubunda da kök taze ağırlığında bir azalma olmuştur. 15 °C'de sadece 5 mM'de kontrol üzerinde oldukça önemli bir artış gözlenmiştir. Tüm sıcaklıklardaki deney grupları karşılaştırıldığında en yüksek klorofil a, b ve toplam klorofil değerleri 35 °C'de tespit edilmiştir. Her üç sıcaklıkta da kontrole göre SOD aktivitesinin azaldığı, CAT ve APX aktivitesinin ise arttığı belirlenmiştir. Kontrole kıyasla 25 ve 35°C'de her iki tiyoüre uygulamasında da NR ve GS aktivitesinde önemli bir artış tespit edilmiştir.

Anahtar Kelimeler: Antioksidan enzimler, nohut, morfolojik parametreler, sıcaklık stresi, azot metabolizması

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1. Introduction

Today, global warming causes an increase in temperature and drought throughout the world, and changing climatic conditions pose a serious threat to plant yield and production (Ahmad et al., 2014). In cases where the temperature is below or above the optimum degree, plants create one or more of the escape, avoidance, tolerance, resistance, adaptation, and adaptation responses by creating physiological, biochemical, metabolic, and molecular changes (Bohnert et al., 2006). High temperature causes acceleration of molecular movements in the cell, loosening of intermolecular bonds, and more fluidity of the cell membrane (Kabay and Şensoy, 2017). Apart from these, when the temperature rises above 40 °C, the photosynthesis level of most plants is seriously affected. In addition, it causes protein denaturation, degradation, and enzyme inactivation and adversely affects the protein mechanism (Akladious, 2014). These negative effects cause starvation, growth inhibition, a decrease in ion flow, increase of toxic compounds and reactive oxygen species (ROS) in the plant (Wahid, 2007). It has been reported that the nitrogen source added externally in some plants facilitates the plant to cope with heat stress (Akladious, 2014).

Plants are affected by low temperatures as well as high temperatures. Low-temperature stress is generally observed at temperatures of 15 °C and below (Kumar et al., 2011). Plants under cold stress synthesize various molecules based on carbohydrates and amino acids, which have antifreeze properties in their sap. However, changes occur in the amount of protein and enzyme activity. There are protective enzymes such as SOD, CAT, and APX, which scavenge the reactive oxygen radicals accumulated in plants exposed to both low and high-temperature stress and minimize the resulting stress damage (Aslantaş et al., 2010). Low and high-temperature stress affects almost all physiological events and chemistry of the plant as well as nitrogen metabolism. It has been stated in studies that ambient temperature is effective on nitrogen uptake by plants and that temperature increase reduces nitrate (NO^{-3}) and ammonium ($NH4^{+}$) uptake (Clarkson and Warner, 1979).

Chickpea (*Cicer arietinum* L.), a plant rich in starch, vitamins, and minerals, is one of the most important legumes cultivated in more than 50 countries on all continents of the world. One of the most important features of leguminous plants, of which chickpea is a member, is that it is very rich in nitrogen maintenance. Plants take nitrogen from the soil in the form of inorganic nitrate and ammonium (Nasr Esfahani et al., 2014). Nitrate, which is taken up by the roots and transported to the target organs via the xylem, is reduced to nitrite by cytosolic nitrate reductase (NR), and nitrite, which passes into the chloroplast, is reduced to ammonium by nitrite reductase. Ammonium taken directly from the soil or converted from nitrate is assimilated into organic compounds via glutamine synthetase (GS)/glutamate synthase (Nasr Esfahani et al., 2014).

This study mainly aimed to investigate the eco-physiological changes caused by high and low temperatures in a *Fabaceae* Lindl. Member, *C. arietinum*, which is important in terms of nitrogen content. It also aimed to determine the effects of temperature change on nitrogen metabolism, to compare the morphological and physiological characteristics of the plant with nitrogen supplementation, and to reveal the extent to which the plant can cope with heat stress.

2. Material and Method

2.1. Abiotic stress applications and morphological measurements

In this study, the seeds of the Işık-05 cultivar of the Chickpea plant were used as experimental material. Seeds were kept in 10% NaClO for 5 minutes and then sterilized by passing through distilled water 3 times. 6 seeds were planted in each pot and the experiments were carried out in 3 repetitions. The seeds were irrigated with pure water only until the cotyledons emerged, and then each pot was watered with 100-150 ml of distilled water (control group) and 2 different thiourea solutions, 5 and 10 mM. The sown seeds were grown for 6-8 weeks at 15/10, 25/20, and 35/30˚C, respectively, in a 16-hour light and 8-hour dark photoperiod. Root-stem lengths (cm), and root-stem fresh and dry weights (g) of the grown plant samples were determined.

2.2. Chlorophyll analysis

To determine the chlorophyll content of the plant samples, the fresh leaf sample of each concentration was weighed 0.05 g and homogenized with 15 ml of 80% acetone, filtered through filter paper, and the final volume was completed to 15 ml with 80% acetone. The absorbance values of the homogenate were measured in 3 replicates at 645 nm and 663 nm wavelengths in the spectrophotometer. The chlorophyll a, b, and total chlorophyll contents of the leaf samples were determined as mg/ml (Arnon, 1949).

2.3. Protein extraction of leaf samples

For the extraction process, 0.5 g of the leaf samples were weighed, powdered with liquid nitrogen, and homogenized with 5 ml extraction buffer. The obtained homogenate was centrifuged at 20000 g for 20 min at +4 °C and the supernatant portion was used for enzyme activity determination. Differently from other enzymes, 5 mM ascorbic acid was also added to the extraction buffer to measure APX activity (Sairam et al., 2000).

2.4. Determination of total protein content

Since protein amounts were used while calculating enzyme activities, the protein contents of all leaf extracts were determined. The Bradford method and bovine serum albumin (BSA) were used as standards for determining the protein concentrations of leaf samples (Bradford, 1976).

2.5. Determination of antioxidant enzyme activities

In the study, SOD and CAT activity of plant leaf samples were determined according to Tepe and Aydemir (2011), and APX, NR, and GS activities were determined according to Cervilla et al. (2007) with minor modifications.

2.6. Statistical analysis

Each study with control and experimental groups was performed with at least 3 independent and 3 dependent replications. For statistical analysis, the p-value was calculated by using a two-way analysis of variance (Two-Way ANOVA) in the Graphpad program.

3. Results

3.1. Determination of morphological parameters

In this study, the control temperature of the study was established as 25°C. It is used in 15°C low-temperature applications and 35°C high-temperature applications. When the temperature experimental groups were compared with the plant samples at the control temperature, it was determined that the root length increased while the stem length decreased in the control group and 10 mM thiourea at low temperature ($p<0.05$; $p<0.001$; $p<0.01$, respectively).

In the application of 5 mM thiourea at high temperature, only the root length decreased compared to the experimental groups at control temperature ($p<0.05$); it was determined that the stem length showed a significant decrease in control, 5 and 10 mM thiourea application ($p<0.001$; $p<0.01$; $p<0.001$, respectively) (Table 1).

Compared to the experimental groups at control temperature, the root fresh weight significantly increased at 5 and 10 mM thiourea at low temperature stress ($p<0.001$; $p<0.01$, respectively), while at high temperature stress the control and both thiourea application it was determined that there was a significant decrease ($p<0.001$) (Table 2 and 3). There was no statistically significant change in root dry weights of all experimental groups.

3.2. Photosynthetic pigment amounts

Low and high temperatures experimental groups were compared with the control temperature, and a significant increase was observed in the amount of chlorophyll a, b, and total chlorophyll only in the control group ($p<0.001$) at 15 °C, and in the control, 5 and 10 mM thiourea application at 35 °C ($p<0.001$) (Fig. 1).

3.3. Total amount of protein

It was determined that while low-temperature stress increased the total protein amount ($p<0.001$), high-temperature stress decreased it ($p<0.05$). The thiourea application with low temperature caused a significant decrease in only 5 mM concentration ($p<0.05$). The interactive effect of thiourea application at high temperatures caused a significant decrease in both thiourea concentrations ($p<0.001$; $p<0.01$, respectively) (Fig. 2).

Table 1. Morphological characteristics of plant samples

		Root Length (cm)	Stem Length (cm)	Root Fresh Weight (g)	Root Dry Weight (g)	Stem Fresh Weight (g)	Stem Dry Weight (g)
15°C	Control	27.89±0.16*	24.25±0.82*	1.54±0.01	0.11±0.003	1.63±0.08***	0.25±0.02*
	5 mM	24.40±0.81	25.02±0.69	2.47±0.06***	0.12±0,000	1.83±0.07***	0.28±0.04
	10 mM	21.18±0.64**	19.42±1.44***	1.00±0.10**	0.07±0.006	1.20±0.17*	0.22±0.02
25°C	Control	23.52±1.29	29.59±0.59	1.60±0,10	0.13±0.007	2.22±0.04	0.45±0.01
	5 mM	22.23±0.99	28.77±1.36	0.97±0.16	0.08±0.014	0.95±0.00	0.22±0.01
	10 mM	16.15±0.07	29.40±0.28	0.78±0.04	0.06±0.007	0.78±0.04	0.27±0.01
35°C	Control	22.50±2.83	14.90±0.14***	0.49±0,08***	0.04±0.002	0.71±0.11***	0.10±0.02**
	5 mM	16.45±3.18*	19.39±0.97**	0.32±0.02***	0.03±0.002	0.92±0.01	0.13±0.02
	10 mM	13.75±1.20	17.70±1.84***	0.41±0.01***	0.04±0.003	0.84±0.15	0.12±0.02

*, 15°C and 35°C experimental groups compared with 25°C control groups. * p<0.05; ** p<0.01; *** p<0.001

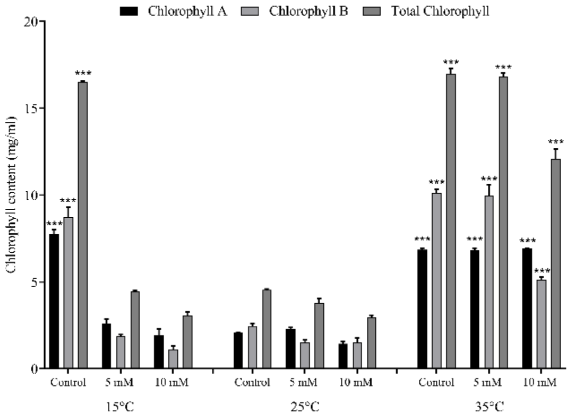

Figure 1. Photosynthetic pigment amounts of chickpea leaves treated with thiourea (mg/ml) (*, 15°C, and 35°C experimental groups compared with 25°C control groups. * p<0.05; ** p<0.01; *** p<0.001)

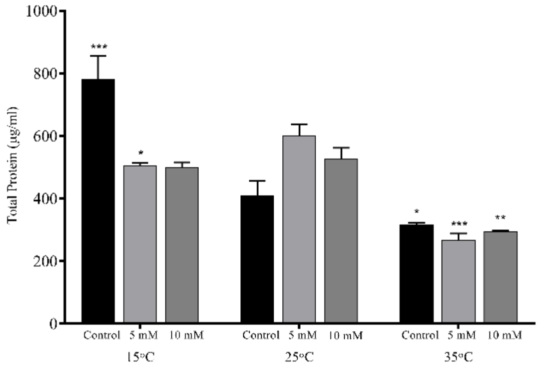

Figure 2. Total protein amounts of chickpea leaves treated with thiourea (mg/ml) (*, 15°C, and 35°C experimental groups compared with 25°C control groups. * p<0.05; ** p<0.01; *** p<0.001)

3.4. Antioxidant enzyme activities

While determining the antioxidant enzyme activities, the experimental groups of plant samples grown at three different temperatures and applied to two different concentrations of thiourea (5 and 10 mM) were compared. When experimental groups in the low and high temperatures were compared with the control temperature, a decrease at low temperature and an increase at high temperature were observed (p<0.01; p<0.001, respectively).

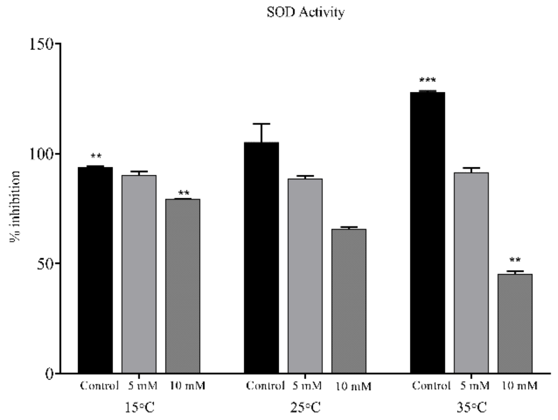

Figure 3. SOD activity of chickpea samples treated with thiourea (%inhibition) (*, 15°C, and 35°C experimental groups compared with 25°C control groups. * p<0.05; ** p<0.01; *** p<0.001)

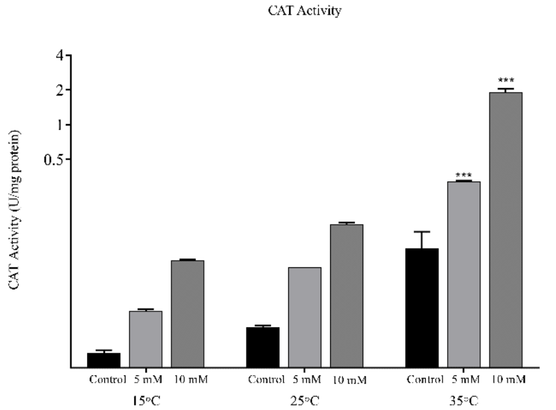

Figure 4. CAT activity of chickpea samples treated with thiourea (U/mg protein) (*, 15°C, and 35°C experimental groups compared with 25°C control groups. * p<0.05; ** p<0.01; *** p<0.001)

Low temperature and 10 mM thiourea application increased SOD activity (p<0.01), and high temperature and 10 mM thiourea application decreased SOD activity (p<0.01 (Fig. 3). The experimental groups of the control temperature were compared, and only the samples applied to 5 and 10 mM thiourea at high temperature showed a quite significant increase at CAT activity (p<0.001) (Fig. 4). While low-temperature application alone decreased APX activity, high-temperature application increased APX activity. (p<0.001; p<0.01, respectively). The interactive effect of thiourea with a low temperature significantly increased the APX activity at both thiourea concentrations (p<0.01;

$p<0.001$, respectively). Similarly, the combined application of high temperature and thiourea increased APX activity only at 5 mM concentration ($p<0.001$) (Fig. 5).

3.5. Nitrogen metabolism enzyme activities

Both low temperature and high-temperature stress alone caused a significant decrease in GS activity compared to the control temperature ($p<0.01$). Both low temperature and thiourea combined application and high temperature and thiourea combined application significantly increased GS activity at 5 mM concentration and decreased it at 10 mM concentration ($p<0.001$; $p<0.01$, respectively) (Fig. 6).

It was determined that there was a significant increase in NR activity at both low and high temperatures compared to the control temperature ($p<0.01$; $p<0.001$, respectively). The combined effect of low temperature and thiourea caused a significant decrease in NR activity in both thiourea concentrations ($p<0.001$), while the combined application of high temperature and thiourea caused a significant increase ($p<0.001$) (Fig. 7).

4. Discussions

4.1. Changes in morphological parameters

Global warming, which is the main subject of the study, causes sudden low temperatures as well as high temperatures. Low temperatures cause changes in the morphology and yield of plants, just like high temperatures. Some plants are extremely sensitive to cold, especially in germination and early seedling development. Cold stress significantly impairs germination and reduces seedling viability, negatively affecting root-stem length and biomass, thereby restricting growth (Croser et al., 2003; Aslantaş et al., 2010; Hussain et al., 2018; Donbaloglu Bozca and Leblebici, 2022). For this reason, the response of plants to stress first emerges in morphological characteristics. In this study, it was determined that low-temperature stress did not significantly affect root length and root fresh-dry weight of chickpea plants. Stem length and stem fresh weight decreased significantly under low-temperature stress. No significant change was detected in the dry weight of the stem (Table 1). In the literature, studies on low-temperature stress, in which different plant species were used as materials, revealed both similar and different results. In a study with chickpea, 12°C cold stress was applied to plant samples and it was found that low temperature reduced root and stem length and total dry weight (Kaur et al., 2008). Another study also reported that low-temperature stress reduced stem length and stem fresh and dry weight in chickpeas (Turan and Ekmekçi, 2011, 2014). The decrease in morphological features observed in the roots and stems of plants under cold stress can be attributed to the fact that cold stress prolongs the cell cycle and reduces division (Rymen et al., 2007; Wu et al., 2022). In addition, cold stress causes a significant reduction in root growth, branching, and root surface area. This, in turn, affects the uptake and transport of water, nutrients, and minerals. For this reason, a significant decrease is observed in the above-ground biomass of the plant (Hassan et al., 2021; Wu et al., 2022).

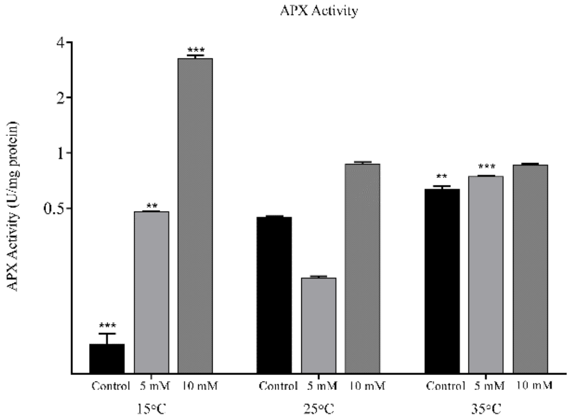

Figure 5. APX activity of chickpea samples treated with thiourea (U/mg protein) (*, 15°C, and 35°C experimental groups compared with 25°C control groups. * $p<0.05$; ** $p<0.01$; *** $p<0.001$)

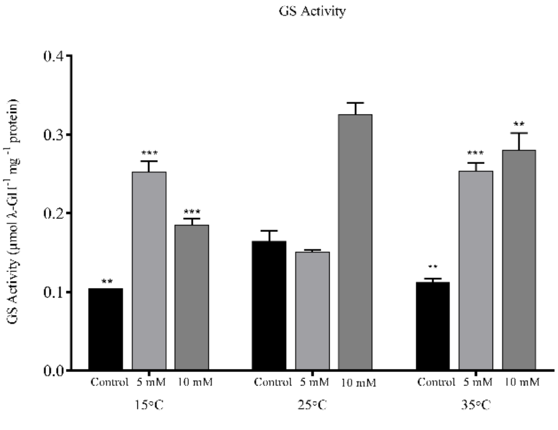

Figure 6. GS activity of chickpea samples treated with thiourea (µmol λ-GH^{-1} mg^{-1} protein) (*, 15°C, and 35°C experimental groups compared with 25°C control groups. * $p<0.05$; ** $p<0.01$; *** $p<0.001$)

The increase in global temperature due to climate change is the biggest concern and is known to have harmful effects on many agricultural products (Yadav et al., 2018). Chickpea is a winter-growing legume, and when it is exposed to heat stress, significant yield losses are experienced. For chickpeas, a temperature of 35°C is a critical temperature for plant growth and yield (Gaur et al., 2014). Therefore, 35°C was chosen for high-temperature stress application in this study. As a result, it was observed that high-temperature stress significantly reduced stem length, root fresh weight, and stem fresh-dry weight.

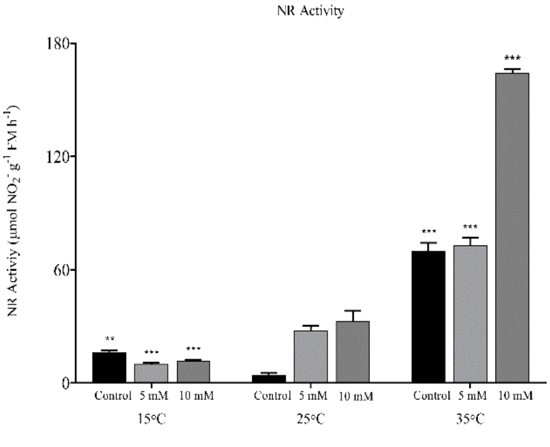

Figure 7. NR activity of chickpea samples treated with thiourea (µmol NO_2^- g^{-1} FM h^{-1}) (*, 15°C, and 35°C experimental groups compared with 25°C control groups. * $p<0.05$; ** $p<0.01$; *** $p<0.001$)

Contrary to this, root length and dry weight were not affected by high-temperature stress (Table 1). In another study with chickpeas, three different heat stresses were applied to the plants at the point of 35, 40, and 45°C. It was reported that heat stress reduced root-stem length (Kaushal et al., 2013). In a study with rice and corn, high-temperature stress was applied to the plants (35, 40, 45°C). It was stated that high-temperature stress reduced root-stem length in both plants (Kumar et al., 2012). Similar to cold stress, heat stress elongates the cell cycle and decreases cell division. This may be the reason for the decrease in morphological parameters in plants exposed to heat stress (Carvalho et al., 2018).

Stress tolerance can be given to the plant by administering some stress-reducing chemicals to the plant. Among the stress-reducing compounds, thiourea; is an important molecule with two functional groups: “thiol”, which is an oxidative stress response, and “imino”, which partially meets the nitrogen requirement under abiotic stress conditions. It provides tolerance to abiotic stresses because it dissolves in water and is easily absorbed in living tissues (Wahid et al., 2007; Akladious, 2014). Therefore, in our study, the role of thiourea in mitigating and eliminating the effects of heat stress on plants was investigated and the combined effects of heat stress and nitrogen supplementation in chickpea plants were demonstrated. In this study, thiourea application to plants subjected to low-temperature stress further reduced root and stem length. In other words, the interactive effect of the two applications stopped the division in the growing parts of the plant and delayed the plant development. Thiourea application at low temperature increased root and stem fresh weight at 5 mM concentration and decreased it at 10 mM concentration. There was no significant change in root and stem dry weights (Table 1). In addition to high-temperature stress, the interactive effect of thiourea on the root was greater than the effect of high temperature alone. Similarly, root wet weight was further reduced by the combined effect of both high temperature and thiourea application. On the contrary, the application of thiourea in plants subjected to high-temperature stress increased the stem length at both concentrations. There was no significant change in root dry weight, stem fresh and dry weight (Table 1). In the literature, there are studies that reveal the effects of thiourea application on plants and support the results of our study. In a study, thiourea was applied to chickpeas in two different ways, with irrigation water (500 ppm) and foliar application for spraying (1000 ppm). It was reported that the total plant weight and plant length increased in both applications compared to the control group (Choudhary et al., 2020). In another study with chickpeas, two different nitrogen applications, 20 and 100 kg ha^{-1}, were applied to the plants. It was stated that the root and stem dry weight of the samples decreased compared to the control group in both nitrogen applications (Kurdali, 1996). While there are studies on only heat stress or only nitrogen supplementation in the literature, there are very limited studies on the combined effect of heat stress and thiourea. The only article to the researchers’ knowledge about chickpeas is the work of Laurie and Stewart (1993). In this study, nitrate supplementation was applied to plant samples subjected to high-temperature stress for 40 days. It was reported that the total dry weight of the plant increased until the 15th day, but decreased afterward. At the end of the study, it was determined that the samples had the lowest plant growth rate. It was determined that the fresh-dry weight ratio of the stem and leaves decreased, while it increased in the root (Laurie and Stewart, 1993). In a study with wheat, the seeds were pretreated with 6 mmol thioureas. Then, salt (NaCl) stress was applied to the plants, and the effect of thiourea on salt stress was investigated. As a result, it was stated that thiourea increased stem length and stem fresh-dry weight of plant samples with salt stress (Baqer et al., 2020). In other studies, using corn and mung beans, it was determined that the root and stem length, root and stem fresh-dry weights of thiourea application to plants with salt stress increased compared to the control group (only NaCl applied samples) (Kaya et al., 2015; Perveen et al., 2016). Khanna et al. (2017) investigated the combined effect of thiourea and heat stress in their study and used corn plants grown at 40°C and applied 2-20 mM thiourea as a material. They found that thiourea application increased root and stem length, and root and stem dry weight in samples under high-temperature stress (Khanna et al., 2017). In the study of Akladious (2014) with sunflower, plant seeds were impregnated with 10 and 20 mM thiourea and left to germinate for 14 days. High-temperature stress (35 and 45°C) was applied to the plants at the end of germination. Root-stem length, root-stem fresh and dry weight and stem diameter were found to increased in both thiourea applications in samples exposed to high-temperature stress (Akladious, 2014). The negative effect of stress on the morphological characteristics of the plant decreased with the effect of thiourea as mentioned above. In other words, the application of thiourea to the plant under stress regulated the cell division and cell cycle of the plant and reduced the negative effect of stress on the morphological characteristics of the plant.

4.2. Changes in physiological parameters

The effects of stress on the electron transport system, photosystems, pigments, photosynthesis-related enzyme activities, gas exchange, and chlorophyll fluorescence in plants have been investigated in many studies. It has been reported that photosynthesis is adversely affected by both low and high-temperature stress (Ibrahimova et al., 2021; Lu et al., 2014). It was observed that both low and high-temperature stress increased the chlorophyll a, b, and total chlorophyll content according to the optimum temperature (Fig. 1). Unlike our results, it has been reported in the literature that high-temperature stress reduces the total chlorophyll content in studies with chickpeas, beans, corn and rice (Kaushal et al., 2011, 2013; Awasthi et al., 2017; Bhandari et al., 2020). It has been reported that the amount of chlorophyll a, b and total chlorophyll decreases with the effect of low temperature in studies conducted with chickpea plants with low-temperature stress (Turan and Ekmekçi, 2011). Thiourea application to plants with low-temperature stress did not cause a significant change in chlorophyll a, b, and total chlorophyll levels of plants. However, thiourea application to plants with high-temperature stress significantly increased chlorophyll a, b and total chlorophyll content at both concentrations compared to optimum temperature and thiourea application (Fig. 1). In studies with corn and mung beans, different concentrations of thiourea were applied to plants with salt stress. The results showed that thiourea application reduced the amount of chlorophyll a, b, and total chlorophyll in plants with salt stress (Kaya et al., 2015; Perveen et al., 2016). In the study of Akladious (2014) with sunflowers, high-temperature stress and two different concentrations of thiourea were applied to the plants. As a result, the amount of chlorophyll a, b, and total chlorophyll increased (Akladious, 2014). No study has been found in the literature investigating the interactive effect of thiourea with heat stress in chickpea plants.

Heat stress at the cellular level (high and low temperature) causes damage such as membrane damage, denaturation of proteins, improper synthesis, and folding, and inactivation of enzymes in mitochondria and chloroplasts (Kaushal et al., 2013; Donbaloglu Bozca and Leblebici, 2022). This study found, that low-temperature stress significantly increased the total protein amount compared to the control temperature. Considering the interactive effect of low-temperature stress and thiourea application, it was determined that the application of 5 mM thiourea significantly reduced the amount of total protein compared to the control temperature (Fig. 2). In their study conducted with chickpeas to which they applied low-temperature stress, reported that low temperature decreased total protein. On the other hand, in another study conducted at low temperatures, it was stated that low-temperature stress increased the amount of total protein (Kazemi-Shahandashti et al., 2014). The current study revelaed that the total protein amount decreased significantly under high-temperature stress compared to the control temperature. Similarly, both thiourea concentrations applied in addition to high-temperature stress significantly reduced the total protein amount (Fig. 2). In the literature, no study has been found that explains the effect of thiourea, which is made with chickpea plant and applied with heat stress, on the total protein of chickpea plant. However, there are studies in which different plants are used as materials and the interactive effects of different stresses are studied. For example, in a study conducted with coffee plants, it was shown that nitrogen application decreased the total protein amount (Reis et al., 2009). Another study established that thiourea application increased the total protein level in mung beans exposed to salt stress (Perveen et al., 2016). Both high and low temperatures also affect protein metabolism closely. Although heat stress stimulates the production of stress-related proteins, it causes the inactivation and degradation of proteins by negatively affecting the synthesis and folding of proteins in the continuation of stress (Gulen and Eris, 2003; Kaushal et al., 2013; Donbaloglu Bozca and Leblebici, 2022). The decrease in total protein content can be attributed to this.

Abiotic stresses such as drought, salinity, and low and high temperature cause an increase in ROS formation in plants due to disruption of cellular homeostasis (Mittler, 2002). Increasing ROS is also cleared by enzymatic antioxidants such as SOD, CAT, and APX (Mittler, 2002). It was observed that SOD and APX activity decreased significantly at low-temperature stress compared to the control temperature. It was established that 10 mM thiourea concentration applied in addition to low-temperature stress further reduced SOD activity (Fig. 3 and 5). In studies conducted with chickpeas under low-temperature stress in the literature, it was found that low-temperature stress reduced APX activity and increased SOD activity (Turan and Ekmekçi 2011, 2014). In another study conducted with chickpeas, it was observed that low-temperature stress increased SOD and APX activity and decreased CAT activity (Arslan et al., 2018; Karami-Moalem et al., 2018). In other studies, in which chickpea was used as the experimental material, it was reported that low-temperature stress increased CAT and APX activity, but there was no change in SOD activity (Nazari et al., 2012; Yousefi et al., 2018). Contrary to these results, in this study, it was reported that only high-temperature stress significantly increased SOD, CAT, and APX activities compared to the control temperature (Fig. 3, 4, and 5). Several studies also supported these results, in the literature, it has been stated that SOD, CAT, and APX activities increase depending on the temperature increase in chickpea, bean, rice, and corn plants exposed to high-temperature stress (Awasthi et al., 2017; Kabay and Şensoy, 2017; Bhandari et al., 2020). Unlike this study, in a study conducted with chickpeas, high-temperature stress at 35, 40, and 45 °C was applied to the plants. It was determined that high-temperature stress increased SOD and APX activity at 35 and 40°C, and decreased at 45°C. It was determined that CAT activity increased at all temperatures compared to the control (Kaushal et al., 2011). It was determined that the thiourea application in addition to the low temperature significantly increased the APX activity at both 5 mM and 10 mM concentrations and did not affect the SOD and CAT activities. However, it was shown that 10 mM thiourea concentration applied in addition to high-temperature stress significantly decreased SOD activity, while 5 mM thiourea concentration significantly increased APX activity. Also it was suggested that the activity of CAT, another of the antioxidative enzymes, increased significantly at both thiourea concentrations (Fig. 3, 4 ve 5). While there are studies on only heat stress or only nitrogen supplementation in the literature, a very limited number of studies on the combined effect of heat stress and thiourea have been found (Awasthi et al., 2017; Kabay and Şensoy, 2017; Bhandari et al., 2020). The effect of thiourea was investigated in a study on wheat samples exposed to salt stress. As a result, it was stated that thiourea increased the SOD, CAT, and APX activities of plants with salt stress (Baqer et al., 2020). In another study conducted with wheat, all thiourea applications increased SOD and CAT activities while decreasing APX activity in both the drought-stressed and non-stressed plant groups (Hassanein et al., 2015). In the study of Khanna et al. (2017) with maize, the combined effect of thiourea and heat stress increased root and stem CAT and SOD activities and reduced APX activity (Khanna et al., 2017). In a study on sunflowers, it was reported that the interactive effect of thiourea application and high-temperature stress increased SOD and CAT activities (Akladious, 2014). The decrease in NR and GS activity in plants exposed to heat stress acts as a biochemical adaptation to conserve energy by stopping nitrate assimilation (Hayat et al., 2009; Akladious, 2014). Both high and low temperature stress causes excessive ROS accumulation in the plant. The increase in ROS causes an increase in ROS-scavenging enzyme activities in the plant (Rymen et al., 2007; Aslantaş et al., 2010; Wu et al., 2022). This may be the reason for the increase in CAT and APX enzyme activities in our study. However, it has been reported that applying external substances to plants under stress reduces the negative effects of stress (Wahid et al., 2007; Akladious, 2014). This can be explained by the fact that the application of thiourea at increasing concentration reduces the SOD enzyme activity.

In this study, both low-temperature stress and high-temperature stress significantly reduced GS activity compared to optimum temperature. Application of thiourea in addition to heat stress significantly increased GS activity at 5 mM concentration at both low and high temperatures. Conversely, 10 mM thiourea application significantly reduced GS activity at both low and high temperatures (Fig. 6). The application of low-temperature stress alone increased the NR activity significantly compared to the optimum temperature. In addition to low-temperature stress, the application of thiourea at 5 mM and 10 mM concentrations significantly reduced NR activity. High-temperature stress, on the other hand, increased the NR activity according to the optimum temperature, while the application of 5 mM and 10 mM thiourea further increased the NR activity (Fig. 7). In a study with chickpeas, it was stated that NR activity in the leaves increased with nitrogen supplementation at high temperature (Laurıe and Stewart, 1993). Another study also reported that NR activity increased in sunflowers, which was applied in both high-temperature stress and thiourea (Akladious, 2014). In a study conducted with coffee, N application at three different concentrations (0, 150, and 300 kg ha^{-1}) was used and decreased NR and GS activities in fruit development were reported (Reis et al., 2009). The decrease in the activity of nitrate metabolism enzymes in plants exposed to heat stress is related to the inhibition of nitrate assimilation in the first stage, thus saving energy (Akladious, 2014). The fact that thiourea application to plants under stress causes a significant increase in both NR and GS activity can be explained by the fact that plants can overcome the negative effects of heat stress more effectively with thiourea application.

5. Conclusion

As a result, when the effects of thiourea application at low temperature and high temperature in chickpeas, which is a protein-rich legume, were compared. It was established that nitrogen supplementation was more effective in plant growth and tolerance of cold stress at low temperature stress than high temperature stress. Especially in the study at 15°C, it was determined that 5 mM, which was a low thiourea application, positively affected plant growth when both morphological and physiological parameters were taken into account. Thus, it is thought that more agricultural production can be made with low concentration nitrogen supplementation in areas with a lower temperature than the optimum temperature required by chickpeas, which is a cool climate grain. It has been revealed that both 5 and 10 mM thiourea applications at 35°C, which are high-temperature applications, does not affect the plant's tolerance to heat stress.

Conflict of interest

The authors have declared no conflict of interest.

Authors' contribution

SL contributed conception and design of the study. SL and FDB performed the experiments, analyzed the data, and wrote the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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