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Salicylic Acid and γ-Aminobutyric Acid Enhance Freezing Tolerance in Damask Rose (*Rosa damascena*): Physiological and Biochemical Insights

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ABSTRACT

Salicylic acid (SA) and γ-aminobutyric acid (GABA) enhance freezing tolerance in Damask rose (Rosa damascena) through distinct biochemical and physiological mechanisms. This study evaluated foliar applications of SA (1 and 2 mM), GABA (20 and 50 mM), and distilled water (control) on five-year-old Rosa damascena plants under spring freezing stress in Bardsir, Iran, using a completely randomized design with three replications. SA at 2 mM significantly reduced membrane injury indices and malondialdehyde (MDA) levels in leaves and stems, while increasing proline, soluble carbohydrates, and total chlorophyll content for osmotic and photosynthetic stability. In flowers, SA at 2 mM increased petal weight, phenolic content, anthocyanins, and flavonoids. GABA at 20 mM provided moderate protection, but 50 mM was less effective, often comparable to the control. No significant effects were observed on flower diameter or essential oil content. Correlation analysis showed strong negative relationships between protective traits (e.g., proline, carbohydrates) and damage indicators (e.g., injury indices, MDA). SA at 2 mM exhibited superior efficacy in enhancing freezing tolerance, offering potential for improving yield and quality in cold-prone regions.

Introduction

Rosa damascena Mill., commonly known as the Damask rose, is a valuable species in the Rosaceae family, and is prized for its aesthetic value and industrial applications. Its essential oil is renowned for therapeutic properties, including memory enhancement, relief of chest and abdominal pain, cardiovascular support, and alleviation of digestive disorders (Kant et al., 2023). The Damask rose exhibits remarkable resilience to environmental stresses such as high salinity, low temperatures, and nutrient-poor soils, enabling it to thrive in harsh climates (Charoimek et al., 2023). However, during its budding phase in late winter to early spring, the plant is particularly vulnerable to freezing stress, which can impair flowering, cause leaf wilting, discoloration, and premature leaf drop, and lead to branch dehydration due to disrupted physiological and biochemical processes (Wang et al., 2023).

Freezing temperatures significantly impact plant distribution and productivity by affecting germination, growth, and reproductive success (Theocharis et al., 2012). Plants employ adaptive strategies, including synthesis of protective metabolites, modulation of phytohormone signaling, and enhancement of antioxidant defenses (Mareri et al., 2022). Phytohormones, such as salicylic acid (SA), are critical signaling molecules that regulate stress responses, improve tolerance, and promote growth under adverse conditions (Saadati et al., 2021). Exogenous application of hormones like SA can mitigate stress-induced damage and enhance plant resilience (Granaz et al., 2022).

Salicylic acid, a phenolic phytohormone, regulates plant growth and stress responses by influencing nitrogen metabolism, photosynthesis, water relations, antioxidant defenses, proline metabolism, and glycine betaine production (Khan et al., 2015). SA priming in wheat increases proline and sucrose levels, improving leaf water potential and reducing cell death under freezing stress (Wang et al., 2022). SA also enhances antioxidant systems by promoting reactive oxygen species (ROS) scavengers and increasing flavonoid and anthocyanin production, which mitigate oxidative damage (Saleem et al., 2021).

γ-Aminobutyric acid (GABA), a non-protein amino acid, is integral to plant growth, development, and stress responses (Li et al., 2021). It links amino acid metabolism to the tricarboxylic acid (TCA) cycle, regulating carbon and nitrogen resources (Guo et al., 2023). GABA levels rise under biotic and abiotic stresses, enhancing plant resilience (Ramesh et al., 2015; Hussein et al., 2024). GABA application in tomato seedlings reduces chilling injury by modulating antioxidant enzyme activity and lowering ROS, MDA, and hydrogen peroxide levels (Abd Elbar et al., 2021). GABA also enhances phenolic content in wheat under drought stress, strengthening antioxidant defenses (Zhao et al., 2023). In Medicago ruthenica, GABA improves cold tolerance by promoting growth and physiological stability (Li et al., 2022). When combined with nitric oxide, GABA enhances cold resistance in tea plants by regulating proline and other protective compounds (Wang et al., 2020).

Spring freezing stress poses a significant challenge to Damask rose production in cold-prone regions like Bardsir, Iran, where subzero temperatures during early spring damage vegetative tissues and reduce floral yield and quality. This study investigates the efficacy of SA and GABA in enhancing freezing tolerance to mitigate these adverse effects, aiming to improve plant resilience and productivity.

Materials and Methods

Plant materials and experimental design

The study was conducted in Bardsir, Kerman province, Iran (29°31'N, 56°50'E; altitude 2,860 m). Average minimum and maximum temperatures for 2023–2024 are shown in Table 1, and soil properties are presented in Table 2. Five-year-old *Rosa damascena* plants, spaced 1.5 m between rows and 0.75 m within rows, were used. Fertilizers (100 kg nitrogen, 20 kg phosphorus, 40 kg potassium) were applied at the beginning of the season, using urea, single superphosphate, and muriate of potash. Drip irrigation was provided every 10 d.

Table 1. Average minimum and maximum temperatures during 2023-2024.

	Jul.	**Aug.**	**Sep.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	**Apr.**	**May**	**Jun.**
Min temp. (°C)	21.2	10.3	9.4	5.1	-2.0	-4.2	-13.5	-15.1	-7.3	-7.2	7.2	12
Max temp. (°C)	41.3	36.2	30.0	24.4	17.5	15.6	8.4	11.4	15.4	19.8	37.3	39.1

Table 2. Soil physical and chemical properties of experimental site.

Depth (cm)	**Texture**	**Sand**	**Silt**	**Clay**	**pH**	**Organic carbon (%)**	**Ec (ds m^{-1})**	**N (%)**	**P (mg kg^{-1})**	**K (mg kg^{-1})**
0-30	Sandy loam	74	12	48	7.8	0.87	0.49	1.84	8.0	272
30-60	Sandy loam	72	12	16	7.7	0.70	0.40	1.35	3.9	196

In November 2023, before the first frost, uniform shrubs were selected and randomly assigned to treatments: foliar sprays of SA (1 and 2 mM), GABA (20 and 50 mM), and distilled water (control). Each treatment was replicated three times, with six shrubs per replication (n =18 per treatment). This resulted in a total of 90 shrubs used across all treatments. Treatments were applied in three stages starting November 11, every 3 d, using motorized spray equipment until runoff. In late April 2024, during full bloom, fully expanded, mature leaves from the middle portion of each shrub, along with stem and flower samples, were collected. The collected samples were divided into two groups: one group was used to assess the index of injury in leaves and stems, while the other group was immediately stored at −80 °C in an ultra-low temperature freezer to preserve biochemical integrity for subsequent biochemical analyses.

Index of injury in leaf and stem

Freezing tolerance was assessed by measuring the index of injury, which quantifies membrane damage through electrolyte leakage (ion leakage) in leaf and stem tissues. Five leaf and stem samples per treatment were cooled from 0 to −20 °C at 5 °C h^{-1}, with six replicates. Control samples (referred to as reference samples) were maintained at 4 °C to serve as a baseline for natural ion leakage. After reaching −20 °C, samples were returned to room temperature (approximately 22 °C) for 20 h. Leaf discs and 0.5-cm stem segments were rinsed with deionized water, placed in 10 mL distilled water, and incubated on a shaker for 24 h. Initial electrical conductivity (EC_1) was measured using an electrical conductivity meter to assess ion leakage due to membrane damage. Samples were then boiled in a water bath for 1 h to maximize ion leakage, cooled to room temperature, and final electrical conductivity (EC_2) was recorded to determine total ion content. The index of injury was calculated using the formula from Ouyang et al. (2019), where EC_1/EC_2 for reference represents the ratio of the initial to the final electrical conductivity for control samples maintained at 4 °C, accounting for baseline ion leakage:

$$\text{Index of injury (\%)} = 100 \times \frac{\left(\frac{EC1}{EC2} sample - \frac{EC1}{EC2}\text{reference}\right)}{1 - \frac{EC1}{EC2}\text{reference}}$$

Malondialdehyde (MDA) in leaf and stem

Lipid peroxidation, indicating oxidative damage, was measured as malondialdehyde (MDA) content (Heath and Packer, 1968). Leaf or stem samples were homogenized in 3 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 10 min. To the supernatant, 4 mL of 20% TCA containing 0.5% thiobarbituric acid (TBA) was added. The mixture was heated at 95 °C for 60 min, cooled rapidly on ice, and centrifuged again at 10,000 rpm for 10 min. Supernatant absorbance was measured at 532 nm and 600 nm. MDA content was calculated by subtracting non-specific absorbance at 600 nm, using an extinction coefficient of 155 mM^{-1} cm^{-1} for the MDA-TBA complex.

Proline content in leaf and stem

Proline content was quantified using ninhydrin as a reagent, following the method of Bates et al. (1973). Then, 0.5 g of fresh leaf or stem samples were homogenized in 3% sulfosalicylic acid and centrifuged at 3500 rpm for 10 min. Two mL of supernatant was mixed with 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid, incubated in a boiling water bath for 1 h, and cooled in an ice bath to stop the reaction. Absorbance was measured at 520 nm. Proline concentration was calculated using a calibration curve and expressed as micromoles per gram of fresh weight (μmol g^{-1} FW).

Soluble carbohydrates in leaf and stem

Total soluble carbohydrate content was measured using the anthrone method (Irigoyen et al., 1992). Briefly, 0.5 g of plant tissue was ground in liquid nitrogen, extracted with ethanol three times at 20 °C, and centrifuged at 3,500 rpm for 15 min. To 100 µL of the ethanolic extract, 3 mL of anthrone reagent (150 mg anthrone in 100 mL of sulfuric acid 72%) was added. The mixture was heated in a boiling water bath for 10 min, cooled rapidly on ice, and absorbance was measured at 625 nm. Carbohydrate content was calculated using a glucose standard curve and expressed as milligrams per gram of fresh weight (mg g^{-1} FW).

Total chlorophyll in leaf and stem

Total chlorophyll was determined per Lichtenthaler (1987). Leaf or stem samples (1 g) were homogenized in 10 mL of 80% acetone, centrifuged at 4,000 rpm for 10 min, and absorbance measured at 663 nm and 646 nm. Total chlorophyll was calculated as:

$$Total\ Chl = 7.15\, A663 + 18.71\, A646\ (mg\ g^{-1}\ FW)$$

Total phenol content in flower

Total phenol content in petal samples was measured using a modified Folin-Ciocalteu method (Folin and Ciocalteu, 1927). One gram of sample was homogenized in 10 mL of methanol and centrifuged. Then, 125 µL of methanolic extract was mixed with 375 µL of distilled water and 2.5 mL of Folin-Ciocalteu 10% reagent. After 6 min, 2 mL of Na_2CO_3 7.5% was added. The mixture was incubated in the dark at room temperature for 30 min, and absorbance was measured at 760 nm. Phenol content was quantified using a gallic acid calibration curve.

Total anthocyanin content in flower

Total anthocyanin content was determined using the pH differential method (Wrolstad, 2000). Extracts were mixed with 2.5 mL of buffers at pH 1.0 and 4.5, and absorbance was measured at 530 nm and 700 nm. Total absorbance was calculated as:

$$A = (Abs_{530} - Abs_{700})\, pH1 - (Abs_{530} - Abs_{700})\, pH4.5$$

Anthocyanin content was expressed in milligrams per gram of fresh weight (mg g^{-1} Fw).

Total flavonoid content in flower

Total flavonoid content was measured using a colorimetric assay (Shin et al., 2007). To 10 µL of methanolic extract, 150 µL of sodium nitrite 5%, 300 µL of aluminum chloride, and 1,000 µL of 1 M acetate were added. The mixture was incubated at room temperature for 30 min, and absorbance was measured at 380 nm. Flavonoid content was quantified using a quercetin calibration curve and expressed as milligrams of quercetin per gram of fresh weight.

Essential oil content in flowers

Essential oil was extracted from 100 g of dried flowers using a modified Clevenger apparatus via water distillation at 100 °C for 4–5 h. Anhydrous sodium sulfate was added to remove residual water, and oil yield was quantified in mg per 100 g of dry flower (Visakh et al., 2022).

Statistical analysis

The experiment followed a completely randomized design (CRD). Data were analyzed using one-way analysis of variance (ANOVA) in SAS (version 9.4). Means were compared using the Least Significant Difference (LSD) test at $P \leq 0.05$.

Results

Effects of treatments on leaves and stems of damask rose

Treatments with SA and GABA significantly affected leaf and stem injury indices, malondialdehyde, proline, soluble carbohydrates, and chlorophyll content under freezing stress (Table 3). Foliar application of SA and GABA significantly reduced the injury index in Damask rose leaves and stems (Fig. 1a and b). The 2 mM SA treatment, followed by 20 mM GABA, yielded the lowest injury index in leaves, while the control showed the highest. In stems, 2 mM SA, 1 mM SA, and 20 mM GABA resulted in the lowest injury indices, with no significant difference between 50 mM GABA and the control. The 2 mM SA treatment reduced the injury index by 111–116% compared to the control.

Malondialdehyde (MDA), an indicator of membrane lipid peroxidation, was lowest in leaves treated with 1 and 2 mM SA, while the control exhibited the highest levels. GABA treatments (20 and 50 mM) showed lower MDA levels than the control but higher than SA treatments (Fig. 1c). In stems, the control had the highest MDA, followed by 50 and 20 mM GABA, 1, and 2 mM SA. Notably, the 2 mM SA treatment resulted in a 234% reduction in MDA compared to the control (Fig. 1d).

Proline an osmotic regulator was highest in leaves treated with 2 mM SA, followed by 1 mM SA, 20 and 50 mM GABA, with the control showing the lowest levels (Fig. 2a). In stems, 2 mM SA and 50 mM GABA yielded the highest proline content, with other treatments showing elevated levels compared to the control (Fig. 2b). Proline levels in 2 mM SA-treated leaves and stems were 12 and 44% higher respectively than the control.

Soluble carbohydrates were highest in leaves treated with 2 mM SA, followed by 1 mM SA, 20 and 50 mM GABA, with the control showing the lowest levels (Fig. 2c). In stems, 1 and 2 mM SA treatments resulted in the highest carbohydrate content, while GABA treatments and the control showed similar levels (Fig. 2d). The 2 mM SA treatment increased soluble carbohydrates by 163% in leaves and 83% in stems compared to the control.

Total chlorophyll content was highest in leaves treated with 1 and 2 mM SA, while 50 mM GABA showed the lowest levels, and 20 mM GABA with the control exhibited intermediate levels (Fig. 3a). In stems, 2 mM SA yielded the highest chlorophyll content, followed by 1 mM SA and 20 mM GABA, while 50 mM GABA and the control showing the lowest levels (Fig. 3b).

Effects of treatments on flowers of damask rose

Treatments with SA and GABA significantly influenced petal phenolic content, anthocyanins, flavonoids, and petal weight, but not flower diameter or essential oil content (Table 3). No significant differences were observed in flower diameter or petal essential oil content across treatments (Fig. 3c and d). However, petal weight was highest in the 2 mM SA treatment (388.70 g plant^{-1}), 44% higher than the control (269 g plant^{-1}) and 50 mM GABA (268 g plant^{-1}) treatments (Fig. 4a). Total phenolic content was highest in the 2 mM SA treatment, with no significant differences compared to 1 mM SA and 20 mM GABA, while 50 mM GABA and the control showed the lowest levels (Fig. 4b). Anthocyanin content was highest in 1 and 2 mM SA treatments, over threefold higher than the control and 50 mM GABA treatments (Fig. 4c). Flavonoid content was highest in the 2 mM SA treatment, followed by 1 mM SA, 20 and 50 mM GABA, while the control showed the lowest levels (48% lower than 2 mM SA) (Fig. 4d).

Table 3. Analysis of Variance (ANOVA) for Physiological and Biochemical Traits of Damask rose under freezing stress.

SOV	**df**	**Mean squares**															
		LII	**SII**	**LMA**	**SMA**	**LPR**	**SPR**	**LSC**	**SSC**	**TLC**	**TSC**	**FDI**	**PEO**	**PPC**	**PAN**	**PFL**	**PWP**
Between	4	145.90^{**}	380.65^{***}	3.98^{***}	319.68^{***}	113.98^{***}	1498.04^{***}	51.15^{***}	1.62^{**}	18.46^{***}	0.04^{***}	0.09^{ns}	0.003^{ns}	8646.60^{***}	2.59^{***}	0.58^{***}	1106.23^{***}
Within	10	9.53	2.42	0.01	0.15	0.76	8.37	0.60	0.07	0.45	0.001	0.15	0.00	165.53	0.05	0.02	31.80
Total	14																
Cv (%)		10.75	3.75	1.81	1.82	0.61	2.11	6.29	10.82	4.30	7.50	7.95	13.86	3.96	4.24	21.15	4.28

Traits: (LII) Leaf index of injury, (SII) Stem index of injury, (LMA) Leaf malondialdehyde, (SMA) Stem malondialdehyde, (LPR) Leaf proline, (SPR) Stem proline, (LSC) Leaf soluble carbohydrates, (SSC) Stem soluble carbohydrates, (TLC) Total leaf chlorophyll, (TSC) Total stem chlorophyll, (FDI) Flower diameter, (PEO) Petal essential oils, (PPC) Petal phenolic content, (PAN) Petal anthocyanin, (PFL) petal flavonoids, (PWP) petal weight per plant. ** and *** denote significance at 0.01 and 0.001, respectively; ^{ns} indicates no significance.

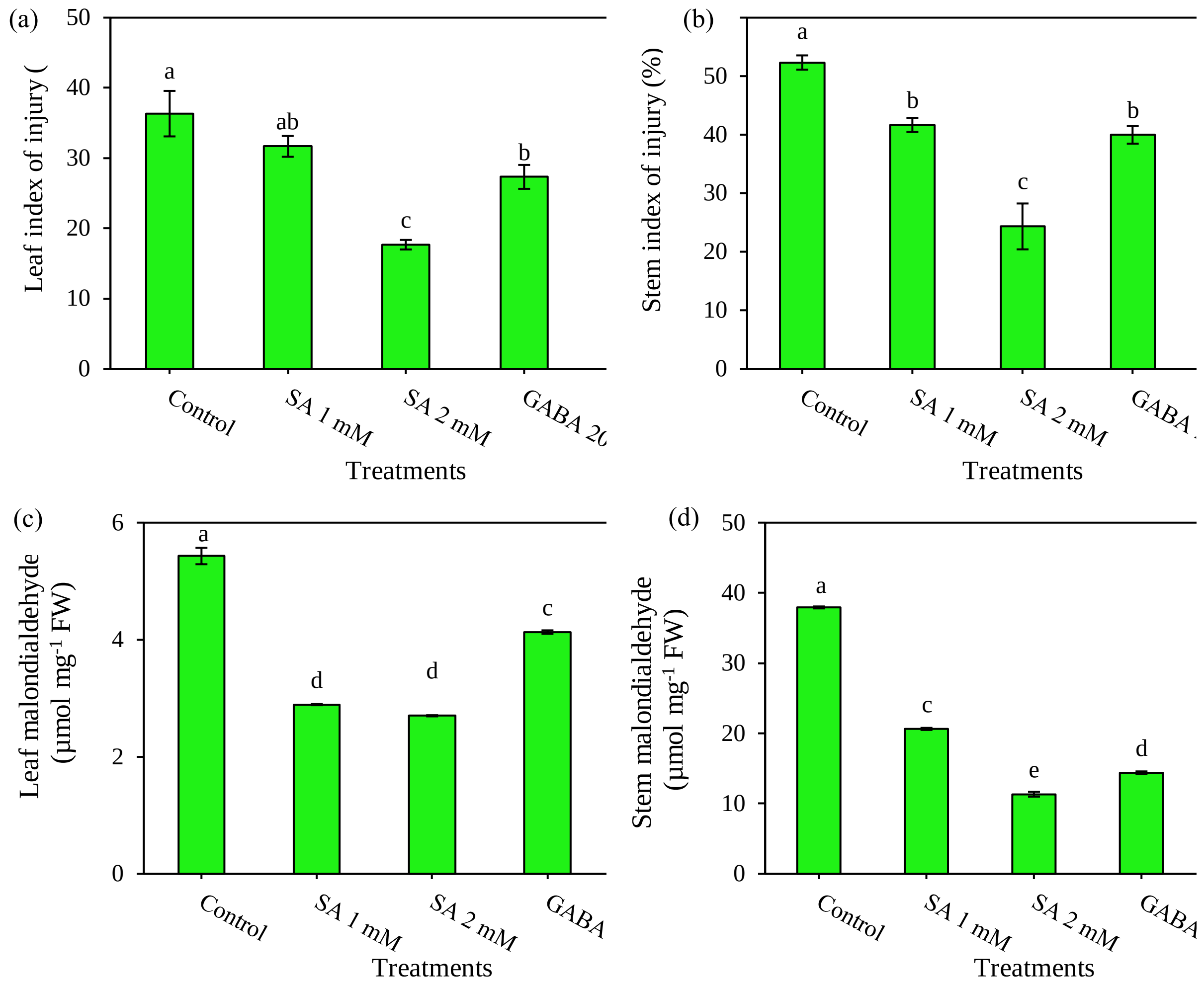
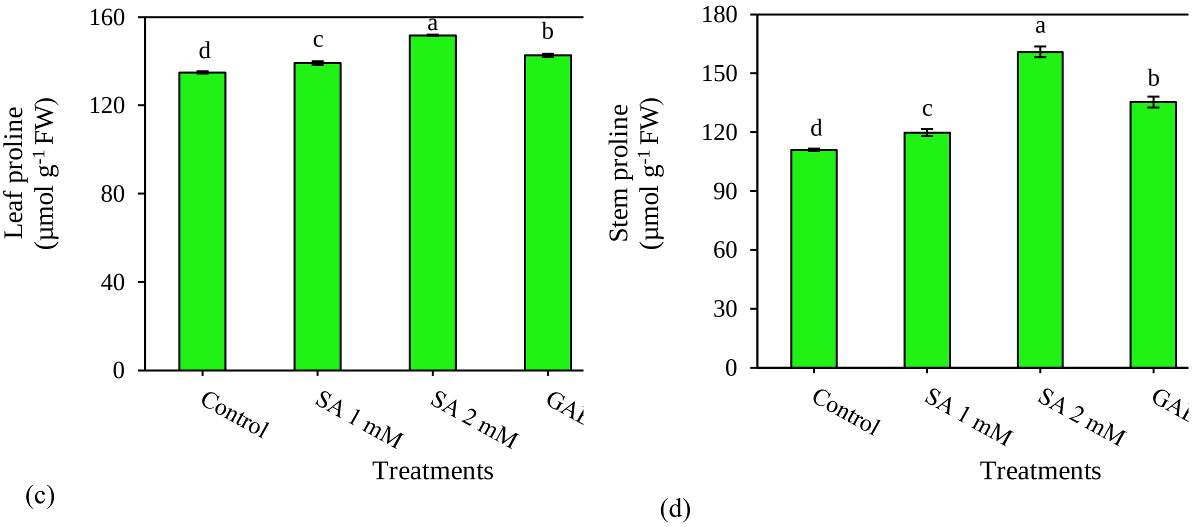


Fig. 1. The impact of salicylic acid (SA) and gamma-aminobutyric acid (GABA) on (**a**) leaf index of injury; (**b**) stem index of injury, (**c**) leaf malondialdehyde, (**d**) stem malondialdehyde of Damask rose under freezing stress. The data present the mean of three replicates ± standard error. Letters that are the same indicate no significant difference at the 5% level, as determined by the LSD test.



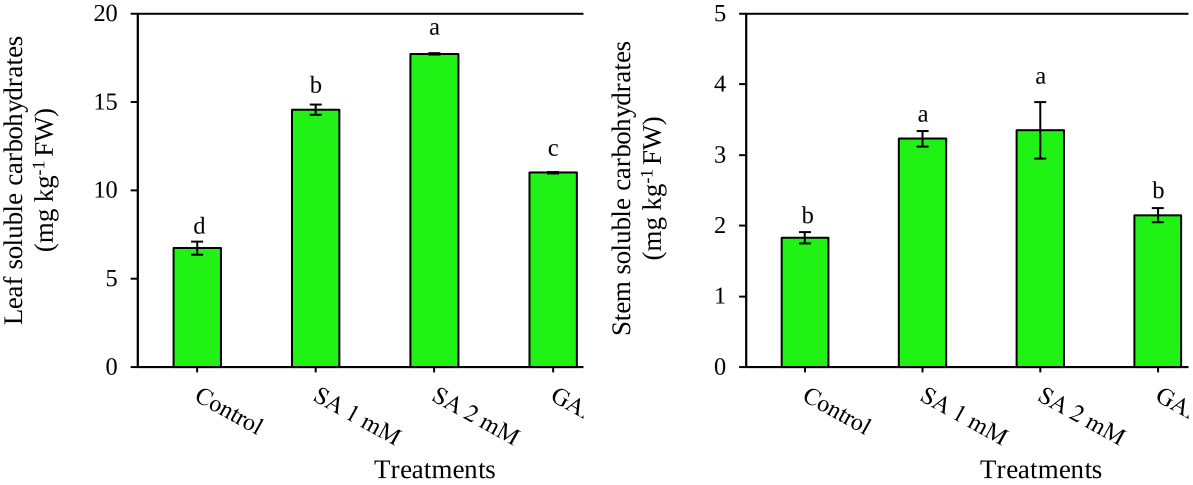


Fig. 2. The impact of salicylic acid (SA) and gamma-aminobutyric acid (GABA) on (**a**) leaf proline, (**b**) stem proline, (**c**) leaf soluble carbohydrates, (d) stem soluble carbohydrates of Damask rose under freezing stress. Data present the mean of three replicates ± standard error. Similar letters indicate no significant difference at the 5% level, as determined by the LSD test.

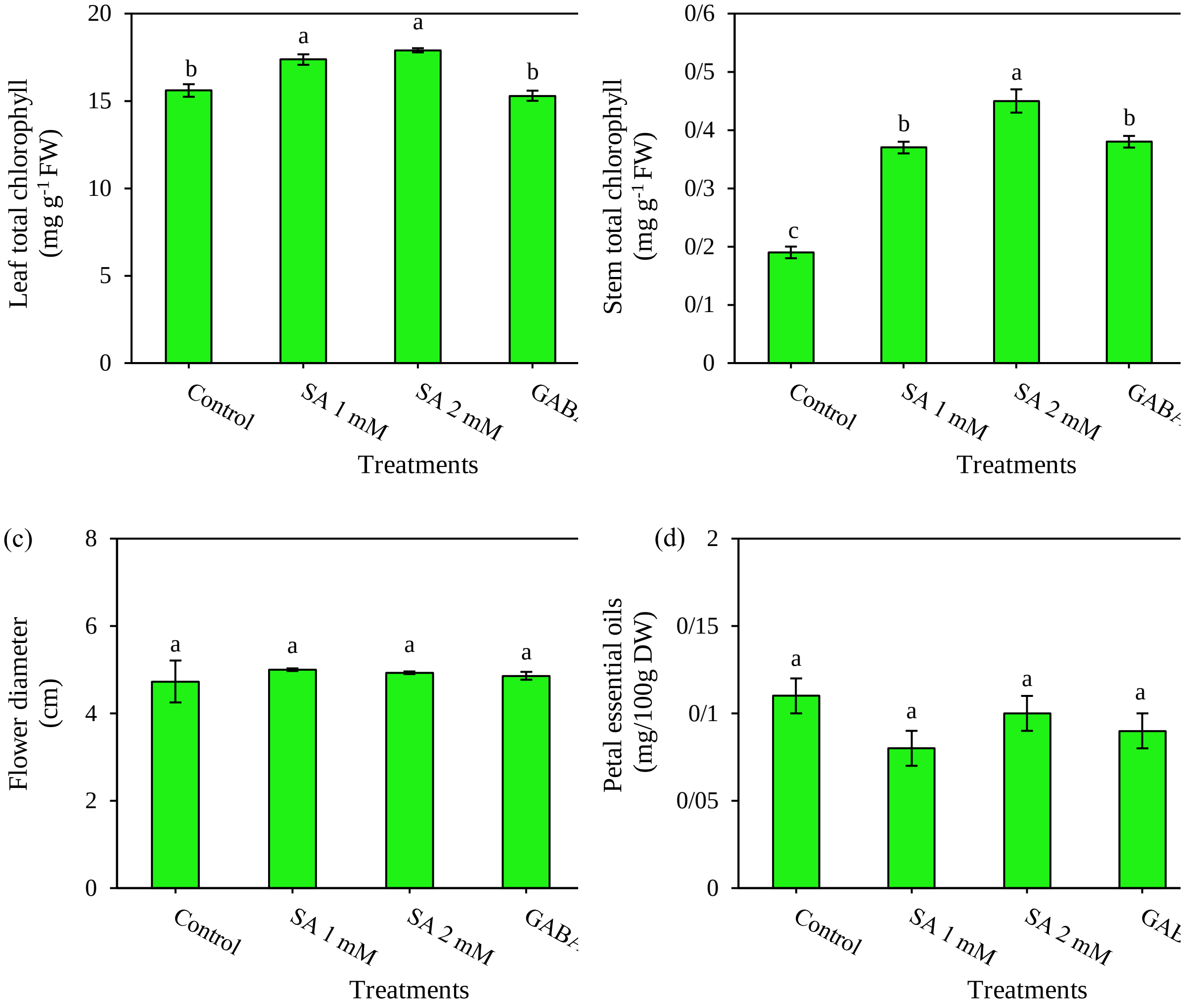


Fig. 3. The impact of salicylic acid (SA) and gamma-aminobutyric acid (GABA) on (**a**) total chlorophyll of leaf, (**b**) total chlorophyll of stem, (**c**) flower diameter, (**d**) petal essential oils of Damask rose under freezing stress. The data present the mean of three replicates ± standard error. Letters that are the same indicate no significant difference at the 5% level, as determined by the LSD test.

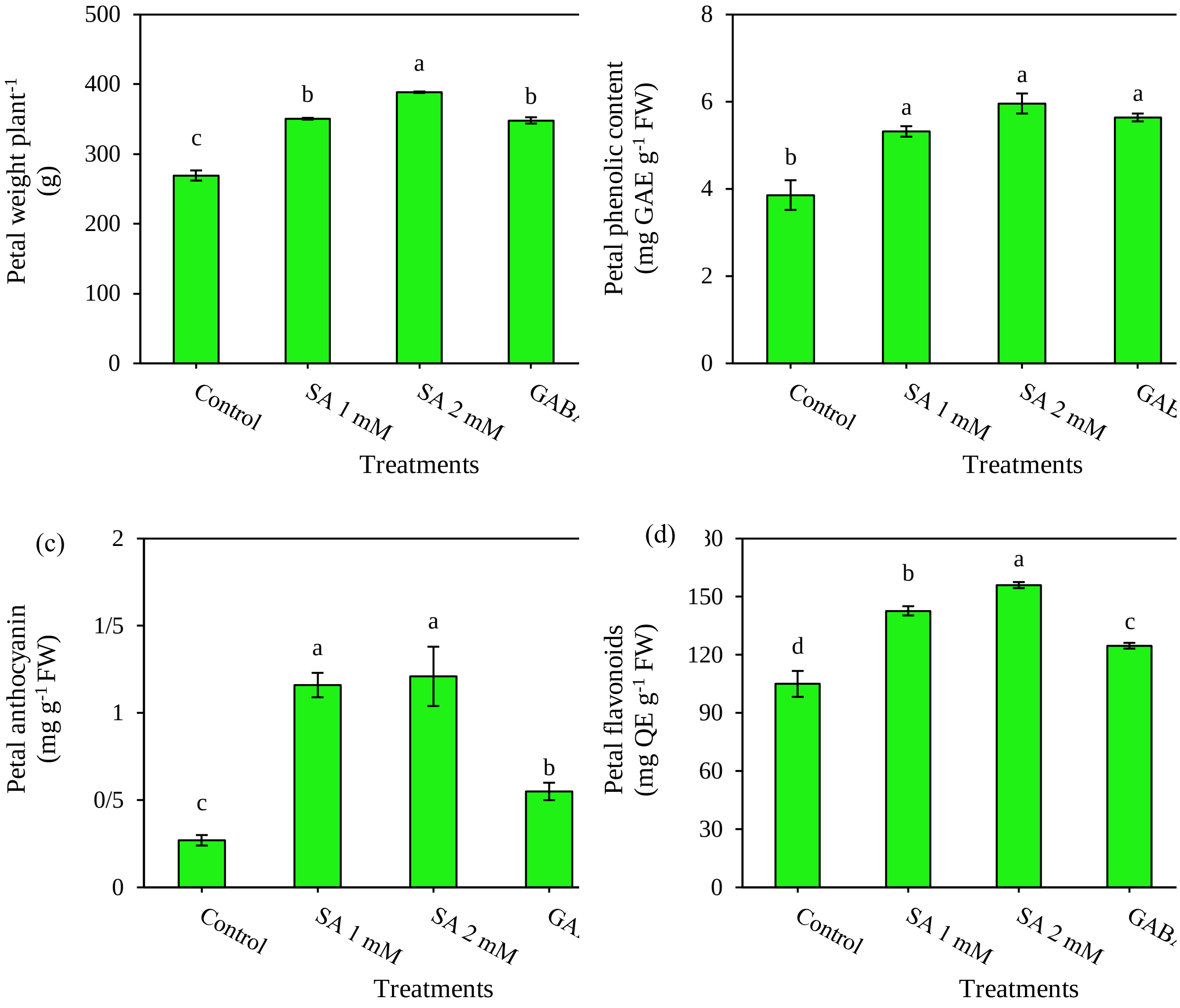


Fig. 4. The impact of salicylic acid (SA) and gamma-aminobutyric acid (GABA) on (**a**) petal phenolic content, (**b**) petal anthocyanin, (**c**) petal flavonoids, (**d**) petal weight per plant of Damask rose under freezing stress. The data presentrt the mean of three replicates ± standard error. Similar letters indicate no significant difference at the 5% level, as determined by the LSD test.

Discussion

This study demonstrated that SA and GABA effectively mitigated freezing stress in Damask rose by reducing electrolyte leakage, an indicator of membrane injury (Fig. 1a and b). Elevated electrolyte leakage reflects cellular damage, and its reduction indicates improved membrane stability and plant health (Bajji et al., 2002). Salicylic acid enhances plasma membrane stability by maintaining ion balance and reducing leakage, crucial for cellular integrity under stress (Mohammadi et al., 2023). It also bolsters antioxidant defenses, protecting cells from oxidative damage (González-Villagra et al., 2022). However, it should be noted that ion balance was not directly measured in this study; the effects of SA and GABA on plasma membrane stability were inferred from reduced malondialdehyde (MDA) levels (Fig. 1c and d) and reduced membrane damage, as indicated by lower electrolyte leakage through the index of injury (Fig. 1a and b). These findings align with studies on apricot flower buds, where SA reduced electrolyte leakage and organ damage under cold stress (Noghondar et al., 2013). Similarly, SA also reduced chilling injury in bird-of-paradise (*Strelitzia reginae*) by lowering transpiration and maintaining floral stem freshness (Pereira et al., 2018). GABA stabilizes cell membranes and reduces oxidative damage by protecting chloroplast ultrastructure and enhancing antioxidant enzyme activity, supporting membrane stability (Kinnersley and Turano, 2000). These effects are consistent with studies on chilled tomato plants (*Solanum lycopersicum*; Abd Elbar et al., 2021), gerbera cut flowers (*Gerbera jamesonii*; Mohammadi et al., 2021), and tea plants (*Camellia sinensis*; Wang et al., 2020), where GABA reduced oxidative damage and enhanced stress tolerance.

A key hallmark of cold-induced oxidative stress is lipid peroxidation, commonly assessed via malondialdehyde (MDA) accumulation (Gaweł et al., 2004). Both SA and GABA treatments significantly lowered MDA levels in Damask rose tissues, indicating reduced oxidative membrane damage (Fig. 1c and d). The robust effect of SA in decreasing MDA is attributable to its capacity to upregulate ROS-scavenging enzymes and promote the synthesis of protective metabolites, as observed in canola (*Brassica napus* L.) and Hami melons (*Cucumis melo* var. saccharinus) under cold stress (Keshavarz et al., 2012; Song et al., 2022). Similarly, GABA reduced MDA accumulation, in line with its documented role in enhancing antioxidant enzyme activity and alleviating oxidative stress across various plant species ((Li et al., 2019; Priya et al., 2019; Salah et al., 2019).

Proline accumulation is a well-established adaptive response to abiotic stress, functioning both as an osmoprotectant and a signaling molecule (Mohammadrezakhani et al., 2019). Our results show that both SA and GABA markedly increased proline content in leaves and stems, with SA exerting a stronger effect (Fig. 2a and b). SA promotes proline accumulation in cold-stressed plants, potentially by upregulating stress-responsive transcription factors like CBF (C-repeat Binding Factor), which enhance downstream pathways leading to increased expression of proline biosynthesis genes such as P5CS, as observed in wheat and consistent with our findings in Damask rose (Sha et al., 2022; Wang et al., 2022). GABA, by modulating carbon and nitrogen metabolism, also promoted proline accumulation, corroborating findings in Medicago and wheat (Hayat et al., 2023; Yuxing Li et al., 2022).

Increased soluble carbohydrate content is another critical mechanism by which plants counteract cold-induced osmotic stress. Both SA and GABA treatments significantly elevated soluble sugar levels, which help maintain cell turgor and energy supply during stress (Fig. 2c and d). SA achieves this by upregulating sugar biosynthetic enzymes (Khan et al., 2015), while GABA's effect is linked to its regulatory role in primary metabolism (Al-Khayri et al., 2024; Zhou et al., 2021). These findings are consistent with studies in grape, white clover, and snap bean, where both compounds enhanced carbohydrate accumulation and stress resilience (Abd El-Gawad et al., 2021).

Photosynthetic performance, as indicated by total chlorophyll content, was also preserved by both SA and GABA (Fig. 3a and b). SA's ability to protect photosystem II and prevent photoinhibition under stress is well documented (Alam et al., 2022; Moustakas et al., 2022), while GABA's stabilizing effect on chloroplasts further supports its role in maintaining photosynthetic efficiency (Geng et al., 2025; Ying Li et al., 2022). Petal weight was significantly increased by SA and GABA (Fig. 4a). SA enhances biomass accumulation by boosting antioxidant defenses and water retention (Liu et al., 2022; Yang et al., 2023). GABA supports biomass production by stabilizing membranes, enhancing antioxidant activity, and regulating metabolism (Shang et al., 2011).

Importantly, both SA and GABA treatments led to significant increases in accumulation of phenolic compounds, flavonoids, and anthocyanins. These secondary metabolites are essential for antioxidant protection and are associated with enhanced tolerance to oxidative stress (García-Pastor et al., 2020). SA's stimulation of the phenylpropanoid pathway and GABA's regulation of phenolic biosynthesis were evident in the elevated levels of these compounds, supporting the observed improvements in floral quality and stress resilience (Ren et al., 2021).

Pearson correlation analysis (Table 4) revealed strong negative correlations between protective traits and damage indicators. Leaf soluble carbohydrates correlated with reduced leaf MDA ($r = -0.95^{*}$, $r^2 = 0.90$), proline with reduced leaf injury ($r = -0.87^{***}$, $r^2 = 0.76$), and stem chlorophyll with reduced stem injury ($r = -0.91^{***}$, $r^2 = 0.83$). Petal phenolic content ($r = -0.92^{***}$, $r^2 = 0.85$) and anthocyanins ($r = -0.89^{***}$, $r^2 = 0.79$) were strongly associated with reduced damage, indicating systemic protection. Flower diameter and essential oil content showed weak correlations ($r = -0.21$ ns to -0.65^{**}, $r^2 = 0.04$–0.42), consistent with their lack of treatment effects (Fig. 3c and d).

SA (2 mM) was the most effective treatment. It significantly reduced injury indices and MDA while enhancing proline, carbohydrates, chlorophyll, and floral quality. These findings suggest that SA application can protect *Rosa damascena* against freezing stress, improving yield and quality in cold-prone regions. Future research should optimize application methods and explore molecular mechanisms.

Table 4. Pearson's correlation coefficients for physiological and biochemical traits of damask rose under freezing stress.

	LII	SII	LMA	SMA	LPR	SPR	LSC	SSC	TLC	TSC	FDI	PEO	PPC	PAN	PFL	PWP
LII	1															
SII	0.87^{***}	1														
LMA	0.67^{*}	0.81^{***}	1													
SMA	0.80^{***}	0.82^{***}	0.79^{***}	1												
LPR	-0.87^{***}	-0.83^{**}	-0.63^{**}	-0.81^{***}	1											
SPR	-0.72^{***}	-0.53^{*}	-0.38^{ns}	-0.67^{**}	0.89^{***}	1										
LSC	-0.78^{***}	-0.83^{***}	-0.95^{***}	-0.81^{***}	0.79^{***}	0.59^{*}	1									
SSC	-0.52^{*}	-0.74^{***}	-0.89^{***}	-0.53^{*}	0.47^{ns}	0.16^{ns}	0.81^{***}	1								
TLC	-0.30^{ns}	-0.64^{**}	-0.63^{**}	-0.27^{ns}	0.17^{ns}	-0.23^{ns}	0.50^{ns}	0.77^{***}	1							
TSC	-0.73^{***}	-0.91^{***}	-0.86^{***}	-0.84^{***}	0.63^{**}	0.31^{ns}	0.79^{***}	0.75^{***}	0.72^{***}	1						
FDI	-0.41^{ns}	-0.26^{ns}	-0.36^{ns}	-0.21^{ns}	0.01^{ns}	-0.08^{ns}	0.27^{ns}	0.37^{ns}	0.35^{ns}	0.42^{ns}	1					
PEO	-0.63^{**}	-0.65^{**}	-0.32^{ns}	-0.23^{ns}	0.51^{*}	0.25^{ns}	0.42^{ns}	0.40^{ns}	0.53^{*}	0.45^{ns}	0.27^{ns}	1				
PPC	-0.68^{**}	-0.92^{***}	-0.86^{***}	-0.80^{***}	0.63^{**}	0.27^{ns}	0.77^{***}	0.79^{***}	0.72^{***}	0.95^{***}	0.26^{ns}	0.45^{ns}	1			
PAN	-0.76^{***}	-0.89^{***}	-0.83^{***}	-0.87^{***}	0.64^{**}	0.34^{ns}	0.77^{***}	0.73^{***}	0.62^{**}	0.96^{***}	0.37^{ns}	0.39^{ns}	0.93^{***}	1		
PFL	-0.55^{*}	-0.74^{***}	-0.94^{***}	-0.63^{**}	0.52^{*}	0.24^{ns}	0.87^{***}	0.92^{***}	0.72^{***}	0.80^{***}	0.39^{ns}	0.39^{ns}	0.80^{***}	0.75^{***}	1	
PWP	-0.65^{**}	-0.79^{***}	-0.91^{***}	-0.79^{***}	0.79^{***}	0.60^{*}	0.94^{***}	0.77^{***}	0.44^{ns}	0.73^{***}	0.04^{ns}	0.31^{ns}	0.77^{***}	0.71^{***}	0.85^{***}	1

Traits: (LII) Leaf index of injury, (SII) Stem index of injury, (LMA) Leaf malondialdehyde, (SMA) Stem malondialdehyde, (LPR) Leaf proline, (SPR) Stem proline, (LSC) Leaf soluble carbohydrates, (SSC) Stem soluble carbohydrates, (TLC) Total leaf chlorophyll, (TSC) Total stem chlorophyll, (FDI) Flower diameter, (PEO) Petal essential oils, (PPC) Petal phenolic content, (PAN) Petal anthocyanin, (PFL) petal flavonoids, (PWP) petal weight per plant. *, ** and *** denote significance at 0.05, 0.01 and 0.001, respectively; ^{ns} indicates no significance.

Conclusion

Foliar application of SA and GABA significantly enhanced physiological and biochemical responses to freezing stress in Damask rose. Both treatments reduced injury indices and MDA levels, increased proline, soluble carbohydrates, and chlorophyll in leaves and stems, and elevated phenolic compounds, anthocyanins, and flavonoids in petals. The 2 mM SA treatment was most effective, yielding the lowest injury indices and MDA levels, and the highest proline, carbohydrate, and chlorophyll content, while improving petal weight and quality. GABA, particularly at 20 mM, showed moderate improvements but was less effective than SA. These treatments can be integrated into stress management strategies to enhance plant resilience. Future research should optimize application methods, elucidate molecular mechanisms, and explore their efficacy against other stresses.

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Author Contributions

Designed the research, carried out the experiment, and reviewed the manuscript, NZ; performed the data analyses, drew the curves, and wrote the manuscript, SS. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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