Acta Physiologiae Plantarum (2025) 47:78
https://doi.org/10.1007/s11738-025-03821-5

ORIGINAL ARTICLE

The effect of different elicitor treatments on cold stress in *V. Vinifera* L. cv. 'Victoria'

Selda Daler[1] · **Adem Yağcı**[2] · **Rüstem Cangi**[2]

Received: 26 August 2023 / Revised: 6 October 2024 / Accepted: 29 July 2025 / Published online: 23 August 2025

Abstract

In the study carried out as a two-year greenhouse experiment, the effects of salicylic acid (SA; 0.5, 1.0 and 2.5 mM), methyl jasmonate (MJ; 5, 10 and 15 mM) and sodium nitroprusside (SNP; 0.5, 1.0 and 1.5 mM) treatments at different concentrations against cold stress (4 °C, 16 h) in Victoria variety grapevine saplings were investigated, and the most effective concentration ranges were investigated. 1.0 mM SA was found to be the most effective treatment to promote cold stress resistance of grapevines by increasing superoxide dismutase (114.23 U mg^{-1} protein), catalase (1.024 U mg^{-1} protein) and ascorbate peroxidase (20.43 U mg^{-1} protein) enzyme activities while decreasing electrolyte leakage (14.44%) and lipid peroxidation (6.07 nmol g^{-1}) levels. Moreover, 10 mM MJ and 1.0 mM SNP treatments also contributed to the improvement of the osmotic adjustment capacity of grapevines by increasing proline content (MJ, 0.185 µmol g^{-1}; SNP, 0.435 µmol g^{-1}) and relative water content (MJ, 90.06%; SNP, 89.78%), and decreasing electrolyte leakage (MJ, 14.71%; SNP, 16.06%) and lipid peroxidation (MJ, 4.10 nmol g^{-1}; SNP, 5.96 nmol g^{-1}). Additionally, principal component analysis, heatmap and comprehensive evaluation based on the analytic hierarchy process indicated that 1.0 mM SA, 10 mM MJ and 1.0 mM SNP treatments performed better than other treatments in terms of both increasing plant resistance and reducing the severity of damage. This study contains important information that can provide a reference for researchers to enhance the adaptation ability of grapevines to cold stress and can enhance the success of future studies.

Keywords Victoria (*V. vinifera* L.) · Cold stress · Methyl jasmonate · Salicylic acid · Sodium nitroprusside

Introduction

Due to the deteriorating ecological balance, the negative changes in climate and environmental conditions from day to day, as well as heatwaves, cause extreme weather events such as droughts, floods, frosts, hail and severe hurricanes to become more common and severe, and this sometimes leads to irreparable economic losses in many viticulture regions around the world (Venios et al. 2020). The viticulture sector, which has a production volume of 75 million tons and a surface area of 7 million hectares in more than 90 countries around the world, is among the main agricultural branches that will be affected by climate change (FAO 2023). The most important environmental factor determining the geographical boundaries of viticulture is temperature (Huang et al. 2012). Low temperatures at high northern and southern latitudes limit the regional distribution of grapevine species and varieties and significantly affect production performance (Ren et al. 2021).

In recent years, the use of cost-effective, environmentally friendly, time-efficient, easily applicable and reproducible elicitors, which are naturally produced by organisms themselves and have significant effects on physiological and biochemical functions in organisms, has been on the agenda in the regulation of plant defense responses against various stress factors to which plants are exposed. It has been reported that elicitors treated exogenously to plants under

---

Communicated by L. Bavaresco.

✉ Selda Daler
selda.daler@kocaeli.edu.tr

✉ Rüstem Cangi
rustem.cangi@gop.edu.tr

Adem Yağcı
adem.yagci@gop.edu.tr

[1] Department of Horticulture, Faculty of Agriculture, Kocaeli University, 41285 Kocaeli, Turkey

[2] Department of Horticulture, Faculty of Agriculture, Tokat Gaziosmanpaşa University, 60240 Tokat, Turkey

environmental stress conditions can reduce the inhibitory effects of stress by eliminating the deficiency in the concentration of endogenous hormones (Ashraf et al. 2008).

One of the treatments that can be used for this purpose is salicylic acid (SA), which has important effects on plant growth and defense responses. SA, which acts as an important phytohormone in the regulation of plant growth and development, supports plants in avoiding or resisting various environmental stress factors by affecting plant physiology and metabolism, including the synthesis of several defense proteins (Cai et al. 2015). Methyl jasmonate (MJ) is one of the elicitors that have recently been widely used exogenously (Wang et al. 2015). MJ functions as a key signaling molecule that modulates morphological, physiological, biochemical and molecular processes to activate the plant defense system against both biotic and abiotic stress factors and to improve plant tolerance to stressful environments (Wasternack and Song 2017). Nitric oxide (NO) has been another elicitor of great interest to researchers in the last decade. NO has important roles in the regulation of cellular mechanisms because it is a free radical that is small in size, uncharged, short-lived and highly diffusible through biological membranes (Li et al. 2013). It has been reported that sodium nitroprusside (SNP), an external NO donor, can improve morphological, physiological and biochemical characteristics in many species under stress (Małgorzata Kopyra 2004).

There are significant gaps in the existing literature due to the lack of studies on the ability of SA, MJ and SNP elicitors to reduce cold stress in grapevines. Although the effects of SA, one of the elicitors associated with cold stress tolerance in grapevines, have been reported in a limited number of studies (Jalili et al. 2023), no study was found to evaluate the effects of MJ and SNP treatments. In this study, the effects of SA, MJ and SNP treatments were investigated comparatively for the first time in grapevines under cold stress. Within the scope of the study, the responses of elicitor-treated grapevine saplings to cold stress were analyzed and because of the study, it was revealed that exogenous treatments have important roles in terms of improving physiological and biochemical processes in grapevine saplings; significant differences were found between treatments and concentrations.

Materials and methods

Place and year the research was conducted

This study was conducted at Yozgat Bozok University, Faculty of Agriculture (39°46′ N, 34°48′ E, 1340 m altitude, Yozgat, Turkey) for two consecutive seasons in 2021–2022. The experiment was established in the research greenhouse. Cold stress treatments were carried out in the climate rooms of the faculty building. Measurements and analyzes of the samples were carried out in the research laboratories within the faculty.

Plant materials

Rootless cuttings of the plant materials were obtained from the collection vineyard of the Middle Black Sea Transitional Zone Agricultural Research Institute (Tokat, Turkey) under the Republic of Turkey Ministry of Agriculture and Forestry. In the experiment, 1-year-old grapevine saplings of *V. vinifera* L. species 'Victoria' (Cardinal × Hafızali) grafted on *V. berlandieri* × *V. rupestris* hybrid '1103 Paulsen' (1103 P) American grapevine rootstock were used.

Planting and cultivation of saplings

Grafted grapevine saplings were planted in 11 × 11 × 22-cm pots containing an equal volume of sterile peat:perlite (1:1) medium. Immediately after planting, the growing media were regularly irrigated with the nutrient solution recommended by Ollat et al. (1998) for grapevine sapling cultivation, with a drainage rate of 30%, and the pH of the solution was adjusted to 6,5. During the vegetation period, the greenhouse environment was kept at 25 ± 5 °C, 60–70% relative humidity and natural day length conditions with the help of a heater and fan & pad system to ensure root and shoot development in grafted saplings. After sufficient root and shoot development was achieved 4 weeks after planting, grapevine saplings with similar characteristics in terms of stem diameter and leaf area were selected and elicitor treatments were performed with the selected saplings.

SA, MJ and SNP treatments

The study was carried out with 5 different treatments (CNT (+), CNT (−), SA, MJ and SNP) on 330 grapevine saplings with 10 plants in each replicate according to the random plot experimental design with 3 replicates (Fig. 1). The SA (Merck, CAS: 69-72-7); MJ (Sigma, CAS: 39924-52-2); and SNP (Merck, CAS: 13755-38-9) elicitors used in the study were prepared as 1000 mM L^{-1} stock solutions using distilled water, and then these stock solutions were diluted to different concentrations. The concentration ranges of the elicitors were determined according to the results of our previous study on grapevine saplings, considering the efficacy-toxicity onset limits (Daler 2021).

In this study, the "12th phenological developmental stage" according to the E-L scale defined by Eichhorn and Lorenz (1977), when the shoots reach a length of about 10 cm and have 5–6 opened leaves on them 4 weeks after planting, was used. Elicitors of different concentrations were treated by the pulverization method to wet all green parts of the plants (25 ml/vine sapling) three times in total with 10-day

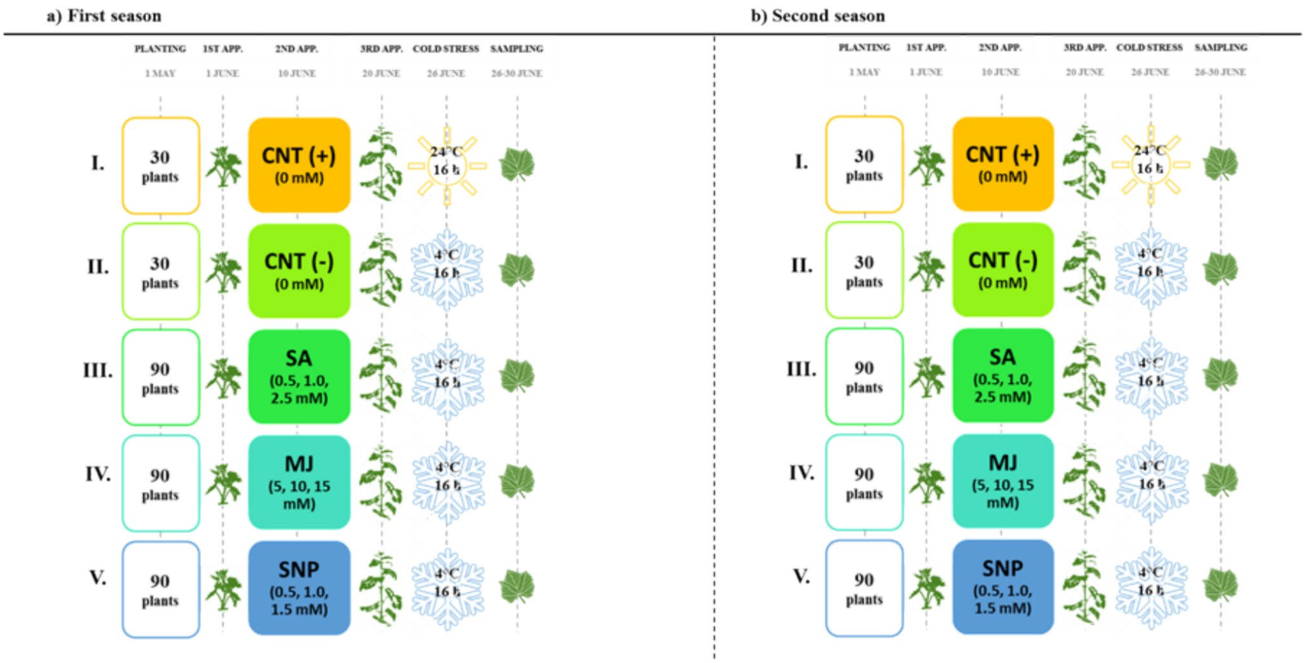

Fig. 1 Implementation scheme. **a** First season: 1 May–30 June 2021; **b** Second season: 1 May–30 June 2022. 1-year old grapevine saplings were used in both growing seasons, and saplings were divided into 5 different treatments. I. CNT (+): positive control, plants treated with pure water and kept at optimum conditions for 16 h at + 24 °C; II. CNT (−): negative control, plants treated with pure water and exposed to cold stress for 16 h at + 4 °C; III. SA: salicylic acid, plants treated with SA at concentrations of 0.5, 1.0 and 2.5 mM and exposed to cold stress for 16 h at + 4 °C; IV. MJ: methyl jasmonate, plants treated with MJ at concentrations of 5, 10 and 15 mM and exposed to cold stress for 16 h at + 4 °C; V. SNP: sodium nitroprusside, plants treated with SNP at concentrations of 0.5, 1.0 and 1.5 mM and exposed to cold stress for 16 h at + 4 °C

intervals. Pure water was used in control groups. Six days after the elicitor treatments were completed under greenhouse conditions, the grapevine saplings were transferred to the Climatic Chambers together with the pots in which they were planted to perform the cold stress treatments.

Exposure of grapevine saplings to cold stress

Negative control CNT (−) plants and elicitor treated plants were placed in climate chamber I with a constant temperature of 19 °C, 65% humidity and 200 µmol m^{-2} s^{-1} PPFD, and then the temperature of the chamber was gradually decreased by 5 °C per hour, reaching 4 °C at the end of the 3rd hour, and exposed to cold stress for 16 h. Positive control CNT (+) plants were simultaneously placed in climate chamber II with a constant temperature of 24 °C, 65% humidity and 200 µmol m^{-2} s^{-1} PPFD and kept under optimum conditions for the same period of time until the cold stress treatments were completed.

Leaf samples for biochemical analyzes were collected immediately after cold stress treatments and stored in an ultra-deep freezer (−80 °C, Thermo Scientific, Forma-89000 Series) until the time of analyzes (Aazami and Mahna 2017). Among the physiological parameters, leaf relative water content and electrolyte leakage were analyzed using leaf samples collected immediately after cold stress treatments (Xu et al. 2022). Stomatal conductivity and leaf temperature were measured directly from the leaves on the grapevine saplings during the sunny midday hours (11:00–13:00) after the cold-stressed grapevine saplings were transferred back to the greenhouse environment under natural lighting conditions and completed a 1-day recovery period (Xu et al. 2022). The SPAD value was measured directly from the leaves on the saplings after the completion of the 4-day recovery period in the grapevine saplings transferred to the greenhouse environment to observe the chlorophyl losses that occurred (Londo et al. 2018).

Obtaining the data

In both growing seasons, the experiment was terminated at the "15th phenological development stage" according to the E-L scale, when 8 leaves on the shoots were opened after 60 days of growing, covering 01 May–30 June. Physiological and biochemical analyzes were performed to determine the changes caused by elicitor treatments against cold stress in grapevine saplings. Sampling was carried out using the fourth leaf from the top of grapevine saplings.

Chlorophyl content (CHL)

Two regions close to the main vein of five leaves in each repetition were measured using a portable chlorophyl meter (Konica Minolta SPAD-502). The mean values obtained were expressed in SPAD (Geravandi et al. 2011).

Electrolyte leakage (EL)

It was calculated by measuring the electrolyte exhaled from the cell (Lutts et al. 1996). Three leaf disks with a diameter of 6 mm were taken from 3 leaves of each repetition with the help of a cork borer. After the leaf disks were kept in 20 ml deionized distilled water (ddH$_2$O) for 4 h, EC1 values were measured with an EC meter (Jenway 470 condimeter), and EC2 values were measured after the same disks were incubated at 100 °C for 10 min. From the values obtained, EL was calculated as percentage (%) using the following formula:

$$\mathrm{EL}\% = \mathrm{EC}_1/\mathrm{EC}_2 \times 100.$$

Stomatal conductance (SC)

The SC value of 3 leaves for each repetition was measured by placing the reading sensor of the porometer (SC-1 Leaf Porometer, Decagon, Pullman, WA) between the veins so that the reading sensor was placed on the lower part of the leaf. The values obtained were recorded in mmol m^{-2} sec^{-1}.

Leaf temperature (LT)

A leaf porometer was used to measure between the veins of three leaves for each repetition. The values obtained were recorded in °C.

Relative water content (RWC)

According to the method of Yamasaki and Dillenburg (1999), the RWC of 3 leaves of each repetition were calculated in percentage according to the following formula by taking into account the fresh weight (FW) measured immediately after harvesting, the turgor weight (TW) determined by soaking in pure water for 6 h, and the dry weight (DW) determined after 48 h in an air-circulating oven at 70 °C:

$$\mathrm{RWC}\% = \left[(\mathrm{FW}-\mathrm{DW})/(\mathrm{TW}-\mathrm{DW})\right] \times 100.$$

Proline content (PRO)

PRO was determined according to the procedure of Bates et al. (1973). Absorbance values were read in a UV/Vis spectrophotometer (Perkin Elmer Lambda 25) at 520 nm and the calculations were recorded as µmol g^{-1} with the help of the calibration curve formed with the proline standard.

Lipid peroxidation (MDA)

Analyzed according to the method of Lutts et al. (1996). Absorbance values were measured spectrophotometrically at 532 and 600 nm, and the results were recorded as nmol g^{-1} tissue.

Total soluble protein content (TSP)

Determined according to the Bradford method (Bradford 1976). The absorbance values were measured at 595 nm in a UV/Vis spectrophotometer and protein concentrations were calculated for each sample using the standard curve.

Antioxidant enzyme activities

Enzyme extracts were prepared according to the method of Özden et al. (2009).

> *Superoxide dismutase (SOD; EC 1.15.1.1):* The SOD was determined according to the procedure of Agarwal and Pandey (2004). At 560 nm, the amount of enzyme causing 50% inhibition of NBT reduction was considered 1 enzyme unit, and values were presented as U mg^{-1} protein.
> *Catalase (CAT; EC 1.11.1.6):* The method treated by Gong et al. (2001) was used. Absorbance values were read against the blind at 240 nm wavelength for 2 min using a UV/Vis spectrophotometer. At 25 °C, the amount of enzyme that decreased the absorbance by 1 µmol in 1 min was considered 1 enzyme unit, and the results were recorded in U mg^{-1} protein.
> *Ascorbate peroxidase (APX; EC 1.11.1.11)*: Analyzed according to the method of Nakano and Asada (1981). The amount of ascorbate oxidized by the enzyme per minute per 1 mg of total protein was considered 1 unit, and the results were recorded in U mg^{-1} protein.

Evaluation of data

The numerical data of the variations between different elicitor treatments and concentrations against cold stress in grapevine saplings were subjected to the analysis of variance (ANOVA) using IBM SPSS vrs. 20.0 package program, and Duncan's Multiple Comparison Test ($p < 0.05$) was used to determine the differences between means. The original data on the elicitor treatments and parameters examined were recorded and processed using Microsoft 365 Excel program and XLSTAT. The relationships among the investigated elicitor treatments and the parameters examined were evaluated by correlation analysis and principal component analysis (PCA); they were also explained with a biplot and hierarchical clustering heatmap (Evgenidis et al. 2011). To provide a comprehensive evaluation of the elicitor treatments and the parameters examined, the methods specified by Wang et al. (2022) and Kılıç (2023a, b) were used. The data means were normalized through variable transformation (min–max scaling) and their weight coefficients were calculated using the analytic hierarchy process (AHP). The consistency ratio of elicitor treatments and parameters examined was 0.09.

Results

Changes in physiological and biochemical parameters

In this study, it was investigated whether exogenous SA, MJ and SNP elicitors could reduce cold stress damage in grapevine saplings and screened for the most effective treatment and optimum concentration that could be a guide for viticulture. Vines without cold stress without elicitor treatment were used as CNT (+), while vines with cold stress without elicitor treatment were used as CNT (−). The results of the analysis of variance showed that the 'Treatment' factor was statistically significant for all parameters examined. In this study, in which the effects of different elicitors and their concentrations treated against cold stress were examined, the findings of physiological changes in grapevines after analyzes are presented in Table 1 and Fig. 2, and the findings of biochemical changes are presented in Table 2 and Fig. 3.

Cold stress shows physiological symptoms such as leaf yellowing (chlorosis) and discoloration caused by the degradation of CHL in leaf tissues (Guo et al. 2018). The findings confirmed that CHL was significantly reduced in the CNT (−) (25.28 SPAD) exposed to cold stress compared to the CNT (+) (37.26 SPAD) grown under normal conditions. Among the elicitor treatments against cold stress, the most effective results in terms of CHL were obtained from 1.0 and 2.5 mM concentrations of SA (33.37 and 33.57 SPAD, respectively), followed by 0.5 mM SA (31.33 SPAD) and 1.0 mM SNP (30.35 SPAD). According to the results, the highest dose of SA (2.5 mM) caused a significant increase in CHL, the highest dose of SNP (1.5 mM; 25.52 SPAD) was in the same group with the CNT (−), and the highest dose

Table 1 The average values of physiological changes in the leaves of grapevine saplings caused by elicitor treatments against cold stress in the first and second growing seasons

Treatment	Concentration	Physiological Parameters				
		CHL (SPAD)	EL (%)	SC (mmol m^{-2} sec^{-1})	LT (°C)	RWC (%)
CNT (+)	0 mM	37.26 ± 1.26a[*]	2.27 ± 0.99f	243.39 ± 14.84a	29.58 ± 0.41 h	93.42 ± 0.58a
	Mean	37.26 ± 1.26A	2.27 ± 0.99D	243.39 ± 14.84A	29.58 ± 0.41D	93.42 ± 0.58A
CNT (−)	0 mM	25.28 ± 2.15f	21.45 ± 1.21a	86.80 ± 5.22 g	31.85 ± 0.94a	85.43 ± 2.68f
	Mean	25.28 ± 2.15E	21.45 ± 1.21A	86.80 ± 5.22D	31.85 ± 0.94A	85.43 ± 2.68C
SA	0.5 mM	31.33 ± 3.41c	15.95 ± 1.43de	157.40 ± 48.02d	30.65 ± 0.50de	87.06 ± 1.86e
	1.0 mM	33.37 ± 4.78b	14.44 ± 1.77e	206.95 ± 6.35b	30.80 ± 0.56 cd	89.44 ± 1.63bc
	2.5 mM	33.57 ± 5.30b	18.80 ± 1.36bc	180.85 ± 19.72c	30.55 ± 1.16e	89.12 ± 1.82bd
	Mean	32.76 ± 4.42B	16.40 ± 2.35C	181.73 ± 35.19B	30.67 ± 0.76B	88.54 ± 1.99B
MJ	5 mM	28.02 ± 1.13e	14.52 ± 2.63de	164.65 ± 20.76d	30.25 ± 0.39f	88.12 ± 1.09de
	10 mM	27.62 ± 0.97e	14.71 ± 2.04de	168.85 ± 15.21 cd	29.90 ± 0.23 g	90.06 ± 1.28b
	15 mM	24.03 ± 0.42 g	18.57 ± 0.63bc	104.00 ± 12.69f	30.05 ± 0.49 fg	88.41 ± 2.83 cd
	Mean	26.56 ± 2.02D	15.93 ± 2.66C	145.83 ± 34.23C	30.07 ± 0.39C	88.86 ± 1.99B
SNP	0.5 mM	29.83 ± 0.83d	17.60 ± 1.84c	132.00 ± 14.02e	31.15 ± 1.03b	89.03 ± 0.40bd
	1.0 mM	30.35 ± 0.88 cd	16.06 ± 0.60d	180.75 ± 27.46c	30.20 ± 0.77f	89.78 ± 1.66b
	1.5 mM	25.52 ± 1.76f	19.30 ± 2.37b	139.10 ± 10.39e	30.95 ± 1.37bc	88.12 ± 2.40de
	Mean	28.57 ± 2.51C	17.65 ± 2.15B	150.62 ± 28.30C	30.77 ± 1.10B	88.98 ± 1.74B
Overall Mean		29.65 ± 4.59	15.79 ± 5.06	160.43 ± 46.97	30.54 ± 0.96	88.91 ± 2.55

CHL: chlorophyl content; EL: electrolyte leakage; SC: stomatal conductance; LT: leaf temperature; RWC: relative water content; CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

[*] The difference between the means shown with different letters in the same column is significant ($P < 0.05$). Data are presented as mean values ± standard deviation (SD) (Daler 2024)

The difference between the means shown with different letters in the same column is significant ($P < 0.05$), according to Duncan's multiplerange test

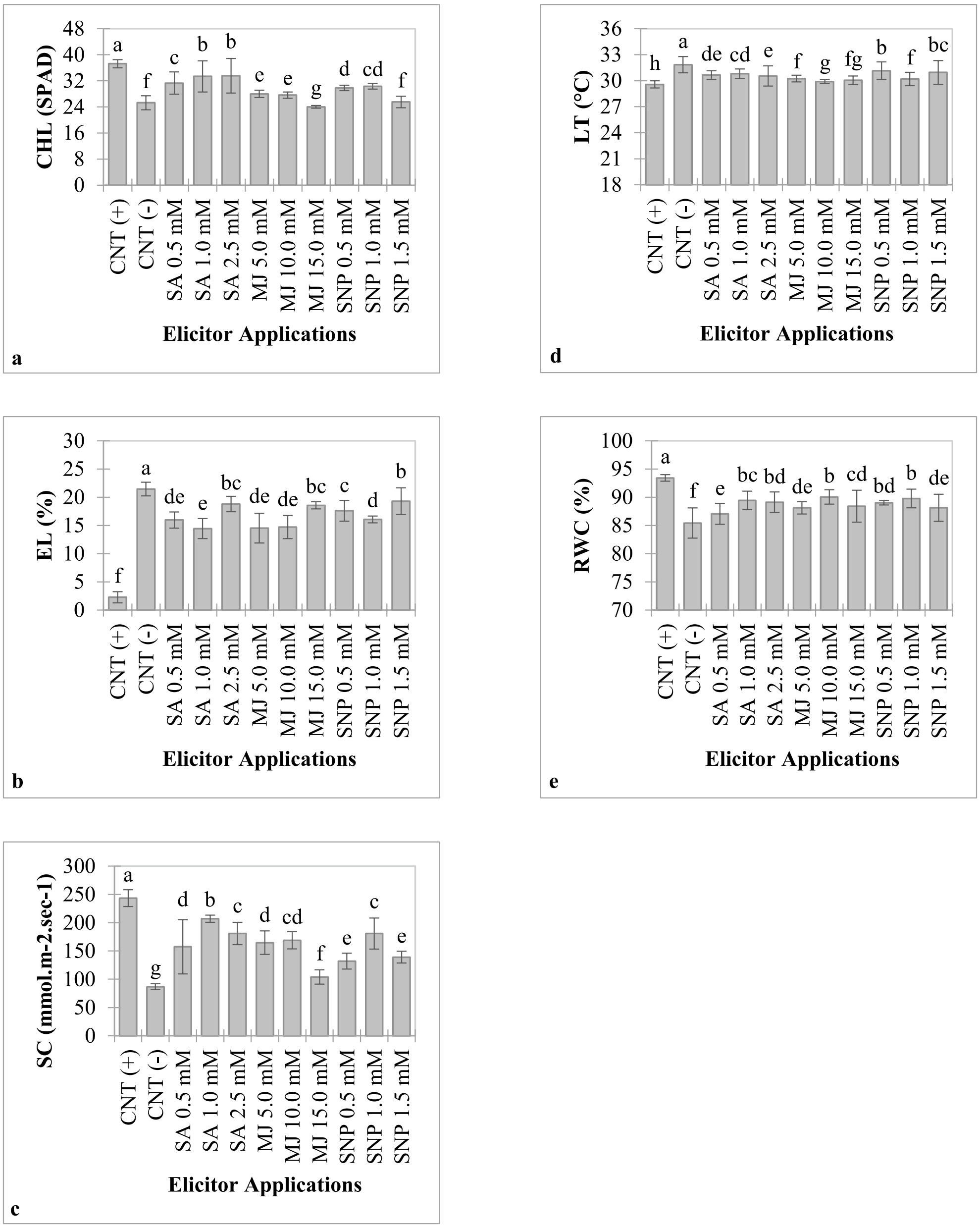

Fig. 2 Effects of elicitor treatments on the physiological characteristics of grapevines subjected to cold stress (average of 2021 and 2022). The data presented are the mean ± standard deviation (SD) of three replicates. Histogram chart represents CHL: chlorophyl content; EL: electrolyte leakage; SC: stomatal conductance; LT: leaf temperature; RWC: relative water content; CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

of MJ (15 mM; 24.03 SPAD) had a lower average than the CNT (−) (Table 1, Fig. 2).

The plasma membrane is the primary site of damage affected by cold stress in plants (Theocharis et al. 2012). Therefore, EL and malondialdehyde (MDA) are the most important stress indicators used to detect membrane damage caused by cold stress (Hasanuzzaman et al. 2019). In this study, it was found that the CNT (+) (2.27%) represented the lowest mean EL and the CNT (−) (21.45%) represented the highest mean EL, and all elicitor treatments contributed to the reduction of EL. However, 0.5 and 1.0 mM SA (15.95% and 14.44%, respectively) and 5 and 10 mM MJ (14.52% and 14.71%, respectively) were found to be the most effective treatments that reduced membrane damage by approximately 1.4-fold compared to CNT (−). In addition, 2.5 mM SA (18.80%), 15 mM MJ (18.57%) and 1.5 mM SNP (19.30%) treatments caused less membrane damage than the CNT (−), but statistically represented the closest averages to the CNT (−) (Table 1, Fig. 2). It was found that MDA content caused the

Table 2 The average values of biochemical changes in the leaves of grapevine saplings caused by elicitor treatments against cold stress in the first and second growing seasons

Treatment	Concentration	Biochemical parameters					
		PRO (μmol g^{-1})	MDA (nmol g^{-1})	TSP (mg)	SOD (U mg^{-1} TSP)	CAT (U mg^{-1} TSP)	APX (U mg^{-1} TSP)
CNT (+)	0 mM	0.036 ± 0.004 g[*]	3.11 ± 0.39 h	0.713 ± 0.077i	1.00 ± 0.31i	0.015 ± 0.002c	2.45 ± 0.23 h
	Mean	0.036 ± 0.004D	3.11 ± 0.39E	0.713 ± 0.077E	1.00 ± 0.31E	0.015 ± 0.002B	2.45 ± 0.23D
CNT (−)	0 mM	0.109 ± 0.058f	13.54 ± 3.98a	0.948 ± 0.079 h	10.02 ± 0.84 h	0.041 ± 0.031c	6.22 ± 3.08 g
	Mean	0.109 ± 0.058C	13.54 ± 3.98A	0.948 ± 0.079D	10.02 ± 0.84D	0.041 ± 0.031B	6.22 ± 3.08C
SA	0.5 mM	0.116 ± 0.046ef	7.09 ± 0.59e	1.989 ± 0.653b	43.61 ± 22.24 cd	0.373 ± 0.388b	11.78 ± 1.92 cd
	1.0 mM	0.191 ± 0.076c	6.07 ± 0.65f	2.214 ± 0.601a	114.23 ± 37.25a	1.024 ± 0.962a	20.43 ± 0.31a
	2.5 mM	0.251 ± 0.032b	8.16 ± 1.46d	1.351 ± 0.232f	35.76 ± 8.73de	0.416 ± 0.424b	10.54 ± 1.27de
	Mean	0.186 ± 0.077B	7.11 ± 1.27C	1.851 ± 0.624A	64.53 ± 43.53A	0.604 ± 0.681A	14.25 ± 4.70A
MJ	5 mM	0.115 ± 0.068ef	6.38 ± 0.91ef	1.504 ± 0.259d	27.85 ± 25.16ef	0.097 ± 0.136c	10.28 ± 0.99de
	10 mM	0.185 ± 0.059 cd	4.10 ± 0.64 g	1.876 ± 0.622c	56.26 ± 10.89b	0.277 ± 0.301bc	16.45 ± 3.30b
	15 mM	0.169 ± 0.145ce	8.56 ± 3.80d	1.449 ± 1.062e	48.22 ± 16.25c	0.237 ± 0.216bc	12.15 ± 2.46 cd
	Mean	0.156 ± 0.097B	6.35 ± 2.85D	1.610 ± 0.709B	44.11 ± 21.22B	0.203 ± 0.228B	12.96 ± 3.51A
SNP	0.5 mM	0.166 ± 0.087ce	9.36 ± 3.14c	1.454 ± 0.346e	20.19 ± 18.65 fg	0.110 ± 0.118c	8.04 ± 3.52f
	1.0 mM	0.435 ± 0.296a	5.96 ± 0.47f	1.868 ± 0.376c	18.60 ± 11.32 g	0.104 ± 0.089c	9.20 ± 4.37ef
	1.5 mM	0.134 ± 0.044df	10.88 ± 4.68b	1.215 ± 0.818 g	14.68 ± 7.74gh	0.090 ± 0.046c	13.43 ± 11.12c
	Mean	0.245 ± 0.219A	8.73 ± 3.73B	1.512 ± 0.592C	17.82 ± 12.78C	0.101 ± 0.084B	10.22 ± 7.16B
Overall mean		0.173 ± 0.143	7.56 ± 3.66	1.507 ± 0.670	35.49 ± 34.15	0.253 ± 0.433	11.00 ± 6.01

PRO: proline content; MDA: malondialdehyde; TSP: total soluble protein content; SOD: superoxide dismutase activity; CAT: catalase activity; APX: ascorbate peroxidase activity; CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

[*] The difference between the means shown with different letters in the same column is significant ($P < 0.05$). Data are presented as mean values ± standard deviation (SD) (Daler 2024).

The difference between the means shown with different letters in the same column is significant (P < 0.05), according to Duncan's multiplerange test

highest increase in CNT (−) (13.54 nmol g^{-1}) compared to CNT (+) (3.11 nmol g^{-1}) kept under optimum conditions. However, it was determined that the lowest average was obtained from 10 mM MJ (4.10 nmol g^{-1}), which decreased the MDA content approximately 3.3 times compared to the CNT (−), and this value was followed by 1.0 mM SNP (5.96 nmol g^{-1}), 1.0 mM SA (6.07 nmol g^{-1}) and 5 mM MJ (6.38 nmol g^{-1}) treatments. Among the elicitor treatments, 1.5 mM SNP (10.88 nmol g^{-1}) caused the least decrease in MDA content (Table 2, Fig. 3).

Cold stress caused a significant decrease in SC in CNT (−) (86.80 mmol m^{-2} sec^{-1}) compared to CNT (+) (243.39 mmol m^{-2} sec^{-1}) grown under normal conditions, while all elicitor treatments caused a significant increase in SC. The most effective treatment was 1.0 mM SA (206.95 mmol m^{-2} sec^{-1}), which caused a 2.4-fold increase compared to the CNT (−), followed by 2.5 mM SA (180.85 mmol m^{-2} sec^{-1}), 1.0 mM SNP (180.75 mmol m^{-2} sec^{-1}) and 10 mM MJ (168.85 mmol m^{-2} sec^{-1}). However, the lowest values among the elicitor treatments were obtained from 15 mM MJ (104.00 mmol m^{-2} sec^{-1}) (Table 1, Fig. 2).

In inverse proportion to SC, the highest mean values were obtained from the CNT (−) (31.85 °C) and the lowest mean values were obtained from the CNT (+) (29.58 °C) in terms of LT. Among the elicitor treatments, CNT (−) was followed by 0.5 and 1.5 mM SNP (31.15 and 30.95 °C, respectively). The closest averages to the CNT (+) were obtained from 10 and 15 mM MJ (29.90 °C and 30.05 °C, respectively) treatments (Table 1, Fig. 2).

The RWC of plant cells is an important indicator of physiological water status in plant tissues (Kohli et al. 2017). The values obtained from the CNT (−) (85.43%) compared to the CNT (+) (93.42%) grown under normal conditions proved that cold stress significantly reduced RWC. However, the highest value in terms of RWC was obtained from the 10 mM MJ (90.06%) treatment, and these averages were in the same statistical group with the 1.0 and 2.5 mM SA (89.44% and 89.12%, respectively) and 0.5 and 1.0 mM SNP (89.03% and 89.78%, respectively) treatments (Table 1, Fig. 2).

The main functions of proline as a compatible osmolte in plants under cold stress conditions are: maintaining redox homeostasis; protecting cells against osmotic stress;

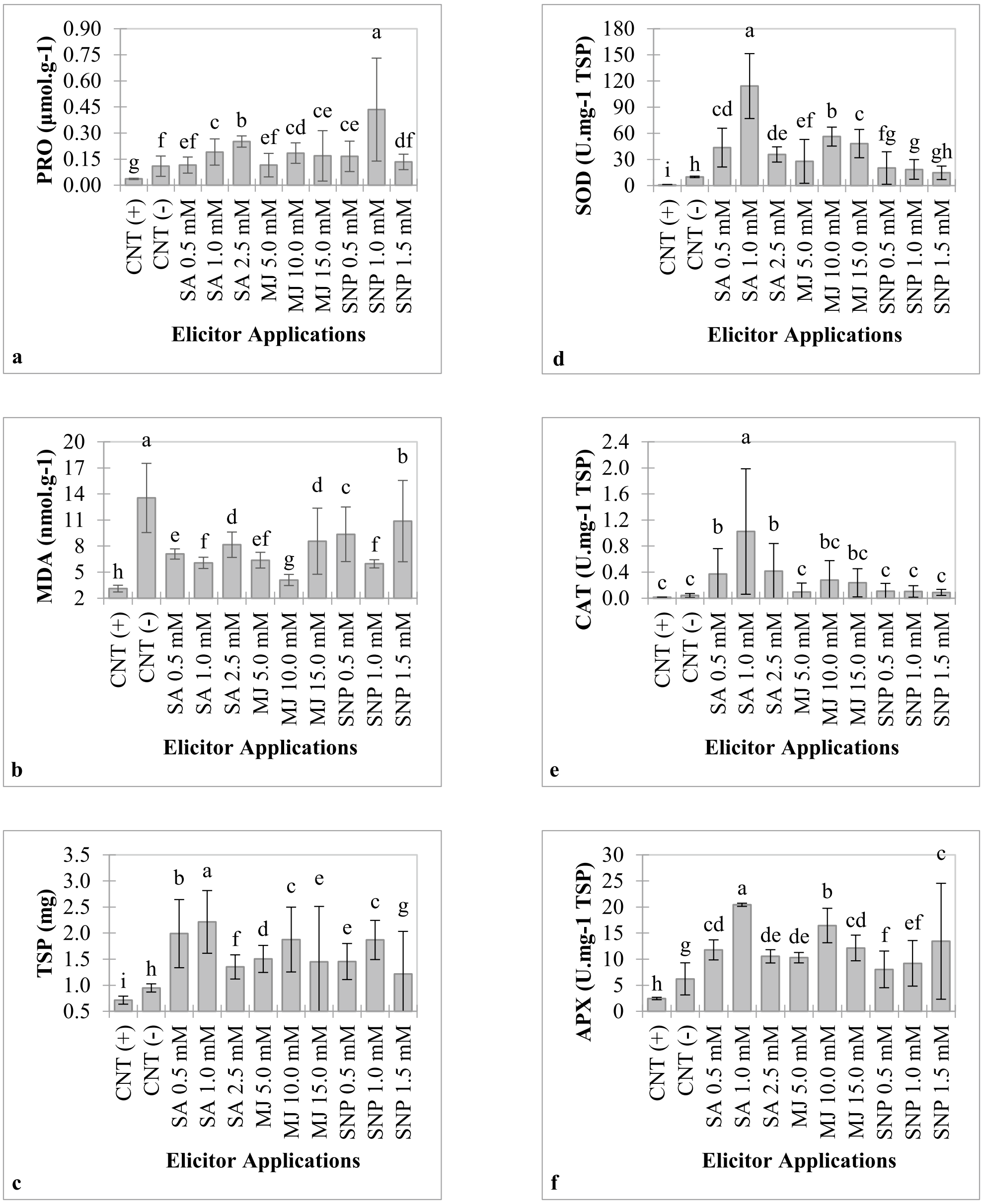

Fig. 3 Effects of elicitor treatments on the biochemical characteristics of grapevines subjected to cold stress (average of 2021 and 2022). Data presented are the mean ± standard deviation (SD) of three replicates. Histogram chart represents PRO: proline content; MDA: malondialdehyde; TSP: total soluble protein content; SOD: superoxide dismutase activity; CAT: catalase activity; APX: ascorbate peroxidase activity; CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

ensuring membrane integrity; stabilizing the structure of proteins; and protecting cellular enzymes (Szabados and Savouré, 2010; Akhkha et al. 2011). The findings confirmed that cold stress significantly increased PRO in the CNT (−) (0.109 µmol g^{-1}) compared to the CNT (+) (0.036 µmol g^{-1}). However, 1.5 mM SNP (0.134 µmol g^{-1}), 0.5 mM SA (0.116 µmol g^{-1}) and 5 mM MJ (0.115 µmol g^{-1}) treatments were in the same statistical group as the CNT (−). It was determined that the highest PRO among the elicitor treatments was obtained from 1.0 mM SNP (0.435 µmol g^{-1}) treatment, which provided a fourfold increase compared to the CNT (−), and this value was followed by 2.5 mM SA (0.251 µmol g^{-1}) treatment (Table 2, Fig. 3).

It was determined that the TSP showed a significant increase with cold stress compared to the CNT (+) (0.713 mg) kept under optimum conditions; the highest average was obtained from 1.0 mM SA treatment with 2.214 mg, which provided a 2.3-fold increase compared to the CNT (−) (0.948 mg), and this value was followed by 0.5 mM SA treatment with 1.989 mg. Among the elicitor treatments, 1.5 mM

SNP (1.215 mg) followed by 2.5 mM SA (1.351 mg) caused the lowest increases in TSP (Table 2, Fig. 3).

Redox homeostasis in plants under stress is provided by antioxidant enzymes, such as SOD, CAT, and APX, which are effective between cells, in addition to low molecular weight compounds, such as proline (Aazami et al. 2021). Although it was determined that SOD increased significantly in the CNT (−) (10.02 U mg^{-1} TSP) under low temperature stress compared to CNT (+) (1.00 U mg^{-1} TSP) under optimum conditions, it was determined that SNP treatments had the lowest average among elicitor treatments with values between 14.68 and 20.19 U mg^{-1} TSP. 1.0 mM SA with 114.23 U mg^{-1} TSP was found to be the treatment with the highest mean 11.4-fold increase in SOD compared to the CNT (−), followed by 10 mM MJ treatment with 56.26 U mg^{-1} TSP and 5.6-fold increase compared to the CNT (−). In terms of CAT, it was determined that the highest value was obtained from 1.0 mM SA (1.024 U mg^{-1} TSP) treatment, and this value increased approximately 25 times compared to the CNT (−) (0.041 U mg^{-1} TSP). It was determined that APX increased significantly in the CNT (−) (6.22 U mg^{-1} TSP) with low temperature stress compared to the CNT (+) (2.45 U mg^{-1} TSP), while the lowest values among the elicitor treatments were obtained from 0.5 mM SNP treatment with 8.04 U mg^{-1} TSP. The highest averages were obtained from 1.0 mM SA (20.43 U mg^{-1} TSP) treatment, and this average caused a 3.3-fold increase compared to the CNT (−) (Table 2, Fig. 3).

Table 3 Factor loadings and correlations between examined parameters and factors

Parameters	Components				
	F1	F2	F3	F4	F5
CHL	**0.80**[*]	–	–	0.52	–
EL	**-0.89**	0.36	–	–	–
SC	**0.95**	–	–	–	–
LT	**-0.79**	–	-0.34	0.44	–
RWC	**0.90**	–	–	–	–
PRO	–	0.38	**0.82**	0.41	–
MDA	**-0.94**	–	–	–	–
TSP	–	**0.90**	–	–	0.33
SOD	–	**0.93**	–	–	–
CAT	–	**0.87**	-0.34	–	–
APX	–	**0.93**	–	–	–
Eigenvalue	4.78	3.63	1.15	0.83	0.29
Variability (%)	43.49	33.04	10.48	7.57	2.60
Cumulative (%)	43.49	76.53	87.00	94.57	97.17

The correlation table represents CHL: chlorophyl content; EL: electrolyte leakage; SC: stomatal conductance; LT: leaf temperature; RWC: relative water content; PRO: proline content; MDA: malondialdehyde; TSP: total soluble protein content; SOD: superoxide dismutase activity; CAT: catalase activity; APX: ascorbate peroxidase activity

[*] Values in bold correspond for each variable to the factor for which the correlation is the largest (Kılıç 2023b)

The bold values indicate the factor with the highest correlation for each parameter, determined based on the results of the Analytic HierarchyProcess (AHP) and correlation analysis

Relationships between elicitor treatments and the parameters examined

Principal Component Analysis (PCA) was performed to show the maximum amount of variation in the data profile in a few principal components and to better explain the relationships between the treated elicitors and the parameters examined. The PCA results are presented both as correlation table (Table 3) and biplot (Fig. 4). The first three components with eigenvalue coefficients greater than 1; Factor 1: 43.49%, Factor 2: 33.04% and Factor 3: 10.48% explained 87% of the cumulative variance.

Principal Component Analysis (PCA) was performed to show the maximum amount of variation in the data profile in a few principal components and to better explain the relationships between the treated elicitors and the parameters examined. PCA of the eleven parameters examined was performed with elicitor treatments, and the results are shown in the Biplot in Fig. 4. The biplot graph supported the relationships obtained from the correlation table. In the biplot graph, examining the relationships between the variables loaded on the first two components was found to be meaningful in terms of evaluating the relationships between physiological and biochemical characteristics. Because a significant portion of the physiological and biochemical characteristics were loaded onto Factor 1 and Factor 2, these components explained 76.53% of the cumulative variance. The variance contribution rate of the first component was 43.49%, and the positive value of the load was the SC, CHL and RWC, while the load value in EL, LT and MDA was larger in the negative direction. The variance contribution rate of the second component was 33.04%, and APX, PRO, TSP, SOD and CAT had larger positive load values. The findings obtained from the biplot graph are in parallel with the results of analysis of variance and confirm that the biggest difference is between CNT (−) and CNT (+). The ranking of elicitor treatments from highest to lowest in terms of the parameters examined was as follows: CNT (+) > 1.0 mM SA > 10 mM MJ > 1.0 mM SNP > 2.5 mM SA > 5 mM MJ > 0.5 mM SA > 0.5 mM SNP > 15 mM MJ > 1.5 mM SNP > CNT (−) (Fig. 4).

To group the parameters examined and treatments, a hierarchical clustering heatmap was constructed in which the rows and columns of the data matrix were ranked according to the output obtained from clustering (Fig. 5). The heatmap basically categorized elicitor treatments at different concentrations into two main clusters. CNT (+) treatment in the

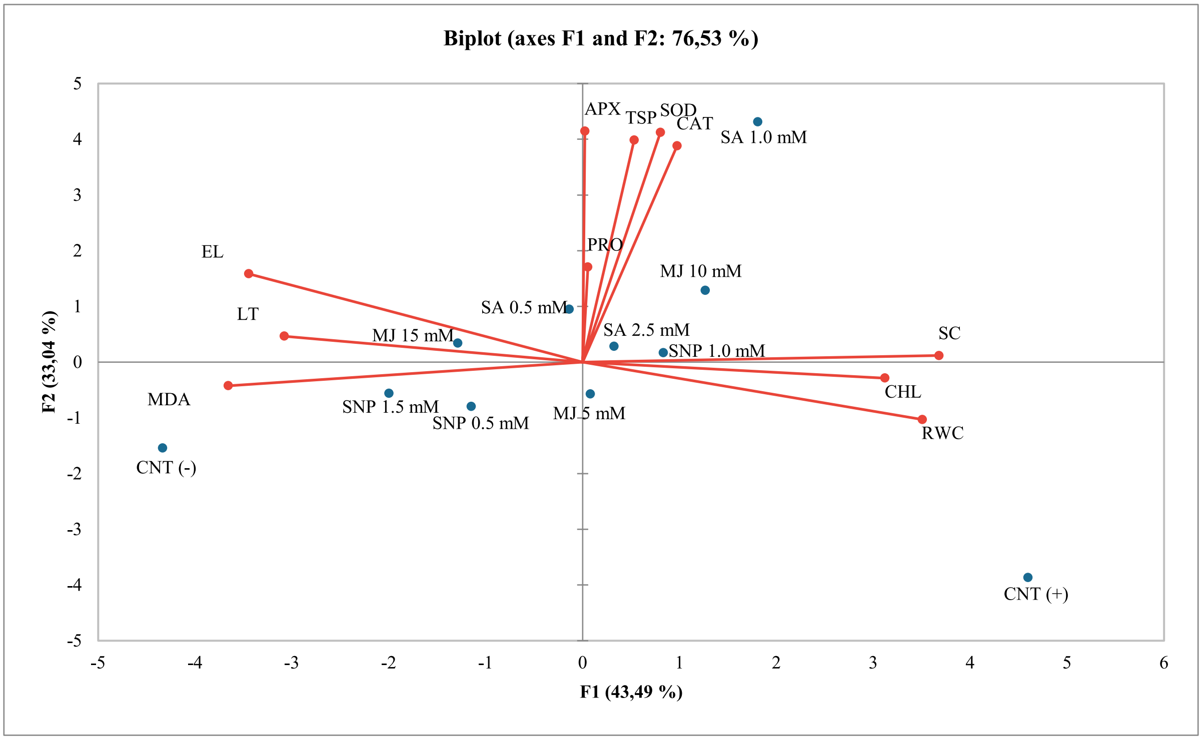

Fig. 4 Biplot graph of elicitor treatments and parameters examined produced by principal component analysis (PCA). The closeness of the lines indicates a high correlation between the variables. The brackets indicate the percentage variation calculated by both principal components. Blue dots indicate the treatments, while red dots indicate the characteristics analyzed. PCA represents CHL: chlorophyl content; EL: electrolyte leakage; SC: stomatal conductance; LT: leaf temperature; RWC: relative water content; PRO: proline content; MDA: malondialdehyde; TSP: total soluble protein content; SOD: superoxide dismutase activity; CAT: catalase activity; APX: ascorbate peroxidase activity; CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

leftmost column of the map was clearly separated from the other treatments by forming the 1st main cluster alone. Here, it was thought that the CNT (+), which was grown under cold stress-free conditions, was clustered differently because it had lower values in terms of stress parameters such as TSP, SOD, CAT, APX and PRO, but higher averages in terms of SC, CHL and RWC compared to the other groups under low temperature stress. The 2nd main cluster represented the 1st subcluster consisting of 15.0 mM MJ, 0.5 and 1.5 mM SNP treatments together with the CNT (−) treatment, and the 2nd subcluster consisting of the other elicitor treatments. In the 1st sub-cluster, in which positive parameters are represented by red color tones and negative parameters are represented by green color tones, it was observed that 15.0 mM MJ, 0.5 and 1.5 mM SNP treatments, which were included together with the CNT (−) treatment, had a lower effect in terms of overcoming low temperature stress compared to other elicitor treatments, and within the same sub-cluster, first the CNT (−) and then 15 mM MJ were separated from 0.5 and 1.5 mM SNP treatments by showing a separate branching. In the 2nd subset in the right columns of the map, in which positive parameters are represented by green color tones and negative parameters are represented by red color tones, the 1.0 mM SA treatment alone formed the 1st group and separated markedly from the other treatments. In the 2nd group, first 1.0 mM SNP and then 10 mM MJ were separated from the other elicitor treatments by showing separate branching.

A comprehensive evaluation of elicitor treatments effective on low temperature stress parameters

From the load and contribution value of each index determined in the principal component analysis, the effects of the elicitor treatments on the parameters examined were calculated, and the weights were determined. Using the weighting coefficients obtained, the comprehensive evaluation of the elicitor treatments according to the composite index values was interpreted separately in terms of positive and negative parameters according to the outcomes obtained from PCA and the results are presented in Figs. 6 and 7, respectively. To facilitate a clearer assessment of the effectiveness of the treatments, the composite index value was requested to be higher for positive parameters and lower for negative parameters.

While the composite index values of control groups according to the positive parameters examined varied

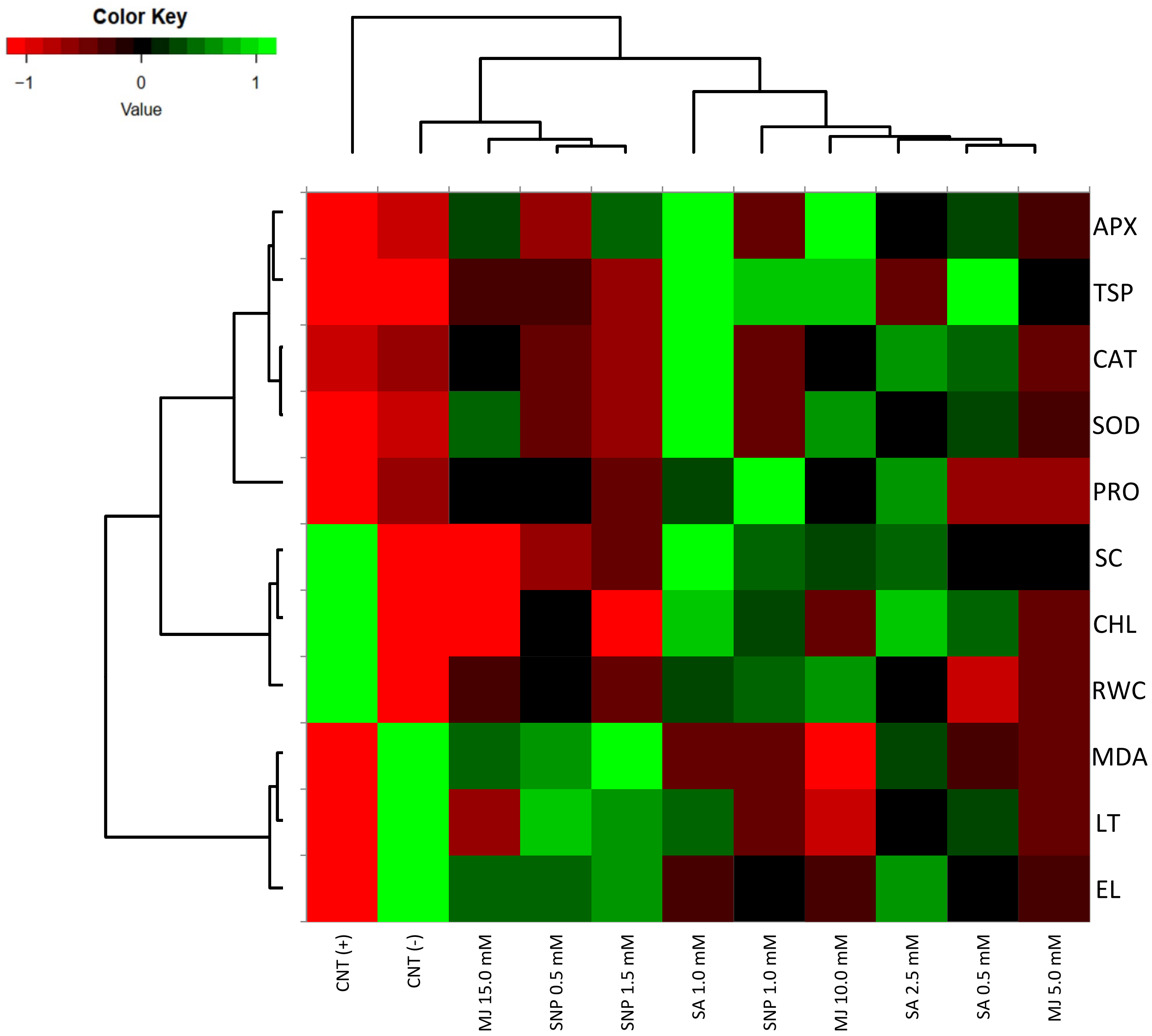

Fig. 5 Heatmap that groups the features and treatments reviewed. Color scale: from red to black and then green. Heatmap represents CHL: chlorophyl content; EL: electrolyte leakage; SC: stomatal conductance; LT: leaf temperature; RWC: relative water content; PRO: proline content; MDA: malondialdehyde; TSP: total soluble protein content; SOD: superoxide dismutase activity; CAT: catalase activity; APX: ascorbate peroxidase activity; CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

between 0.56 (CNT (−)) and 0.95 (CNT (+)), that of the elicitor treatments varied from 1.28 to 3.65. While the highest composite indexes as the elicitor treatments were found in 1.0 mM SA (3.65), 1.0 mM SNP (2.66) and 10 mM MJ (2.36), the lowest indexes were determined in 1.5 mM SNP (1.28), 5 mM MJ (1.42) and 0.5 mM SNP (1.46).

While the composite index values of control groups according to the negative parameters examined varied between 0.00 (CNT (+)) and 5.38 (CNT (−)), that of the elicitor treatments varied from 1.93 to 4.33. While the highest composite indexes as the elicitor treatments were found in 1.5 mM SNP (4.33), 0.5 mM SNP (3.76), 2.5 mM SA (3.54) and 15 mM MJ (3.54), the lowest indexes were determined in 10 mM MJ (1.93), 1.0 mM SA (2.50), 5 mM MJ (2.50) and 1.0 mM SNP (2.60).

Discussion

This study focused on the effect of different elicitor treatments on grapevines subjected to cold stress. The elicitors used affected the physical and biochemical properties of the grapevine to different degrees depending on the

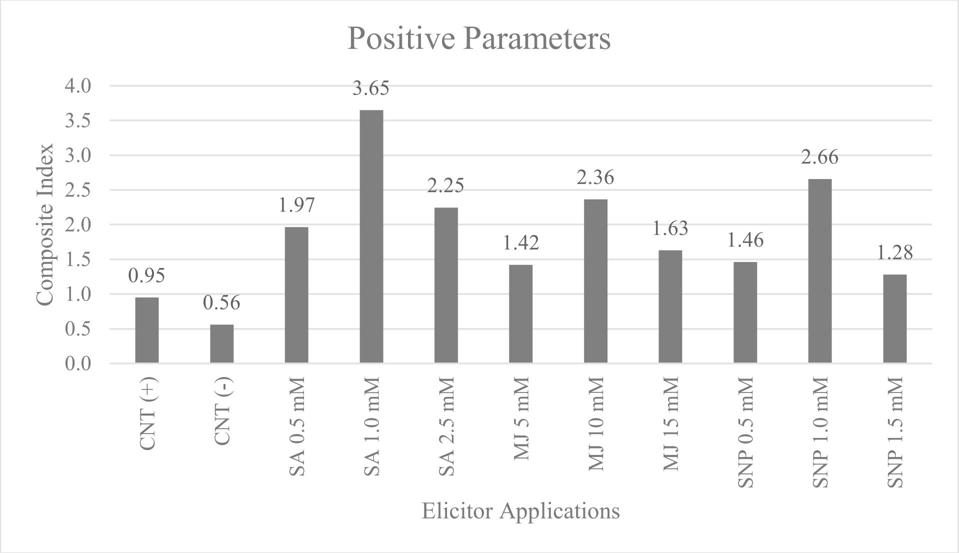

Fig. 6 Comprehensive evaluation of the elicitor treatments according to positive parameters examined (CHL: chlorophyl content; SC: stomatal conductance; RWC: relative water content; PRO: proline content; TSP: total soluble protein content; SOD: superoxide dismutase activity; CAT: catalase activity; APX: ascorbate peroxidase activity). Composite Index represents CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

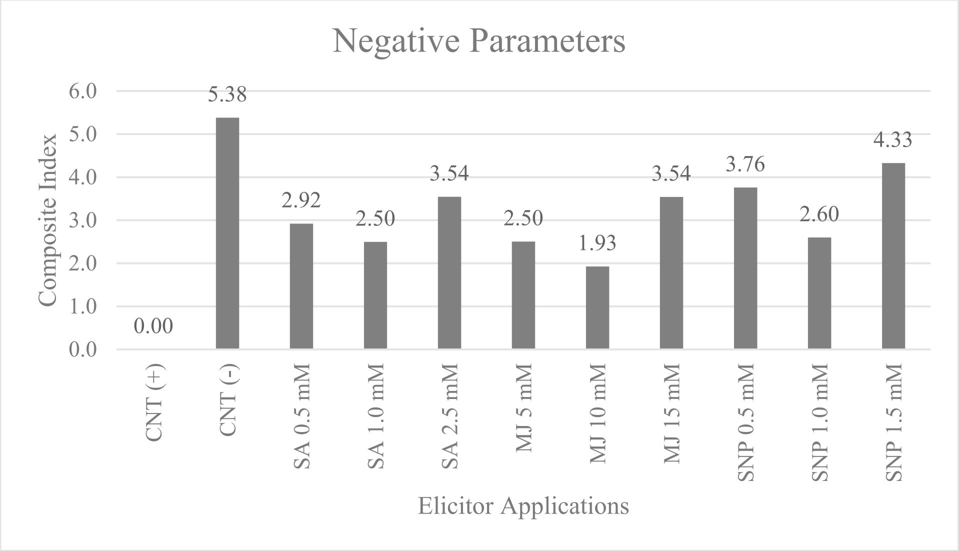

Fig. 7 Comprehensive evaluation of the elicitor treatments according to negative parameters examined (EL: electrolyte leakage; LT: leaf temperature; MDA: malondialdehyde). Composite Index represents CNT (+): positive control; CNT (−): negative control; SA: salicylic acid; MJ; methyl jasmonate; SNP; sodium nitroprusside

concentration, and the grapevines responded to cold stress with defense mechanisms triggered due to these changes.

In this study, in terms of CHL, the best results after CNT (+) were obtained from 1.0 and 2.5 mM concentrations of SA, followed by 0.5 mM SA and 1.0 mM SNP treatments. Similarly, Zhang et al. (2020) reported that SA treatment at 50 mg/L to the leaves of maize saplings below 4 °C improved photosynthesis through increased CHL.

Membrane damage, the main mechanism of low-temperature stress, occurs due to the accumulation of MDA and overproduction of ROS such as O_2^- and H_2O_2, which are critical components of stress response regulation in plants (Hasanuzzaman et al. 2020). In this study, all treatments contributed to the reduction of EL. However, 0.5 and 1.0 mM SA and 5 and 10 mM MJ were found to be the most effective treatments, which reduced membrane damage by approximately 1.4-fold compared to the CNT (−). MJ was found to effectively alleviate chilling injury in tomato (Zhang et al. 2012) and loquat (Jin et al. 2014), and activate some defense compounds to protect the cell membrane against low temperature damage in these crops.

Under extreme conditions such as cold stress, plants can produce highly toxic ROS in excessive amounts, leading to the oxidative destruction of cells and lipid peroxidation (Garg and Manchanda 2009). MDA is an important parameter used to understand the extent of membrane lipid peroxidation and can indirectly measure the extent of membrane damage and resistance to stress (Li and Wang 2021). All elicitor treatments used in this study suppressed the increase in MDA under cold-stress conditions. The best results were obtained from the 10 mM MJ treatment, which reduced the MDA content approximately 3.3-fold compared to the CNT (−). In this study, it is thought that the decrease in EL and MDA as a result of MJ treatments is associated with a significant decrease in O_2^- and H_2O_2 content, which disrupts cell membrane integrity and leads to metabolic imbalances, thus protecting the vines from oxidative stress damage and improving cold stress tolerance. Repkina et al. (2021) revealed that 1 µM MJ treated stabilized increased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in wheat in response to low temperatures.

Yadav (2010) argued that dehydration occurs in plants when exposed to low temperatures due to low water uptake resulting from stomatal closure. It is also stated that low temperatures contribute to ROS formation by reducing gas exchange in leaves due to reduced stomatal conductance (Goswami et al. 2022). In this study, all elicitor treatments caused a significant increase in SC. However, it was recorded that the most effective treatment among the elicitors was 1.0 mM SA, which caused a 2.4-fold increase compared to the CNT (−), and the lowest values were obtained from the 15 mM MJ treatment. The increase in SC in response to elicitor treatments treated in our study can probably be explained by increases in SOD, CAT and APX, which cause inhibition of ROS-signalled stomatal closure. According to the results obtained from this study, it was determined that the lowest averages in terms of LT were obtained from 10 and 15 mM MJ treatments.

Under low temperature stress, plant cells lose water, which leads to a water deficit, resulting in osmotic stress and decreased RWC of the cell (Kohli et al. 2017). In this study, it was determined that the highest value in terms of RWC was obtained from 10 mM MJ and 1.0 and 2.5 mM SA, and 0.5 and 1.0 mM SNP treatments. Zhang et al. Zhang et al. 2020) found that SA treatment increased the RWC of leaves in maize saplings exposed to low temperatures (< 4 °C). Researchers have reported that SA increases cold resistance by promoting the growth and physiological characteristics of maize saplings, and this resistance effect is stronger in cold-sensitive varieties than in resistant varieties.

In previous studies, researchers have reported that proline can stabilize the cell membrane, scavenge free radicals and serve as a carbon and nitrogen source (Szabados and Savouré 2010). In this study, it was determined that the highest PRO was obtained from 1.0 mM SNP treatment, which provided approximately fourfold increase compared to the CNT (−), and this value was followed by 2.5 mM SA treatment. Li and Wang (2021) found that PRO in grapevine saplings, which increased to a certain extent with low temperature stress, was significantly increased by the treatment of different concentrations of SA to the leaves. Repkina et al. (2021) found that exogenous 1 µM MJ treated before exposure to 4 °C caused an increase in PRO in wheat.

Under cold stress, the antioxidant enzyme activities of most plants continue to increase, slowing the rate of oxidation and reducing oxidative damage (Bracale and Coraggio 2003; Einset et al. 2007). At temperatures close to freezing, enzymatic activities decrease directly (Thomashow 1999). In this study, it was determined that the best treatments in terms of SOD were 1.0 mM SA, which provided an approximately 11.4-fold increase compared to the CNT (−), and 10 mM MJ, which provided an approximately 5.6-fold increase. Repkina et al. (2021) found that exogenous MJ treatment (1 µM) significantly increased the activities of antioxidant enzymes in wheat leaves and determined that SOD activity was about 53% higher than the initial level on the 7th day of 4 °C cold storage treatment, while MJ treatment provided a 97% increase. In this study, it was determined that the highest average in terms of CAT was obtained from a 1.0 mM SA treatment, with an increase of approximately 25 times compared to the CNT (−). According to the findings of this study, the highest mean APX was obtained from 1.0 mM SA treatment, with an increase of approximately 3.3 times compared to the CNT (−). Li and Wang (2021) found that after exogenous SA treatments, the SOD, CAT and POD activities of grapevine leaves at low temperatures significantly increased continuously with low temperature duration and reached maximum activity at exogenous SA concentrations of 1 and 2 mM. According to the results obtained from this study, the highest TSP was obtained from 1.0 mM SA treatment, which provided a 2.3-fold increase compared to the CNT (−).

According to the correlation table obtained from PCA, all physiological parameters were grouped under Factor 1, while all other biochemical properties except MDA were loaded on Factor 2. Under Factor 1, the increase in EL, LT and MDA negatively affected CHL, SC and RWC. It was expected that the increase in the parameters listed above, which are important indicators of cold stress damage, would encourage the closure of stomata as a defense mechanism of the plant and cause a decrease in RWC by creating osmotic stress as well as CHL loss due to damage to the cells. Under factor 2, PRO, TSP, SOD, CAT and APX were generally positively affected by the increase in the degree of EL. This relationship can be explained by the increase in ROS levels due to the increase in EL in cells under cold stress and the activation of antioxidant defense mechanisms in response to ROS signals. Under factor 3, contrary to what was expected, it was determined that the increase in PRO negatively changed CAT activity. These results suggest that the increase in PRO has a major effect on the elimination of ROS by stabilizing cellular activities, and therefore, enzymatic activity is disabled in the stabilized cell.

The PCA results clearly showed the extent to which the treated elicitors affected the cold stress tolerance of grapevines, with changes in physical and chemical properties.

According to Biplot, the first two components effectively reflect most of the information on the elicitor treatments and parameters examined. It was determined that the SC, CHL and RWC parameters in the positive charge value of the first component were interrelated, and the increase in any of the mentioned parameters positively affected the others. This result proves that the related physiological parameters change in response to low temperature stress. In addition, PRO, TSP, SOD, CAT and APX in the second component were found to be positively correlated and supported each other in coping with low temperature stress. This situation reveals that antioxidant defense systems act together against cold stress in grapevines. In general, 1.0 mM SA treatment, which is closely related to the properties in the positive charge value of the first two components, was the most effective elicitor treatment both in the improvement of physiological parameters and in the activation of plant defense mechanisms. In addition, 1.0 mM SNP and 10 mM MJ treatments were closely associated with the same parameters, suggesting that these treatments contributed to the improvement of the osmotic adjustment capacity of grapevines by increasing PRO and decreasing EL, MDA and LT while improving physiological properties. This shows that 1.0 mM SA, 1.0 mM SNP and 10 mM MJ treatments not only improved the physiological parameters of grapevine but also activated antioxidant mechanisms in response to cold stress and contributed to the development of an effective defense against the damage of stress.

Hierarchical clustering heatmap analysis successfully identified differences between treatments and their concentrations. The results showed that there was a negative correlation between EL, MDA and LT with CHL, SC, RWC, PRO, TSP, SOD, CAT and APX and that the most effective treatment among the treated elicitors to reduce the negative effect of cold stress by improving physiological and biochemical properties was 1.0 mM SA treatment, followed by 1.0 mM SNP and then 10 mM MJ treatments. Similarly, Kılıç (2023a, b) reported that elicitor treatments such as SA caused a negative relationship between EL, MDA and LT parameters and CHL, SC, RWC, PRO, TSP, SOD, CAT and APX parameters in the findings obtained from hierarchical clustering heatmap analysis.

According to the results of AHP analysis, the most favorable treatment in terms of increasing positive parameters was 1.0 mM SA, followed by 1.0 mM SNP and 10 mM MJ. In terms of decreasing the negative parameters, the most favorable treatment was 10 mM MJ, followed by 1.0 mM SA, 5 mM MJ and 1.0 mM SNP. These results suggest that concentrations rather than treatments are more effective in alleviating low-temperature stress damage in grapevines. In this study, as in many other studies in different fields (Wang et al. 2022; Kılıç 2023a, b), the AHP method allowed the inclusive evaluation of several data analyzed and provided useful information on the determination of the most effective treatment combinations.

Conclusions

Low temperature stress during the growing season is a major environmental stress that can damage productivity worldwide. Climate change in recent years has become an important threat to the future of the viticulture sector. Therefore, developing rational and strategic approaches to maintain high plant yields for growers is among the priority objectives of agricultural production. In this study, it was confirmed that exogenous SA, MJ and SNP treatments were effective at varying levels in terms of improving physiological and biochemical processes in grapevines exposed to cold stress and that there were significant differences between treatment concentrations. Among the elicitor treatments, SA treatment at 1 mM concentration effectively reduced the damage of cold stress on the vines and increased the cold resistance of the vines. It was also concluded that 1.0 mM SNP and 10 mM MJ were the other most suitable concentrations following 1.0 mM SA to alleviate cold stress on grapevines. It is thought that the findings obtained from this study will provide important contributions in terms of grapevine cultivation and will also provide a new perspective on elicitor-induced cold tolerance treatments, allowing the development of new strategies for increasing the quality and yield of agricultural products potentially sensitive to cold stress and expanding the geographical boundaries of production. In future studies, in order to further elucidate the mechanisms of cold stress tolerance in grapevines, the effects of the metabolic pathways of SA, MJ and SNP at the transcriptome level should be investigated, and the key genes required for signaling and biosynthesis of these elicitors should be identified.

Acknowledgements We would like to express our gratitude to the Research Square team for their contribution to the publication of our preprint titled "The Effect of Different Elicitor Treatments on Cold Stress in *V. Vinifera* L. cv. 'Victoria'" (https://doi.org/10.21203/rs.3.rs-3298549/v1). The platform has significantly enhanced the visibility of our research and contributed to the broader knowledge base in this field.

Author contribution statement Investigation and methodology S.D., A.Y. and R.C., conceptualization S.D. and A.Y., formal analysis S.D. and R.C., visualization S.D., Supervision A.Y., Writing-original draft and writing-review and editing S.D., A.Y. and R.C. All authors have read and agreed to the published version of the manuscript.

Funding Not applicable.

Data availability Correspondence and requests for materials should be addressed to S.D.

Declarations

Competing interests The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

Consent for publication Not applicable.

Ethics approval and consent to participate Not applicable.

References

Aazami MA, Asghari–Aruq M, Hassanpouraghdam MB, Ercisli S, Baron M, Sochor J (2021) Low temperature stress mediates the antioxidants pool and chlorophyll fluorescence in *Vitis vinifera* L. cultivars. Plants (Basel) 10(9):1877. https://doi.org/10.3390/plants10091877

Aazami MA, Mahna N (2017) Salicylic acid affects the expression of VvCBF4 gene in grapes subjected to low temperature. J Genet Eng Biotechnol 15:257–261. https://doi.org/10.1016/j.jgeb.2017.01.005

Agarwal S, Pandey V (2004) Antioxidant enzyme responses to NaCl stress in *Cassia angustifoli*. Biol Plant 48:555–560. https://doi.org/10.1023/B:BIOP.0000047152.07878.e7

Akhkha A, Boutraa T, Alhejely A (2011) The rates of photosynthesis, chlorophyll content, dark respiration, proline and abscicic acid (ABA) in wheat (*Triticum durum*) under water deficit conditions. Int J Agric Biol 13:215–221

Ashraf M, Athar HR, Harris PJC, Kwon TR (2008) Some prospective strategies for improving crop salt tolerance. Adv Agron 97:45–110. https://doi.org/10.1016/S0065-2113(07)00002-8

Bates L, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/bf00018060

Bracale M, Coraggio I (2003) Chilling and freezing stresses in plants: cellular responses and molecular strategies for adaptation, in: di Toppi, L.S., Pawlik-Skowrońska, B. (Eds.), Abiotic stresses in plants. Springer, Dordrecht, 2, pp. 23–51.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye-binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Cai H, He M, Ma K, Huang Y, Wang Y (2015) Salicylic acid alleviates cold-induced photosynthesis inhibition and oxidative stress in *Jasminum sambac*. Turk J Biol 39:241–247. https://doi.org/10.3906/biy-1406-35

Daler S (2021) The effects of salicylic acid (SA), methyl jasmonate (MJ) and sodium nitroprusside (SNP) applications on physiological and biochemical properties against high temperature stress. International Cappadocia Scientific Research Congress, Nevşehir, Turkey, 15 - 17 December 2021, pp.37–59.

Daler S (2024) Improving grapevine (Vitis vinifera L., cv. Superior Seedless) drought tolerance with cerium oxide nanoparticles: agronomic and molecular insights. Scientia Horticulturae 338:113606. https://doi.org/10.1016/j.scienta.2024.113606

Eichhorn KW, Lorenz DH (1977) Phaenologischeen Twicklungs Stadien der Rebe, Braunschweig, 29

Einset J, Winge P, Bones A (2007) ROS signaling pathways in chilling stress. Plant Signal Behav 2(5):365–367. https://doi.org/10.4161/psb.2.5.4461

Evgenidis G, Traka-Mavrona E, Koutsika-Sotiriou M (2011) Principal component and cluster analysis as a tool in the assessment of tomato hybrids and cultivars. Int J Agron 9:1–7. https://doi.org/10.1155/2011/697879

Food and Agriculture Organization of the United Nations (FAO) (2023) Crops and Livestock Products Data. https://www.fao.org/faostat/en/#data/QCL. Accessed 06 May 2023

Garg N, Manchanda G (2009) ROS generation in plants: boon or bane? Plant Biosyst 143:81–96. https://doi.org/10.1080/11263500802633626

Geravandi M, Farshadfar E, Kahrizi D (2011) Evaluation of some physiological traits as indicators of drought tolerance in bread wheat genotypes. Russ J Plant Physiol 58:69–75. https://doi.org/10.1134/S1021443711010067

Gong Y, Toivonen PMA, Lau OL, Wiersma PA (2001) Antioxidant system level in 'Braeburn' apple in related to its browing disorder. Bot Bull Acad Sin 42:259–264

Goswami AK, Maurya NK, Goswami S, Bardhan K, Singh SK, Prakash J, Pradhan S, Kumar A, Chinnusamy V, Kumar P, Sharma RM, Sharma S, Bisht DS, Kumar C (2022) Physio-biochemical and molecular stress regulators and their crosstalk for low-temperature stress responses in fruit crops: a review. Front Plant Sci 13:1022167. https://doi.org/10.3389/fpls.2022.1022167

Guo X, Liu D, Chong K (2018) Cold signaling in plants: Insights into mechanisms and regulation. J Integr Plant Biol 60(9):745–756. https://doi.org/10.1111/jipb.12706

Hasanuzzaman M, Bhuyan M, Anee TI, Parvin K, Nahar K, Mahmud JA, Fujita M (2019) Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants 8:384. https://doi.org/10.3390/antiox8090384

Hasanuzzaman M, Bhuyan MHMB, Raza A, Hawrylak-Nowak B, MatraszekGawron R, Al Mahmud J, Nahar K, Fujita M (2020) Selenium in plants: Boon or bane? Environ Exp Bot 178:104170. https://doi.org/10.1016/j.envexpbot.2020.104170

Huang GT, Ma SL, Bai LP, Zhang L, Ma H, Jia P, Liu J, Zhong M, Guo ZF (2012) Signal transduction during cold, salt, and drought stresses in plants. Mol Biol Rep 39(2):969–987. https://doi.org/10.1007/s11033-011-0823-1

Jalili I, Ebadi A, Askari MA, KalatehJari S, Aazami MA (2023) Foliar application of putrescine, salicylic acid, and ascorbic acid mitigates frost stress damage in *Vitis vinifera* cv. 'Giziluzum' BMC Plant Biol 10 23(1):135. https://doi.org/10.1186/s12870-023-04126-w

Jin P, Duan Y, Wang L, Wang J, Zheng Y (2014) Reducing chilling injury of loquat fruit by combined treatment with hot air and methyl jasmonate. Food Bioproc Tech 7:2259–2266. https://doi.org/10.1007/s11947-013-1232-3

Kılıç T (2023a) Identifying successful combinations by fertility index in old garden roses and hybrid tea roses crosses. PeerJ 11:e15526. https://doi.org/10.7717/peerj.15526

Kılıç T (2023b) Seed treatments with salicylic and succinic acid to mitigate drought stress in flowering kale cv. 'Red Pigeon F1'. Sci Hortic 313:111939. https://doi.org/10.1016/j.scienta.2023.111939

Kohli SK, Handa N, Sharma A, Gautam V, Arora S, Bhardwaj R, Alyemeni MN, Wijaya L, Ahmad P (2017) Combined effect of 24-epibrassinolide and salicylic acid mitigates lead (Pb) toxicity by modulating various metabolites in *Brassica juncea* L. seedlings. Protoplasma, 255(1):11–24. https://doi.org/10.1007/s00709-017-1124-x

Li B, Wang W (2021) Salicylic acid induces tolerance of *Vitis riparia* × *V. labrusca* to chilling stress by altered photosynthetic, antioxidant mechanisms and expression of cold stress responsive genes. Plant Signal Behav 16:(11). https://doi.org/10.1080/15592324.2021.1973711

Li ZG, Yang SZ, Long WB, Yang GX, Shen ZZ (2013) Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays* L.) seedlings. Plant Cell Environ 36:1564–1572. https://doi.org/10.1111/pce.12092

Londo JP, Kovaleski AP, Lillis JA (2018) Divergence in the transcriptional landscape between low temperature and freeze shock in cultivated grapevine (*Vitis vinifera*). Hortic Res 5:10. https://doi.org/10.1038/s41438-018-0020-7

Lutts S, Kinet JM, Bouharmont J (1996) NaCl-Induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann Bot 78:389–398. https://doi.org/10.1006/anbo.1996.0134

Małgorzata Kopyra EAG (2004) The role of nitric oxide in plant growth regulation and responses to abiotic stresses. Acta Physiol Plant 26:459-472. https://doi.org/10.1007/s11738-004-0037-4

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232

Ollat N, Geny L, Soyer JP (1998) Grapevine fruiting cuttings: validation of an experimental system to study grapevine physiology. I. Main vegetative characteristics. J Int Sci Vigne Vin 32:1–9. https://doi.org/10.20870/oeno-one.1998.32.1.1061

Özden M, Demirel U, Kahraman U (2009) Effects of proline on antioxidant system in leaves of grapevine (*Vitis vinifera* L.) exposed to oxidative stress by H_2O_2. Sci Hortic 119:163–168. https://doi.org/10.1016/j.scienta.2008.07.031

Ren C, Li H, Wang Z, Dai Z, Lecourieux F, Kuang Y, Xin H, Li S, Liang Z (2021) Characterization of chromatin accessibility and gene expression upon cold stress reveals that the RAV1 transcription factor functions in cold response in *Vitis amurensis*. Plant Cell Physiol 62:1615–1629. https://doi.org/10.1093/pcp/pcab115

Repkina N, Ignatenko A, Holoptseva E, MiszalskI Z, Kaszycki P, Talanova V (2021) Exogenous Methyl Jasmonate Improves Cold Tolerance with Parallel Induction of Two Cold-Regulated (COR) Genes Expression in *Triticum aestivum* L. Plants 10(7):1421. https://doi.org/10.3390/plants10071421

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15(2):89–97. https://doi.org/10.1016/j.tplants.2009.11.009

Theocharis A, Clement C, Ait Barka E (2012) Physiological and molecular changes in plants grown at low temperatures. Planta 235:1091–1105. https://doi.org/10.1007/s00425-012-1641-y

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol 50:571–599. https://doi.org/10.1146/annurev.arplant.50.1.571

Venios X, Korkas E, Nisiotou A, Banilas G (2020) Grapevine responses to heat stress and global warming. Plants 9(12):1754. https://doi.org/10.3390/plants9121754

Wang L, Jin P, Wang J, Jiang L, Shan T, Zheng Y (2015) Methyl jasmonate primed defense responses against *Penicillium expansum* in sweet cherry fruit. Plant Mol Biol Rep 33:1464–1471. https://doi.org/10.1007/s11105-014-0844-8

Wang ZL, Yao F, Hui M, Wu D, Wang Y, Han X, Cao X, Li YH, Li H, Wang H (2022) Fertility analysis of intraspecific hybrids in *Vitis vinifera* and screening of superior hybrid combinations. Front Plant Sci 13:940540. https://doi.org/10.3389/fpls.2022.940540

Wasternack C, Song S (2017) Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. J Exp Bot 68:1303–1321. https://doi.org/10.1093/jxb/erw443

Xu H, Huang C, Jiang X, Zhu J, Gao X, Yu C (2022) Impact of cold stress on leaf structure, photosynthesis, and metabolites in *Camellia weiningensis* and *C. oleifera* seedlings. Horticulturae 8:494. https://doi.org/10.3390/horticulturae8060494

Yadav SK (2010) Role of ascorbate-glutathione during salt stress induced oxidative stress tolerance in plantsi in: Oxidative stress in plants: causes, consequences and tolerance. New Delhi: IK International.

Yamasaki S, Dillenburg LC (1999) Measurements of leaf relative water content in *Araucaria angustifolia*. Rev Bras Fisiol Veg 11:69–75

Zhang G, Hou X, Wang L, Xu J, Chen J, Fu X, Shen N, Nian J, Jiang Z, Hu J (2020) PHOTO-SENSITIVE LEAF ROLLING 1 encodes a polygalacturonase that modifies cell wall structure and drought tolerance in rice. New Phytol 229:890–901. https://doi.org/10.1111/nph.16899

Zhang X, Sheng J, Li F, Meng D, Shen L (2012) Methyl jasmonate alters arginine catabolism and improves postharvest chilling tolerance in cherry tomato fruit. Postharvest Biol Technol 64:160–167. https://doi.org/10.1016/j.postharvbio.2011.07.006