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Research Article

Alternating thermal conditions enhance seedling morphophysiology, antioxidant activity and nutrient dynamics in eggplant

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Abstract

Partial dormancy in eggplant (*Solanum melongena* L.) seeds often delays germination and emergence, leading to poor and non-uniform seedlings development. Nonetheless, limited research has examined the effects of alternating temperature regimes on seedling vigour, antioxidant capacity, and nutrient dynamics in eggplant. To address this gap, the present study evaluated the responses of four commercial hybrids ('Brigitte F1', 'Faselis F1', 'Karaefe F1', 'Hünkar F1') and two hybrid rootstocks ('Hawk F1', 'Köksal F1') to two alternating temperature regimes (35/20 °C-16/8 h and 20/35 °C-16/8 h) compared with constant temperature (25 °C). A Completely Randomized Design was employed to assess germination, emergence, morphological and biomass traits, antioxidant enzyme activities (SOD, CAT, APX), and macro-and micronutrient uptake. The genotypes exhibited differential responses: the 35/20 °C-16/8 h regime significantly improved germination (up to 97.7%) and emergence (up to 94.6%), enhanced seedling length and biomass, and increased SOD, CAT, and APX activities compared with constant temperature. Nutrient uptake (Ca, Mg, P, K, Fe, Zn, Cu, Mn, B) was also highest under this regime, while Na accumulation decreased. Correlation and PCA analyses revealed strong positive relationships among germination, emergence, antioxidant activity, and nutrient accumulation, while mean germination and emergence times were negatively correlated with seedling vigour. These findings highlight the role of antioxidant mechanisms in conferring tolerance to thermal fluctuations thereby improving seedling quality and nutrient dynamics of eggplant. Consequently, the 35/20 °C - 16/8 h regime can be readily and economically adopted by commercial producers to enhance germination rates and improve seedling development in eggplant cultivars.

Keywords: nutrient uptake; seed dormancy; seed physiology; seedling quality; *Solanum melongena*; temperature regimes

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Introduction

The eggplant (*Solanum melongena* L.) is an important crop in the tropical and subtropical zones, both economically and nutritionally. Owing to its high content of phenolics, it has received a remarkable attention in the recent years, being grouped as of the top ten vegetables due its potent antioxidant potential (Niño-Medina *et al*., 2017; Gürbüz *et al*., 2018; El-Feky *et al*., 2024; Opoku *et al*., 2024). In addition to being a staple vegetable, it is also considered as a component of grafting systems for sustainable horticulture (Bogoescu and Doltu, 2015; Kyriacou *et al*., 2020; Argento *et al*., 2023). In terms of agricultural production context, the success of eggplant production largely depends on producing uniform, vigorous seedlings (Caruso *et al*., 2017). However, the species is characterized by slow and erratic germination, which reduces seedling quality and stand establishment. Such critical irregularities were considered to be caused by partial dormancy, a condition where only a fraction of viable seeds germinate under favourable conditions. This staggered germination reduces the viability and vigour of the seeds and ultimately affects yield potential, which presents a crucial problem for commercial nurseries (Özden *et al*., 2018).

Among the strategies used to break seed dormancy, alternating temperature regimes are recognized as one of the most effective. These regimes simulate natural day-night temperature fluctuations, accelerating the germination in a diverse species, *viz*. sweet wormwood, aubergine, guar (Kumar *et al*., 2013; Özden *et al*., 2021; Reis *et al*., 2024). Alternating temperatures cascade a series of physiological alterations such as seed coat softening, embryo growth, and enzymatic activation. Such changes are translated into the enhanced germination rate and uniformity (Footitt *et al*., 2014; Batlla and Benech-Arnold, 2015; Burghardt *et al*., 2015; Huo and Bradford, 2015). Importantly, rapid and synchronous germination provides additional advantages beyond uniform seedling stands (Eren *et al*., 2023). For instance, early-emerging seedlings access water and mineral nutrients, which in turn lead to improved growth, biomass accumulation, and stress resilience. On the other hand, the delayed or uneven germination causes in seedlings with varying sizes, which compete unequally for resources available. Ultimately, such circumstances compromise the quality of transplants (Demir *et al*., 2008). Thereby, it is paramount to understand how germination media influence the nutrient uptake in order to optimize the vigour and productivity of seedlings.

In spite of the well-documented functions of alternating temperature in breaking dormancy (Baskin and Baskin, 2001; Li *et al*., 2012; Koutsovoulou *et al*., 2014), little is known about how such regimes affect both seedling quality and mineral nutrient absorption in eggplant. The former reports have largely focused on germination-associated and morphological traits. Therefore, the studies dealing with the link germination physiology with nutrition uptake as well as antioxidant enzyme status are required.

In this study, we hypothesized that initiating germination under high-based alternating temperature regimes would not only accelerate germination and emergence but also enhance antioxidant enzyme activities and mineral nutrient uptake, thereby improving overall seedling quality in eggplant hybrids and rootstocks. In order to test the hypotheses driven, we compared six genotypes under two alternating and one constant temperature regime, evaluating germination performance, seedling morphology, antioxidant responses, and nutrient uptake.

Materials and Methods

Study area and experimental conditions

Genetic materials

In the study, four hybrids and two hybrid rootstock eggplant varieties obtained from different commercial companies ('Faselis F1', 'Brigitte F1', 'Hünkar F1', 'Karaefe F1', 'Hawk F1' and 'Köksal F1', were obtained from Seminis, Rijk Zwaan, Syngenta, Bursa, Vilmorin and Yüksel Seed Company respectively) were used as plant material (Table A1). The seeds were stored in the dark at +4 °C in hermetically laminated aluminium foil packets until used. Among the tested cultivars, 'Hawk F1' and 'Köksal F1' are commercial rootstock cultivars widely used for grafting purposes in eggplant production; however, in the present study they were evaluated directly as seed lots rather than for grafting-related indices. Viability tests of eggplant seeds were conducted in cooled incubators, while emergence tests of seedlings were carried out in a controlled plant growth chamber.

Research design and experimental factors

The study was conducted using a two-factor factorial experiment arranged in a Completely Randomized Design (CRD) with three replications. The experimental factors were: (i) six eggplant cultivars [four commercial hybrids ('Brigitte F1', 'Faselis F1', 'Karaefe F1', and 'Hünkar F1') and two commercial rootstocks ('Hawk F1' and 'Köksal F1')] and (ii) three temperature regimes [T1: alternating high-low (35/20 °C, 16/8 h light/dark), T2: alternating low-high (20/35 °C, 16/8 h light/dark), and T3: constant (25 °C, 24 h)].

Each treatment combination (6 cultivars × 3 temperature regimes = 18) was replicated three times. For germination and emergence assays, each replicate consisted of 50 uniform seeds (150 seeds per treatment).

Imposition of temperature treatment

In the experiment ~3 g of seeds in each petri dishes were dark-incubated at two alternating temperature and time combination regimes (35/20 °C-16/8h and 20/35 °C-16/8h) and constant temperature (25 °C) for 72 hours. The experiment was designed to examine dynamics of germination, emergence, seedling quality parameters and macro-micro plant nutritional elements absorption in seeds during seed incubation different alternating temperatures with constant temperature.

Germination and emergence test

Following the application of different incubation temperatures, germination tests were conducted on three replicates, each consisting of 50 healthy uniform seeds of each cultivar, utilizing the between-paper method over a 14-day period in darkness (ISTA, 2020).

For emergence analysis, 50 healthy uniform sized seeds of each cultivar were hand-sown at 0.6 - 0.9 cm depth below the surface of a plastic nursery pot (45 x 33 x 9 cm) filled with a mixture of peat moss and pertile in a 2:1 ratio arranged in a climatic growth chamber. The medium was characterized by a pH range of 5.5-6.5, an electrical conductivity of 45 µS cm^{-1} g^{-1}, and nutrient concentrations of KCl: 1.2 g L^{-1}, N: 130-300 mg L^{-1}, P_2O_5: 70-170 mg L^{-1}, and K_2O: 140-340 mg L^{-1}. The peat medium had a pH range of 6.5-7.5, was sterile, and had a density of 12 kg/200 L, while the perlite medium had a particle size of 1.5-2.5 mm. Emergence percentages were assessed after 30 days under conditions of 24±2 °C, with a photoperiod of 16/8 hours light/dark (72 µMol m^{-2} s^{-1} of illumination) and a relative humidity of 70±5%.

Data collection

Physiological parameters

After day 30, normal seedling percentages (healthy and well-developed plants) were determined.

MGT/MET: The mean germination time (MGT, days) was calculated using the formula (Demir *et al.*, 2008);

MGT/MET=Σn.t/Σn

where n = number of seeds newly germinated/emerged (2 mm radicle emerged) at time t

t = days from planting, Σn = final germination/emergence.

Morphological parameters

Seedling and root length of randomly selected data plants per treatment was recorded using a hand-held digital calliper (Mitutoyo, Japan). Stem girth was measured above the cotyledon leaves using a digital calliper. Fully opened and developed true leaves of data plants were counted manually and average estimated to represent each treatment.

Biomass parameters

At the end of experiment, plants were harvest and roots carefully waters to avoid damage and breakage. Subsequently, shoots were cut at the collar region of the stem using a sharp blade. Both shoot and root fresh weights were measured using a sensitive digital scale (Mettler Toledo, 0.0001). Shoot and roots were bagged in well labelled enveloped, dried at 80 °C to a constant weight. After drying, samples were allowed to cool at room temperature and subsequently dry weights measured using digital scale (Asante *et al*., 2024).

Antioxidant enzyme analyses

To determine changes in enzyme activities, 0.25 g of seed samples were taken from seeds that had been kept for 72 hours under appropriate temperature combinations and humidity conditions in each replication. The samples were ground in porcelain mortars using liquid nitrogen. They were then homogenized with 5 ml of cold extraction buffer containing 0.1 M sodium phosphate (pH 7.5), 0.5 mM Na-EDTA, and 1 mM ascorbic acid. The homogenized samples were centrifuged at 18.000 rpm for 30 minutes at 4 °C. The supernatants were incubated at room temperature for 1 hour. Catalase (CAT) activity was immediately estimated in a portion of the homogenate, and the remaining extract was stored at -20 °C until Superoxide Dismutase (SOD) and Ascorbate Peroxidase (APX) activities were analysed (Jebara *et al*., 2005).

Superoxide dismutase (SOD) activity

SOD activity was measured based on the inhibition of the reduction of nitro blue tetrazolium chloride (NBT) by superoxide radicals (O_2^-) at 560 nm. The reaction mixture (total volume 2.5 ml) consisted of 50 mM sodium phosphate buffer (pH 7.0), 0.1 mM Na-EDTA, 33 µM NBT, 5 µM riboflavin, and 13 mM methionine. To this mixture, 0.1 ml of enzyme extract was added, and the reaction was initiated by exposing the samples to light at 25 °C (40 W) and 75 µmol m^{-2} s^{-1} for 10 minutes. A control (without enzyme) was kept in the dark for the same period. One unit of SOD activity was defined as the amount of enzyme required to inhibit 50% of the NBT reduction at 560 nm (Rahnama and Ebrahimzadeh, 2005).

Catalase (CAT) activity

CAT activity was determined by monitoring the decomposition of H_2O_2 at 240 nm. The reaction mixture (final volume 1 ml) contained 2.5 ml of 0.05 M KH_2PO_4 buffer (pH 7.0), 1.5 mM H_2O_2, and 0.2 ml of enzyme extract. Enzyme activity was evaluated as the change in absorbance per minute per mg protein, or using an extinction coefficient of 40 mM^{-1} cm^{-1} at 240 nm (Jebara *et al*., 2005).

Ascorbate peroxidase (APX) activity

APX activity was measured based on the decrease in absorbance at 290 nm due to the ascorbate-dependent reduction of H_2O_2. The reaction mixture (final volume 1 ml) contained 3 ml of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM H_2O_2, and 0.1 ml of enzyme extract. The reaction was initiated by the addition of 0.1 ml of extract protein. Enzyme activity was calculated based on the change in absorbance per minute per mg protein, using an extinction coefficient of 2.8 mM^{-1} cm^{-1} at 290 nm (Sairam and Saxena, 2000).

Nutrient analysis

Dried plant samples were ground in porcelain mortar. 0.25 g of plant samples were weighed and burned in an ash furnace at 550 ± 50 °C for 8 hours. After adding some distilled water and 2 ml HNO_3 to the ash obtained, the final volume was completed with the help of distilled water to 50 ml. Subsequently, the samples were filtered with Whatman 42 filter paper and the filtrates were transferred to tubes and made ready for reading in the ICP. Macro and Microelement contents in the obtained filters were determined with the ICP-OES (Perkin Elmer, Optima 2100 DV) device (Kacar and Inal, 2008). In ICP-OES device, each treatment is repeated in three replications, Ca (%), Mg (%), P (%), K (%), Na (%), Fe (ppm), Zn (ppm), Cu (ppm), Mn (ppm) and B (ppm) nutrient contents were determined.

Statistical analysis

Data collected on morpho-physiology, antioxidant and nutrients of plants were initially tested for normality using Shapiro-Wilk test of normality. Data showed normal distribution hence, no transformation was applied prior to analysis. Analysis of variance (ANOVA) was carried to determine variation been experimental factors. Subsequently, where significance was obtained, Duncan's Multiple range test was carried out to determine the least difference between treatment means at $p < 0.05$. A two-way ANOVA was performed using GraphPad Prism (version 10), and significant mean differences among treatments were compared using Duncan's multiple range test at $p < 0.05$. For trait associations, Pearson correlation coefficients were computed in R Studio (version 4.4.0) using the psych and Hmisc packages, while correlation matrices were visualized with the corrplot package. Furthermore, Principal Component Analysis (PCA) was carried out using OriginLab® software on pooled data across all cultivars and temperature regimes to identify the major factors contributing to trait variation. Additionally, broad-sense heritability (H^2) was estimated for each quantitative trait across all treatments to assess the relative contribution of genetic versus environmental variance. It was calculated as:

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_{G\times E}^2/e + \sigma_e^2/re}$$

where σ^2_G is the genotypic variance, $\sigma^2_{G\times E}$ is the cultivar × environment (temperature) interaction variance, σ^2_e is the error variance, r is the number of replications, and e is the number of environments (temperature regimes).

Results

Descriptive statistics and summary ANOVA

The two-way ANOVA results showed that both temperature regimes and cultivar differences had significant effects on all traits considered in the study, with interactions effects varying among the traits (Table A2). Of the parameters, germination percentage was significantly influenced by temperature regimes (42.15% of variation, $p < 0.0001$) and cultivars (47.46%, $p < 0.0001$), whereas emergence was strongly affected by cultivars (47.17%, $p < 0.0001$) and temperature regimes (37.52%, $p < 0.0001$), with a significant interaction effect (5.95%, $p = 0.0336$). Seedling growth traits, including seedling length (SL), root length (RL), seedling diameter (SD), and total leaf number (TLN), were largely affected by cultivar differences (ranging from 55% to 85% of the variation), although temperature regimes and their interactions also contributed significantly. These findings reveal the genotype-dependent responses to environmental conditions. Fresh and dry biomass parameters of both seedlings and roots (SFW, SDW, RFW, RDW) were similarly influenced by cultivar (46-79%), with temperature regimes and interactions accounting for smaller but significant portions of the variance. In the case of nutrient accumulation, Fe, Zn, Cu, Mn, B, Na, P, K, Ca, and Mg were also significantly affected by cultivars and temperature, with cultivar effects dominating for Zn (80.24%), Cu (63.23%), and Mn (72.10%), while temperature explained a higher share of variation in Ca (36.25%) and Mg (41.55%). Significant interactions were also observed for Na (24.40%) and P (29.20%). A strong effect of genotype × environment was observed on nutrient accumulation. Concerning the responses of the enzymes, antioxidant enzyme activities (SOD, CAT, and APX) were markedly affected by both factors, with SOD activity mainly driven by temperature (54.21%) and cultivars (39.41%), CAT activity largely by cultivars (82.27%), and APX activity showing a more balanced contribution from cultivars (52.07%), temperature regimes (37.86%), and interaction effects (9.88%).

Germination and vegetative growth

Germination rates ranged from 100.0% to 87.7%. The highest mean germination rate was obtained under the 35/20 °C-16/8 h regime with 97.7%, while the lowest was recorded under constant temperature conditions with 92.0% (Figure 1).

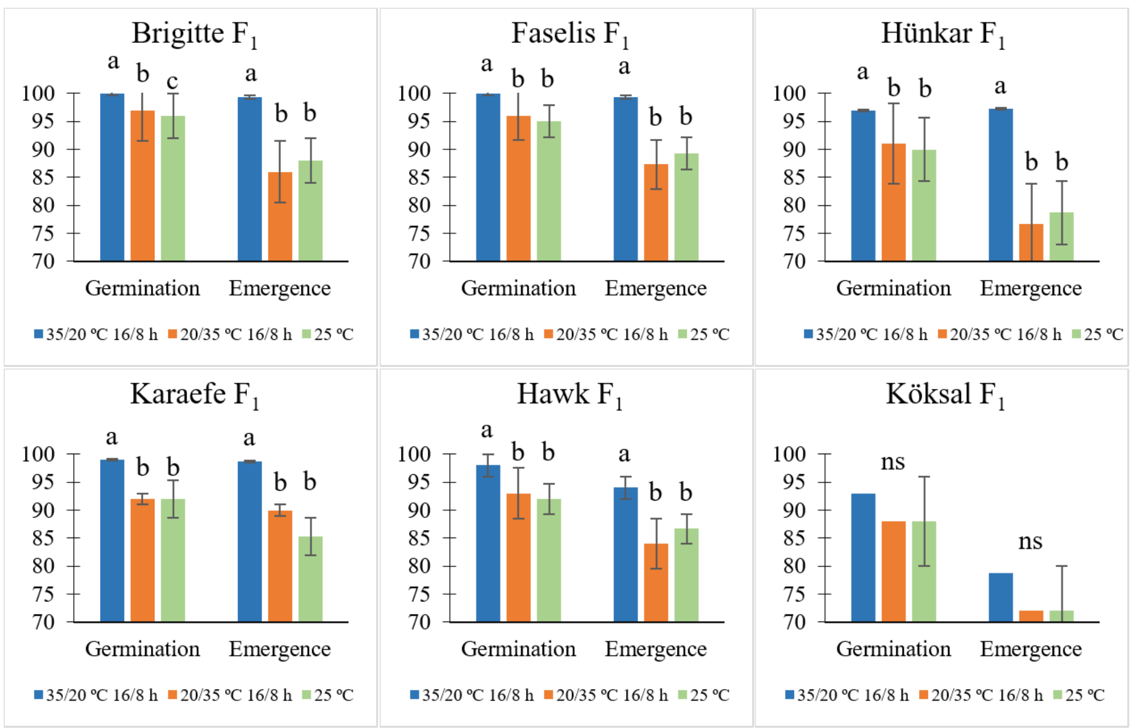

Figure 1. Effects of temperature and duration combinations on seed germination and emergence in different eggplant cultivars ($p < 0.05$)
The error bars represent ± SEM. ns: non-significant

In the 'Brigitte', 'Faselis', Hünkar, and 'Karaefe' cultivars, as well as in 'Hawk' rootstock, the 35/20 °C-16/8 h condition statistically increased seed viability compared to the other alternating temperature regime and the constant temperature condition. In 'Köksal' cultivar, no significant differences were found among the treatments. No statistical difference was observed between the constant temperature and the 20/35 °C-16/8 h application in any of the cultivars except 'Brigitte', among groups showing significant differences (Figure 1).

Seedling emergence rates ranged from 99.3% to 72.0%. Regarding the averages, the highest emergence rate was recorded under the 35/20 °C-16/8 h regime with 94.6%, while the lowest rates were observed under 20/35 °C-16/8 h and constant temperature (25 °C) conditions, with 82.3% and 83.3%, respectively (Figure 1).

In general, seedling emergence results showed similar trends to those observed in germination. While no statistically significant differences were found among treatments in the 'Hünkar' cultivar, the highest emergence rate was recorded under the 35/20 °C-16/8 h regime for all other cultivars. No significant difference was observed between the 20/35 °C-16/8 h and constant temperature treatments (Figure 1).

Morphological response of cultivars to different temperature regimes

Seedling length (SL) varied between 141.7 mm and 38.4 mm. Concerning the average values, the highest SL was obtained under the 35/20 °C-16/8 h condition with 118.5 mm. The lowest average SL was recorded under the control and 20/35 °C-16/8 h treatments, with 94.6 mm and 95.6 mm, respectively. No significant differences were found between the constant temperature and the 20/35 °C-16/8 h treatments in the 'Brigitte', 'Faselis', and 'Hawk' cultivars, whereas variable temperature was more effective in the 'Hünkar' cultivar and constant temperature yielded higher SL in the 'Köksal' cultivar (Table 1).

Table 1. Impact of temperature and photoperiod regimes on growth parameters in various eggplant cultivars

Cultivar	**Measurements**				
	Treatment	**SL (mm/plant)**	**RL (mm/plant)**	**SD (mm/plant)**	**TLN (unit/plant)**
Brigitte	35/20 °C-16/8 h	121.3 ± 4.07 ^{a}	78.9 ± 3.45 ns	1.58 ± 0.03 c	3.0 ± 0.04 ns
	20/35 °C-16/8 h	105.3 ± 1.68 ^{b}	69.3 ± 4.27 ns	1.72 ± 0.02 b	3.0 ± 0.02 ns
	25 °C-Constant	108.4 ± 1.31 ^{b}	73.1 ± 2.86 ns	1.86 ± 0.01 a	3.1 ± 0.00 ns
Faselis	35/20 °C-16/8 h	141.5 ± 0.31 a	70.6 ± 1.08 ab	1.85 ± 0.01 ns	3.3 ± 0.06 a
	20/35 °C-16/8 h	111.6 ± 2.35 b	65.0 ± 3.85 b	1.81 ± 0.04 ns	3.0 ± 0.06 b
	25 °C-Constant	104.0 ± 4.99 b	74.7 ± 1.16 a	1.81 ± 0.02 ns	3.2 ± 0.06 ab
Hunkar	35/20 °C-16/8 h	141.7 ± 3.77 a	76.9 ± 4.17 ns	1.89 ± 0.07 ns	3.1 ± 0.02 a
	20/35 °C-16/8 h	106.6 ± 1.88 b	77.9 ± 8.76 ns	1.79 ± 0.08 ns	2.4 ± 0.10 b
	25 °C-Constant	94.5 ± 2.98 c	83.5 ± 5.59 ns	1.96 ± 0.01 ns	3.1 ± 0.08 a
Karaefe	35/20 °C-16/8 h	129.2 ± 2.88 a	103.0 ± 7.11 a	2.07 ± 0.04 ns	3.3 ± 0.07 ns
	20/35 °C-16/8 h	115.6 ± 7.89 ab	63.4 ± 1.40 b	2.02 ± 0.01 ns	3.2 ± 0.04 ns
	25 °C-Constant	109.0 ± 3.28 b	73.8 ± 4.14 b	1.97 ± 0.10 ns	3.2 ± 0.02 ns
Hawk	35/20 °C-16/8 h	51.1 ± 0.29 a	63.5 ± 1.86 ns	1.21 ± 0.01 a	3.2 ± 0.04 b
	20/35 °C-16/8 h	38.4 ± 1.34 b	61.7 ± 5.57 ns	0.90 ± 0.09 b	2.9 ± 0.02 c
	25 °C-Constant	40.8 ± 0.19 b	63.7 ± 2.01 ns	0.99 ± 0.03 b	3.5 ± 0.02 a
Köksal	35/20 °C-16/8 h	126.3 ± 0.47 a	104.4 ± 6.49 ns	1.61 ± 0.04 b	4.1 ± 0.04 a
	20/35 °C-16/8 h	95.9 ± 1.46 c	94.3 ± 5.31 ns	1.38 ± 0.04 c	3.4 ± 0.04 b
	25 °C-Constant	111.0 ± 2.25 b	89.8 ± 5.42 ns	1.85 ± 0.06 a	4.0 ± 0.00 a

Means with different letters in the same column denote significant difference at $p < 0.05$. ± SEM. ns: non-significant

Root length (RL) ranged from 104.4 mm to 61.7 mm across measurements, with the highest average RL obtained under the 35/20 °C-16/8 h condition (89.9 mm). The lowest average RL was recorded under the 20/35 °C-16/8 h regime with 71.9 mm. No statistically significant differences in RL were found among treatments for all cultivars except 'Faselis' and Karaefe. In the 'Faselis' cultivar, the highest RL was observed under constant temperature, while in the 'Karaefe' cultivar, it was recorded under the 35/20 °C-16/8 h treatment (Table 1).

Stem diameters in seedling samples ranged from 2.07 mm to 0.90 mm. On average, the highest stem diameter (SD) was observed under constant temperature conditions at 1.74 mm, while the lowest was recorded under the 20/35 °C-16/8 h regime at 1.60 mm. No statistically significant differences were observed in SD among treatments for the 'Faselis', 'Hünkar', and 'Karaefe' cultivars. The highest SD was found under constant temperature in the 'Brigitte' and 'Köksal' cultivars, and under the 35/20 °C-16/8 h condition in the 'Hawk' cultivar (Table 1).

The number of true leaves per seedling (TLN) ranged from 4.1 to 2.4 leaves. The highest average TLN (3.30 leaves) was recorded under both constant temperature and the 35/20 °C-16/8 h condition, while the lowest TLN was observed under the 20/35 ° C-16/8 h regime, with an average of 3.0 leaves. The treatments had no significant effect on TLN in the 'Brigitte' and 'Karaefe' cultivars (Table 1).

Interaction of cultivar and temperature on biomass production

Seedling fresh weight varied considerably among cultivars and across temperature regimes. On average, seedlings grown under 35/20 °C accumulated 37% more SFW than at 20/35 °C and 33% more than at 25 °C. For instance, 'Brigitte' recorded 1117.7 mg plant^{-1} at 35/20 °C compared with 893.8 mg plant^{-1} at 20/35 °C, representing a 25% reduction. The most pronounced difference was observed in 'Karaefe', which showed a 61% higher SFW at 35/20 °C compared with 20/35 °C. By contrast, 'Hawk' consistently exhibited the lowest SFW across regimes, averaging 245.3 mg plant^{-1} under 35/20 °C, which was nearly 1.7-fold higher than under 20/35 °C (145.9 mg plant^{-1}) (Table 2).

Table 2. Impact of temperature and duration regimes on biomass parameters in eggplant cultivars

Cultivar	Treatment	SFW (mg/plant)	SDW (mg/plant)	RFW (mg/plant)	RDW (mg/plant)
Brigitte	35/20 °C-16/8 h	1117.7 ± 5.2 a	83.8 ± 1.18 a	154.5 ± 10.62 a	12.2 ± 1.74 a
	20/35 °C-16/8 h	893.8 ± 12.0 b	68.8 ± 4.22 b	116.3 ± 3.64 b	6.7 ± 0.53 b
	25 °C-Constant	999.4 ± 52.2 b	69.7 ± 3.94 b	113.3 ± 8.32 b	8.5 ± 0.67 ab
Faselis	35/20 °C-16/8 h	1168.1 ± 89.4 ns	82.1 ± 4.42 a	155.9 ± 13.35 a	11.2 ± 0.62 a
	20/35 °C-16/8 h	959.6 ± 78.5 ns	62.2 ± 1.23 b	76.8 ± 5.84 b	6.1 ± 0.39 b
	25 °C-Constant	953.4 ± 55.6 ns	61.1 ± 2.01 b	104.4 ± 8.56 b	7.3 ± 1.27 b
Hunkar	35/20 °C-16/8 h	1309.4 ± 93.4 a	95.0 ± 4.85 a	128.8 ± 4.49 a	10.7 ± 0.32 a
	20/35 °C-16/8 h	920.9 ± 4.7 b	67.2 ± 2.31 b	105.5 ± 2.32 b	7.3 ± 0.38 b
	25 °C-Constant	964.4 ± 48.0 b	72.3 ± 3.92 b	123.2 ± 8.63 ab	6.9 ± 1.25 b
Karaefe	35/20 °C-16/8 h	1575.3 ± 32.4 a	126.8 ± 18.27 a	223.6 ± 31.87 a	16.3 ± 1.88 a
	20/35 °C-16/8 h	977.0 ± 10.7 b	63.4 ± 1.68 b	87.0 ± 9.27 b	7.4 ± 0.73 b
	25 °C-Constant	971.1 ± 102.6 b	69.3 ± 3.75 b	75.5 ± 7.58 b	6.9 ± 0.60 b
Hawk	35/20 °C-16/8 h	245.3 ± 14.9 a	26.9 ± 1.06 a	65.2 ± 8.43 a	5.2 ± 0.35 a
	20/35 °C-16/8 h	145.9 ± 1.7 b	14.0 ± 0.20 b	19.7 ± 2.36 b	1.6 ± 0.05 b
	25 °C-Constant	149.9 ± 9.2 b	16.5 ± 1.01 b	18.8 ± 1.95 b	1.5 ± 0.17 b
Koksal	35/20 °C-16/8 h	1314.3 ± 30.6 a	119.0 ± 9.60 ns	148.3 ± 2.05 a	16.0 ± 0.46 a
	20/35 °C-16/8 h	1010.8 ± 1.3 b	101.6 ± 0.48 ns	124.6 ± 1.38 b	13.1 ± 0.80 b
	25 °C-Constant	1005.7 ± 52.4 b	101.3 ± 9.54 ns	117.1 ± 4.93 b	12.2 ± 0.83 b

Means with different letters in the same column denote significant difference at $p < 0.05$. ± SEM. ns: non-significant

Seedling dry weight followed a similar pattern, with the 35/20 °C regime producing 41% higher SDW compared with 20/35 °C. Hünkar showed the largest genotype-specific increase, with SDW at 35/20 °C (95.0 mg plant^{-1}) being 41% higher than at 20/35 °C (67.2 mg plant^{-1}). In 'Hawk', however, the difference was more pronounced: SDW decreased from 26.9 mg plant^{-1} at 35/20 °C to 14.0 mg plant^{-1} at 20/35 °C, representing a 48% reduction. The constant 25 °C condition generally produced intermediate values, closer to those at 20/35 °C (Table 2).

Significantly, it was clearly observed that root biomass was highly sensitive to temperature regimens considered here. Among the cultivars, RFW at 35/20 °C was 66% higher than at 20/35 °C, and 59% higher than at 25 °C. Critically, Karaefe cultivar showed the most dramatic effect, showing 223.6 mg plant^{-1} at 35/20 °C, which was more than 2.5-fold higher than the 87.0 mg plant^{-1} observed at 20/35 °C. Similarly, 'Hawk' cultivar also maintained lower RFW, with an almost 3-fold reduction under 20/35 °C compared to 35/20 °C. Root dry weight (RDW) also showed reduction along with the temperature regimes. With respect to the average values, seedlings at 35/20 °C produced 70% higher RDW than at 20/35 °C and 63% higher than at 25 °C. Of the cultivars, 'Karaefe' and 'Köksal' exhibited the highest RDW under 35/20 °C (16.3 and 16.0 mg plant^{-1}, respectively), which represented a 2.2-fold increase compared with 20/35 °C. On the other hand, RDW of 'Hawk' cultivar decreased from 5.2 mg plant^{-1} at 35/20 °C to only 1.6 mg plant^{-1} at 20/35 °C, reflecting a 69% decline.

Variation in antioxidant enzymes under alternating temperature

Among all three antioxidant enzymes examined, the 35/20 °C-16/8 h treatment exhibited the highest activity levels. Based on averages of the cultivar, the highest superoxide dismutase (SOD) activity was observed under the 35/20 °C-16/8 h condition with 25.795 units g^{-1}, while the lowest was recorded under constant temperature conditions with 13.068 units g^{-1}. In the 'Köksal' cultivar, the lowest statistically significant SOD activity was observed under the 20/35 °C-16/8 h treatment, whereas in all other cultivars, the lowest values were found under constant temperature conditions (Figure 2A).

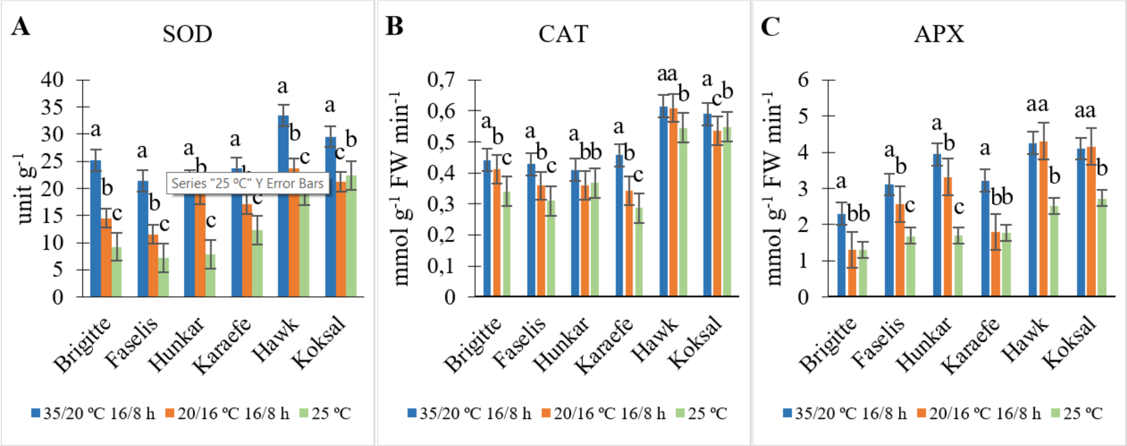

Figure 2. Effects of temperature treatments on antioxidant enzyme activities: A) SOD, B) CAT, and C) APX in different eggplant cultivars ($p < 0.05$)

The error bars represent ± SEM.

Catalase (CAT) activities showed similar trends to SOD. Except for the "Hawk" cultivar, the highest CAT activities were observed under the 35/20 °C-16/8 h treatment in all cultivars. On average, the highest CAT activity was 0.490 mmol g^{-1} FW min^{-1} under the 35/20 °C-16/8 h regime, while the lowest was 0.400 mmol g^{-1} FW min^{-1} under constant temperature conditions (Figure 2B).

Ascorbate peroxidase (APX) activities in rootstock cultivars were statistically similar under both variable temperature regimes and were higher than those under constant temperature. Among cultivars, APX activities were higher under the 35/20 °C-16/8 h treatment. Considering the cultivar averages, the highest APX activity was 3.490 mmol g^{-1} FW min^{-1} under 35/20 °C-16/8 h, whereas the lowest was 1.949 mmol g^{-1} FW min^{-1} under constant temperature (Figure 2C).

Variation in nutrient content under alternating temperature

Regarding Ca and Mg concentrations, significant cultivar × temperature regime interactions were observed ($p < 0.0001$; Tables A2). Being consistent with other findings of physiological and biomass-related traits, plants grown at 35/20 °C exhibited the highest Ca accumulation, particularly in 'Brigitte' (2.31%), while a pronounced reduction was recorded under 25 °C constant conditions, especially in 'Hünkar' (1.39%) (Table 3). In the case of Mg concentration, the highest values of Mg were recorded İn 'Hawk' cultivar (1.2% at 35/20 °C). Constant temperature again decreased the values of Mg, most notably in 'Hawk' and 'Hünkar' cultivars. As in the interactive effects on Ca and Mg, strong interactive effects were also recorded for P and K ($p < 0.0001$; Tables A2). Except 'Hawk', P levels were relatively stable across cultivars at 35/20 °C, constant temperature critically decreased the levels, especially in 'Hawk' and 'Hünkar' cultivars. Interestingly, K exhibited cultivar-specific responses. For instance, K concentration did not differ in 'Brigitte' under varying temperature regimes. In the case of 'Faselis' and 'Hünkar', the concentration did not differ between 35/20 °C and 20/35 °C, but higher than constant temperature. In 'Köksal' cultivar, plants grown at 20/35 °C exhibited the higher concentration in comparison to 35/20 °C and constant temperature.

Table 3. Variation in macroelement in content in eggplant leaves under alternating temperature

Cultivar	Treatment	Macroelements					Microelements				
		Ca (%)	Mg (%)	P (%)	K (%)	Na (%)	Fe (ppm)	Zn (ppm)	Cu (ppm)	Mn (ppm)	B (ppm)
Brigitte	35/20 °C-16/8 h	2.31 ± 0.03 a	0.77 ± 0.03 a	0.64 ± 0.02 a	6.79 ± 0.12 ns	0.21 ± 0.03 a	61.4 ± 4.24 a	13.6 ± 0.63 ns	18.2 ± 1.27 a	64.1 ± 1.29 a	35.9 ± 0.63 a
	20/35 °C-16/8 h	2.10 ± 0.11 a	0.79 ± 0.01 a	0.66 ± 0.02 a	6.83 ± 0.08 ns	0.14 ± 0.02 b	51.2 ± 1.73 b	11.9 ± 0.36 ns	9.8 ± 2.93 b	51.0 ± 2.07 b	38.2 ± 0.57 a
	25 °C-Constant	1.82 ± 0.05 b	0.62 ± 0.02 b	0.57 ± 0.01 b	6.59 ± 0.18 ns	0.24 ± 0.04 a	40.8 ± 1.97 c	11.8 ± 0.93 ns	7.4 ± 0.30 b	34.7 ± 0.91 c	25.4 ± 2.61 b
Faselis	35/20 °C-16/8 h	1.91 ± 0.01 ab	0.77 ± 0.01 a	0.63 ± 0.01 a	6.72 ± 0.04 a	0.13 ± 0.02 ns	55.9 ± 1.31 a	10.7 ± 0.79 a	12.5 ± 2.09 a	38.8 ± 0.74 ns	32.2 ± 1.09 a
	20/35 °C-16/8 h	1.98 ± 0.10 a	0.73 ± 0.01 b	0.65 ± 0.02 a	6.25 ± 0.06 b	0.11 ± 0.02 ns	47.6 ± 1.26 b	8.4 ± 0.34 b	6.2 ± 2.87 b	39.4 ± 1.39 ns	33.0 ± 0.44 a
	25 °C-Constant	1.67 ± 0.06 b	0.68 ± 0.01 c	0.58 ± 0.02 b	6.15 ± 0.03 b	0.16 ± 0.02 ns	46.0 ± 0.43 b	8.3 ± 0.34 b	8.0 ± 2.26 ab	37.6 ± 0.66 ns	26.4 ± 1.51 b
Hünkar	35/20 °C-16/8 h	2.53 ± 0.06 a	0.78 ± 0.01 a	0.64 ± 0.01 a	7.02 ± 0.11 a	0.17 ± 0.02 ns	64.7 ± 2.62 a	13.8 ± 0.33 a	11.7 ± 1.01 a	79.8 ± 1.77 a	32.0 ± 0.89 a
	20/35 °C-16/8 h	1.74 ± 0.05 b	0.57 ± 0.02 b	0.55 ± 0.01 b	6.55 ± 0.13 a	0.21 ± 0.01 ns	46.8 ± 3.10 b	13.2 ± 0.28 a	5.9 ± 1.47 b	68.5 ± 1.77 b	35.2 ± 0.19 a
	25 °C-Constant	1.39 ± 0.13 c	0.48 ± 0.03 c	0.31 ± 0.04 c	5.61 ± 0.22 b	0.18 ± 0.02 ns	34.3 ± 0.34 c	7.6 ± 0.89 b	2.0 ± 0.20 c	35.8 ± 4.19 c	7.6 ± 1.88 b
Karaefe	35/20 °C-16/8 h	1.94 ± 0.01 b	0.82 ± 0.01 a	0.68 ± 0.03 ns	7.36 ± 0.08 a	0.10 ± 0.00 b	67.7 ± 4.57 a	11.5 ± 0.25 ns	8.5 ± 0.81 ns	40.5 ± 1.60 b	36.9 ± 1.50 a
	20/35 °C-16/8 h	2.23 ± 0.01 a	0.84 ± 0.01 a	0.67 ± 0.02 ns	6.27 ± 0.32 b	0.17 ± 0.01 a	51.5 ± 7.23 b	12.1 ± 0.28 ns	5.5 ± 1.59 ns	48.0 ± 1.80 a	37.3 ± 0.87 a
	25 °C-Constant	1.88 ± 0.06 b	0.75 ± 0.01 b	0.63 ± 0.02 ns	6.07 ± 0.09 b	0.16 ± 0.02 a	38.8 ± 0.24 c	11.4 ± 0.15 ns	6.8 ± 0.95 ns	39.0 ± 0.35 b	26.3 ± 1.65 b
Hawk	35/20 °C-16/8 h	3.31 ± 0.21 a	1.12 ± 0.05 a	1.14 ± 0.07 a	7.33 ± 0.23 a	0.20 ± 0.00 b	104.9 ± 8.38 a	27.6 ± 1.41 a	27.0 ± 1.46 a	142.9 ± 8.19 a	45.1 ± 1.74 a
	20/35 °C-16/8 h	1.91 ± 0.01 b	0.77 ± 0.01 b	0.81 ± 0.01 b	5.52 ± 0.07 b	0.30 ± 0.01 a	80.2 ± 1.98 b	24.6 ± 2.85 a	23.8 ± 3.20 a	115.9 ± 1.33 b	38.2 ± 0.43 b
	25 °C-Constant	1.81 ± 0.170b	0.57 ± 0.059 c	0.52 ± 0.03 c	5.50 ± 0.15 b	0.26 ± 0.03 a	67.9 ± 4.13 c	18.3 ± 0.59 b	15.4 ± 1.16 b	76.9 ± 3.47 c	27.1 ± 0.66 c
Köksal	35/20 °C-16/8 h	2.02 ± 0.020b	0.84 ± 0.024 a	0.79 ± 0.02 ab	6.86 ± 0.10 b	0.10 ± 0.01 b	56.3 ± 6.70 ns	14.3 ± 0.19 ns	12.6 ± 0.76 a	75.8 ± 2.81 a	41.9 ± 0.85 a
	20/35 °C-16/8 h	2.28 ± 0.053 a	0.74 ± 0.006 b	0.84 ± 0.02 a	7.56 ± 0.04 a	0.19 ± 0.01 a	57.8 ± 3.97 ns	12.7 ± 0.78 ns	9.6 ± 1.01 ab	77.6 ± 6.86 a	40.8 ± 0.18 a
	25 °C-Constant	1.72 ± 0.053 c	0.69 ± 0.006 b	0.77 ± 0.01 b	6.84 ± 0.17 b	0.09 ± 0.00 b	46.7 ± 4.83 ns	13.3 ± 1.74 ns	7.4 ± 1.06 b	61.5 ± 1.85 b	34.0 ± 5.12 b

Means with different letters in the same column denote significant difference at $p < 0.05$. ± SEM. ns: non-significant

Na concentration showed a marked cultivar × temperature interaction ($p = 0.0002$; Table A2). In 'Brigitte' cultivar, 35/20 °C and constant temperature did not differ with respect to the accumulation. Growing plants at 20/35 °C increased Na in 'Brigitte' cultivar. In 'Faselis' and Hünkar cultivars, temperature regimes were not effective on Na responses. On the other hand, 'Hawk' and 'Karaefe' exhibited same behaviour of Na accumulation, with highest levels at 20/35 °C and constant temperature. In 'Köksal', highest concentration was recorded at 20/35 °C.

Concerning the accumulation of micronutrients, the nutrients also responded significantly to the interactive effects (Tables S2). In the case of Fe, except 'Köksal' cultivar, the highest levels of Fe were recorded at 35/20 °C, with notably levels in 'Hawk' (Table 3). Zn remained comparatively stable, though 'Hawk' again stood out under alternating regimes (27.6 ppm). For Cu, 35/20 °C regime contributed to the levels in 'Brigitte', 'Faselis', and 'Hünkar'. In other cultivars, 35/20 °C and 20/35 °C regimes did not significantly differ. Mn levels were cultivar-specific. Importantly, it reached a higher level at 35/20 °C (79.8 ppm), whereas constant temperature suppressed accumulation to nearly half. B concentration peaked in 'Hawk' (45.1 ppm) under 35/20 °C, but remained the lowest in 'Hünkar' at constant conditions.

Heritability estimation

We further estimated the broad-sense heritability (H^2), reporting marked differences among the traits considered for the study (Table 4). For instance, germination and emergence showed moderate heritability (0.62 and 0.58, respectively). These findings indicate that both genetic and environmental factors influence early seedling establishment. Concerning the growth-related traits, including SL, RL, SD, and TLN, showed moderately high heritability (0.69-0.75) and Biomass traits (SFW, SDW, RFW, and RDW) demonstrated slightly lower heritability (0.64-0.68), indicating the greater effects of environmental conditions on seedling biomass accumulation. On the other hand, we observed that nutrient uptake/accumulation traits were characterized by high heritability, particularly Fe, Zn, Cu, and Mn concentrations (0.79-0.84). These findings reflect those micronutrient uptake/accumulation are largely genotype-dependent, in case of eggplant cultivars considered in the present study. However, macronutrients such as K and Mg showed low across-regime heritability (0.31 and 0.41, respectively) but very high heritability within individual temperature regimes (> 0.90), indicating strong genotype × environment interactions. Additionally, Ca, P, Na, and B concentrations also exhibited high heritability values (> 0.70). Regarding antioxidant enzymes including SOD, CAT, and APX showed high heritability (0.91-0.97).

Table 4: Broad-sense heritability (H^2) for all measured traits in the study

Trait	**H^2 (across regimes)**	**H^2 @ 35/20 °C**	**H^2 @ 20/35 °C**	**H^2 @ 25 °C**
Germination (%)	0.62	0.7	0.55	0.46
Emergence (%)	0.58	0.65	0.5	0.43
Shoot length (SL)	0.71	0.78	0.66	0.59
Root length (RL)	0.69	0.77	0.63	0.57
Shoot diameter (SD)	0.73	0.8	0.69	0.61
Total leaf number (TLN)	0.75	0.82	0.71	0.63
Shoot fresh weight (SFW)	0.64	0.72	0.59	0.53
Shoot dry weight (SDW)	0.68	0.74	0.61	0.56
Root fresh weight (RFW)	0.66	0.73	0.62	0.57
Root dry weight (RDW)	0.67	0.75	0.63	0.58
Fe concentration	0.82	0.9	0.95	0.88
Zn concentration	0.79	0.87	0.94	0.9
Cu concentration	0.84	0.92	0.96	0.91
Mn concentration	0.81	0.9	0.94	0.89
B concentration	0.72	0.95	0.96	0.96
Na concentration	0.76	0.89	0.96	0.85
P concentration	0.66	0.97	0.97	0.98
K concentration	0.31	0.77	0.95	0.92
Ca concentration	0.7	0.95	0.95	0.95
Mg concentration	0.41	0.96	0.99	0.91
SOD activity	0.92	0.99	1	1
CAT activity	0.97	0.99	1	1
APX activity	0.91	1	1	1

Correlation and principal component analysis of the variables

The first two principal components (PCs) explained 100.00% of the total variation, with PC1 contributing 77.81% and PC2 accounting for 22.19% (Figure 3A). With respect to the trait associations and loadings, most nutrients (Ca, Mg, K, P, Fe, Zn, Cu, Mn, and B) loaded positively on PC1, along with antioxidant enzyme activities (SOD, CAT, APX) and several growth traits (SL, GER, EMER, RL, RFW, RDW). By contrast, Na and MGT were positioned negatively on PC1, suggesting an antagonistic relationship between sodium accumulation, mean germination time, and nutrient efficiency. PC2 was mainly explained by seedling biomass traits. SDW exhibited a strong positive loading on PC2 (0.40), whereas SD (-0.42) and MLN (-0.41) were negatively associated. Concerning the cultivar and temperature regime patterns, a clear separation was observed for temperature regimes. For instance, plants under constant 25 °C temperature clustered negatively on PC1, closely associated with higher Na and MGT values. However, alternating temperature regimes (35/20 °C and 20/35 °C) were positioned positively on PC1. Their statuses were driven by enhanced macro- and micro-nutrient accumulation, higher antioxidant enzyme activities, and improved growth performance.

According to the correlation analysis, germination percentage (GER) and emergence rate (EMER) were found to be highly and positively correlated ($r = 0.95$, $p < 0.001$). This indicates that improved emergence is closely associated with higher germination success. Conversely, mean germination time (MGT) showed a strong negative correlation with GER ($r = -0.98$, $p < 0.001$) and EMER ($r = -0.93$, $p < 0.01$). As expected, delayed germination was detrimental to both germination success and seedling establishment. Seedling length (SL) showed significant positive correlations with GER ($r = 0.96$, $p < 0.001$) and EMER ($r = 0.97$, $p < 0.001$). Biomass parameters, including seedling fresh weight (SFW, $r = 0.97$, $p < 0.001$) and seedling dry weight (SDW, $r = 0.94$, $p < 0.001$), were also strongly correlated with GER. Similarly, antioxidant enzyme activities, such as superoxide dismutase (SOD) and catalase (CAT), were positively associated with growth and nutrient status (e.g. SOD with SFW, $r = 0.98$, $p < 0.001$; CAT with RFW, $r = 0.89$, $p < 0.01$). Among nutrients, potassium (K), calcium (Ca), magnesium (Mg) and iron (Fe) showed significant positive correlations with GER and EMER ($r = 0.82$-0.92, $p < 0.01$), while sodium (Na) displayed significant negative correlations with most growth parameters ($r = -0.83$ to -0.87, $p < 0.01$).

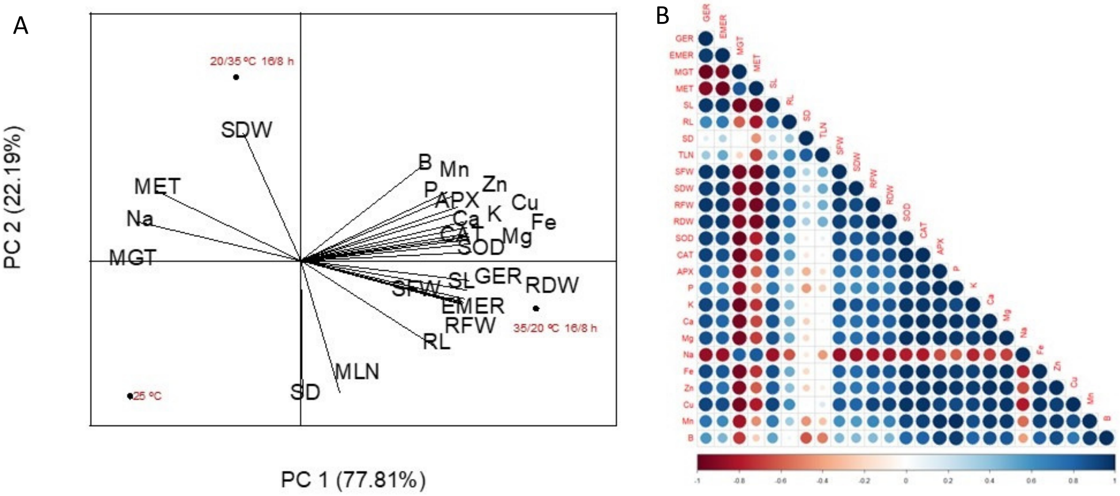

Figure 3. PCA-biplot (A) and Correlation analysis (B) of measured morphophysiological, nutrient, and other traits of eggplant cultivars grown under varying temperature regimes

Discussion

Germination and emergence responses to alternating temperatures

The present study clearly demonstrated that the 35/20 °C-16/8 h temperature regime produced the most favorable results for the germination and emergence of eggplant seeds, whereas the 20/35 °C-16/8 h treatment yielded responses comparable to those under constant temperature (Figure 1). These findings are consistent with previous reports indicating that alternating temperatures enhance seed coat softening, accelerate dormancy release, and promote normal seedling development (Amato *et al.*, 2007; Hu *et al.*, 2012; Reis *et al.*, 2024). However, in species adapted to cooler climates, such as *Thymus munbyanus* Boiss & Reut, lower alternating temperatures can be more effective for germination (Ouahzizi *et al.*, 2024).

Alternating temperature regimes may also strengthen the embryo and shoot, facilitating easier shedding of the seed coat (Özden *et al.*, 2021). This structural advantage often translates into greater seedling vigor and healthier development, as documented by previous studies (Koyano *et al.*, 2005; Shimizu *et al.*, 2008; Inthichack *et al.*, 2014; Yang *et al.*, 2016). Quantitatively, the 35/20 °C regime increased germination rate by approximately 14%, emergence by 37.9%, and shoot length (SL) by 269% compared with the control. Root length (RL) increased by 69.3%, while stem diameter (SD) and true leaf number (TLN) rose by 130% and 70.8%, respectively. These results highlight the pronounced stimulatory effect of initiating germination with a warm phase and maintaining it for an extended duration.

The 35/20 °C-16/8 h regime also enhanced plant height and the fresh and dry weights of both shoots and roots, reflecting improved viability and early growth performance. Conversely, the 20/35 °C-16/8 h treatment neither enhanced these traits nor maintained root length and leaf formation, which were even reduced relative to the constant temperature control (Tables 1 and 2). Similar findings have been reported for *Matthiola incana*, *Antirrhinum majus*, *Viola* × *wittrockiana*, and *Impatiens walleriana*, where extended warm periods promoted seedling height (Itol *et al.*, 1997a-b). Likewise, Inthichack *et al.* (2013) observed that greater day-night temperature differences increased the height of eggplant, pepper, and tomato seedlings, whereas lower daytime temperatures had the opposite effect.

Temperature is a decisive factor in root development, as the radicle is the first organ to emerge (Moss, 1983; Özden *et al.*, 2021). Previous studies demonstrated that extended warm phases under alternating regimes can increase both shoot and root biomass (Jia *et al.*, 2015). However, the impact varies depending on the temperature amplitude and sequence. For eggplant, a warm-climate species, initiating germination with a high-temperature phase and maintaining it for longer periods clearly enhanced both germination efficiency and early seedling growth, as confirmed in the present study.

Antioxidant enzyme responses under alternating temperatures

Regarding antioxidant status, after 72 h of incubation we observed higher activities of SOD, CAT, and APX under alternating temperatures compared with constant temperature conditions, with the highest levels recorded under the 35/20 °C-16/8 h treatment (Figure 2). These results are consistent with the improved seed viability and seedling growth parameters observed under the same regime. In line with our findings, Tesfay *et al.* (2016) reported that in *Moringa oleifera* seeds, both germination rate and speed, as well as SOD and CAT activities, were enhanced under high alternating temperatures. Similarly, Rasheed *et al.* (2016) found that in *Salsola drummondii* seeds, increases in alternating temperature led to elevated germination rates together with higher SOD, CAT, and APX activities. Ducic *et al.* (2003) observed that SOD and CAT activation coincided with the onset of germination in *Chenopodium rubrum* and ceased once germination stopped. Comparable patterns were also reported for *Chenopodium murale* (Bogdanovic *et al.*, 2008) and *Picea omorika* (Prodanovic *et al.*, 2007). Collectively, these findings indicate that seeds with higher germination vigor tend to exhibit elevated antioxidant enzyme activities, as also observed in our study. However, Bailly *et al.* (2002) showed that certain pre-treatments increased germination rates and CAT activity in sunflower seeds, while SOD activity decreased. For species with partial dormancy, such as eggplant, fluctuating temperatures may promote physiological maturation, simultaneously triggering defence mechanisms and enhancing antioxidant enzyme activities. Indeed, as seeds undergo maturation during incubation under fluctuating temperature regimes, cellular degradation decreases while antioxidant capacity increases (Özden, 2022).

Nutrient accumulation under alternating temperatures

Seedlings grown under alternating temperatures exhibited higher nutrient uptake, with elevated levels of Mg, Ca, P, K, Fe, Zn, Cu, Mn, and B compared with those grown at the constant temperature of 25 °C. In parallel with seedling and root development, the highest nutrient contents-except for Na-were recorded under the 35/20 °C-16/8 h regime (Tables 3). Magnesium plays a central role in green leaves, serving as the central atom in the chlorophyll molecule, and is also involved in protein synthesis (Wilkinson *et al.*, 1990; White, 2009). However, prolonged exposure to low temperatures in fluctuating regimes has been reported to reduce dry matter accumulation and photosynthetic rates in pea and other leafy tissues (Stavang *et al.*, 2010). Similar effects have been observed in cucumber (Patil and Moe, 2009; Xiong *et al.*, 2011). These reports align with our findings, where Mg content increased with longer exposure to high temperatures compared with both constant temperature and fluctuating regimes dominated by extended low temperatures (Table 3). Nonetheless, contradictory results have also been reported. For instance, Inthichack *et al.* (2013) showed that under a 15/25 °C-12/12 h regime, Mg content increased in the roots and stems of eggplant and tomato, while no significant change was observed in leaves; in pepper, however, the highest Mg content was recorded in the leaves. These findings suggest that Mg accumulation may vary depending on plant organ and species. Importantly, the Mg contents observed in our study fall within the sufficiency ranges reported by Kacar and Katkat (2011) for young petioles and mature leaves of eggplant.

Calcium is a component of the α-amylase enzyme, which plays a critical role in seed germination and significantly influences its enzymatic activity (Bush *et al.*, 1986). In our study, analysis of seedling leaves showed that the highest average Ca concentrations were recorded under the 35/20 °C-16/8 h and 20/35 °C-16/8 h treatments, while the lowest Ca content was observed under constant temperature conditions (Table 3). These Ca levels fall within the sufficiency ranges defined by Kacar and Katkat (2011). By contrast, Inthichack *et al.* (2013) reported no significant differences in Ca content among alternating temperature treatments in tomato leaves, while in eggplant, the lowest Ca content occurred under 15/25 °C-12/12 h, and the highest under constant temperature and 25/15 °C-12/12 h conditions. Taken together, our findings suggest that alternating temperatures may increase Ca content compared with constant temperatures, thereby supporting seed germination and plant development and indirectly enhancing K uptake.

Regarding P and K, the highest average values in seedling leaves were recorded under the 35/20 °C-16/8 h treatment, while the lowest were observed under constant temperature conditions (Table 3). The measured P contents were within the sufficiency ranges reported by Kacar and Katkat (2011) for young petioles and mature eggplant leaves, whereas K contents under fluctuating temperatures were relatively high. Yang *et al.* (2016) reported that in tomato roots, decreasing night-time temperatures under alternating regimes reduced N, P, and K contents, while increasing daytime temperatures enhanced their uptake. These results are consistent with our findings. By contrast, Xu and Kafkafi (2003) showed that under prolonged high temperatures in alternating regimes, pepper seeds accumulated more K and Cl, whereas P, Mg, and Ca contents decreased. Taken together, these studies suggest that nutrient uptake may increase under alternating temperatures, but the distribution of nutrients can vary depending on exposure duration and the plant organ analysed.

In our study, high temperatures positively influenced P and K uptake. Similarly, Jia *et al.* (2015) reported that greater temperature fluctuations enhanced the uptake of macroelements such as N, P, and K in *Gerbera* seedlings. Potassium is a key mineral for photosynthesis and plays essential roles in plant growth and development (Shah *et al*., 2024). Increased K uptake contributes to a higher net photosynthetic rate and, consequently, greater yield (Ding *et al*., 2006). The high K contents observed under alternating temperatures in our study, compared with the sufficiency ranges reported by Kacar and Katkat (2011), suggest that enhanced photosynthetic activity may contribute to increased biomass production. Indeed, higher seedling fresh and dry weights are often associated with greater photosynthetic potential (Huang *et al*., 2019).

Although statistical differences were observed among the varieties, no significant difference was found in sodium (Na) content based on the average values (Table 3). Proportionally, however, the lowest Na content was observed under the 35/20 °C-16/8 h treatment, while higher values were recorded under constant temperature and 20/35 °C-16/8 h treatments. Na functions as a water-regulating ion under water-deficient conditions but is also a major contributor to salinity stress in plants (Kumlay *et al*., 2023). While some species require only trace amounts of Na, others do not need it at all (Kacar, 2015). Assaha *et al*. (2013) reported that under salinity stress at constant temperatures, Na and Mg contents increased in the leaves, stems, and roots of eggplant (*Solanum melongena* L.) and African nightshade (*Solanum scabrum* Mill.), whereas K and Ca contents decreased. Particularly in eggplant, elevated salinity leads to higher Na/K and Na/Ca ratios, resulting in reduced plant growth.

According to our findings, Fe and Zn concentrations were statistically highest under the 35/20 °C-16/8 h treatment, while the lowest values were recorded under constant temperature (control) conditions (Table 3). The Fe contents observed under alternating temperatures were within the sufficiency ranges reported by Kacar and Katkat (2011) for young petioles and mature eggplant leaves, whereas values under constant temperature fell below these sufficiency thresholds. In contrast, Zn contents across all treatments were below the sufficiency limits. Fe and Zn are essential for catalyzing numerous biochemical processes in plants, including chlorophyll synthesis and respiration, through the activation of various enzymes (Kacar, 2015). Jia *et al*. (2015) reported that in *Gerbera* seedlings, increasing temperature fluctuations elevated Fe content in roots and leaves, while Zn content was unaffected. In line with these results, our study showed that Zn uptake did not differ significantly among treatments in three cultivars, whereas significant variation was observed in the other three. These findings suggest that insufficient Fe and Zn levels may reduce enzymatic activity, potentially impairing plant development.

Cu and Mn are essential for plant development, enzyme reactions, and chlorophyll formation, while B plays a crucial role in RNA synthesis (Kacar, 2015). In our study, Cu, Mn, and B contents in seedling leaves were higher under alternating temperatures than under constant temperature conditions, with Cu and Mn levels being particularly elevated under the 35/20 °C-16/8 h treatment (Table 3). Inthichack *et al*. (2014) reported that in cucumber, melon, and watermelon, a 25/15 °C-12/12 h regime promoted greater Cu uptake compared with 15/25 °C-12/12 h, whereas the highest Mn concentrations in all three species were observed under the 15/25 °C-12/12 h treatment. These results indicate that nutrient uptake varies depending on plant species, temperature regime, and exposure duration. Nevertheless, our findings suggest that alternating temperatures positively influenced Cu, Mn, and B concentrations, potentially enhancing biochemical activation in seedlings.

Taking all findings into account, it is clear that maintaining eggplant seeds under alternating temperatures-beginning with a high temperature-rather than constant temperatures prior to sowing can enhance germination and emergence rates, offering a practical approach for seedling production. Overall, our results are consistent with previous reports, highlighting that alternating temperatures improve not only seedling size and quality in hybrid cultivars but also in rootstock seeds, suggesting effectiveness across genotypes with different genetic backgrounds. Although significant differences were observed among cultivars, antioxidant capacity and mineral nutrient uptake were generally enhanced under alternating temperatures compared with constant conditions (Figure 3). These biochemical and physiological responses are translated into improved plant development across multiple stages, from germination to seedling growth.

Conclusion

This study demonstrated that alternating temperature regimes-particularly those involving high temperatures applied for extended periods-stimulate germination, emergence, seedling development, antioxidant activity, and nutrient uptake more effectively than constant temperature conditions. A positive correlation was observed between seedling emergence rate and seedling growth, which was reflected in improvements in both morphological and physiological parameters. However, it should be noted that antioxidant capacity and the uptake of specific mineral nutrients may vary depending on species, cultivar, plant tissue, and the type of treatment applied.

It is suggested that the 35/20 °C-16/8 h alternating temperature regime can be easily and economically implemented by commercial producers to enhance germination rates and promote seedling development in eggplant cultivars and rootstocks.

Authors' Contributions

Conceptualization: EÖ, MK; Data curation: EÖ, İD; Formal analysis: EÖ, MK; Funding acquisition: EÖ; Investigation: EÖ, İD; Methodology: EÖ, İD; Project administration: EÖ, İD; Resources: EÖ; Software: EÖ, MK; Supervision: İD; Validation: EÖ, İD; Visualization: EÖ, MK; Roles/Writing - original draft: EÖ, MK; and Writing - review & editing: EÖ, MK, İD.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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Appendix

Table A1. The key characteristics of the cultivars or rootstocks utilized in the study

Cultivar/Rootstock	Key traits/notes according to the seed sources	Seed source
Brigitte F1	This cultivar, with a generative growth habit, is well-suited for indoor cultivation. It is easy to manage, reliably sets fruit, and produces uniform, high-quality fruits (200 - 400 g; 4-6 cm diameter; 25 - 30 cm length) with a spineless calyx, greenish flesh, and parthenocarpic nature.	Rijk Zwaan
Faselis F1	This compact, moderately vigorous cultivar produces glossy black fruits (23 - 25 cm × 4 - 6 cm; 200 - 220 g) with excellent transport tolerance and storage quality. It recovers quickly from cold and offers high yield potential.	Seminis
Hünkar F1	This adaptable cultivar has a robust root system and open architecture that limits fungal diseases. It is early-maturing, high-yielding, and can produce extra fruits from lateral branches. Cylindrical fruits (20 - 25 cm × 4 - 6 cm) are dense, brightly coloured, spineless, and have long shelf life. Stable quality across seasons, good cold tolerance, and suitability for greenhouse cultivation enhance its value.	Syngenta
Karaefe F1	This vigorous cultivar suits spring open-field and plastic tunnel cultivation. It produces black, cylindrical fruits (19 - 21 cm) with long shelf life, good transportability, a thornless green stem, and strong Verticillium resistance.	Bursa
Hawk F1	This rootstock forms a strong, resilient root system, ensuring high adaptability. Its vigor reduces the need for excessive nitrogen and irrigation, while strong resistance to diseases and pests eliminates soil pesticide use.	Vilmorin
Köksal F1	This hybrid rootstock variety is resistant to diseases (such as verticillium, fusarium, and nematodes) and has a strong plant structure. In particular, it exhibits excellent root development.	Yüksel

Table A2. Descriptive statistics and summary ANOVA

Traits	Analysis of Variance (ANOVA)			Percentage variance		
	Temperature (T)	Cultivar (C)	T × C	Temperature (T)	Cultivar (C)	T × C
Germination						
Germination (%)	<0.0001	<0.0001	0.183	42.15	47.46	3.04
Emergence (%)	<0.0001	<0.0001	0.034	37.52	47.17	5.95
Morphological						
Shoot length (SL)	<0.0001	<0.0001	<0.0001	13.41	79.84	4.65
Root length (RL)	<0.0001	<0.0001	0.003	10.38	55.21	16.42
Shoot diameter (SD)	<0.0001	<0.0001	<0.0001	2.80	85.43	7.52
Total leaf number (TLN)	<0.0001	<0.0001	<0.0001	17.32	64.55	14.79
Biomass						
Shoot fresh weight (SFW)	<0.0001	<0.0001	0.001	13.16	78.96	4.29
Shoot dry weight (SDW)	<0.0001	<0.0001	0.006	14.12	72.50	6.19
Root fresh weight (RFW)	<0.0001	<0.0001	<0.0001	28.25	46.34	17.06
Root dry weight (RDW)	<0.0001	<0.0001	0.023	28.00	57.95	5.71
Nutrient composition						
Fe concentration	<0.0001	<0.0001	0.026	29.35	52.77	7.17
Zn concentration	<0.0001	<0.0001	<0.0001	7.58	80.24	8.21
Cu concentration	<0.0001	<0.0001	0.005	20.96	63.23	7.48
Mn concentration	<0.0001	<0.0001	<0.0001	14.39	72.10	11.16
B concentration	<0.0001	<0.0001	<0.0001	51.29	28.40	16.07
Na concentration	<0.0001	<0.0001	0.001	6.43	51.49	24.40
P concentration	<0.0001	<0.0001	<0.0001	22.71	43.46	29.20
K concentration	<0.0001	<0.0001	<0.0001	33.09	23.65	32.80
Ca concentration	<0.0001	<0.0001	<0.0001	36.25	14.23	41.24
Mg concentration	<0.0001	<0.0001	<0.0001	41.55	24.17	28.66
Antioxidants						
SOD activity	<0.0001	<0.0001	<0.0001	54.21	39.41	6.11
CAT activity	<0.0001	<0.0001	<0.0001	12.71	82.27	4.51
APX activity	<0.0001	<0.0001	<0.0001	37.86	52.07	9.88

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