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Research Paper

Foliar sprays of 1-MCP, CPPU, or KNO_3 improve mango physiology following chilling by promoting stomatal opening

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ABSTRACT

Chilling is a major abiotic stress that impairs physiological functions and reduces crop productivity, particularly in tropical and subtropical species. Mango trees (Mangifera indica L.) are especially sensitive to chilling events, notably when cold nights are followed by bright, sunny days—a condition referred to as 'Cold Night–Bright Day'. Under these conditions, stomatal opening is impaired, leading to restricted gas exchange, disruption of the photosynthetic machinery, and subsequent physiological damage. The purpose of this study was to evaluate whether foliar sprays of agriculturally approved compounds could restore stomatal function and mitigate chilling-associated damage in mango. Following a night-chilling event (4 °C for 12 h), young and orchard-grown mango trees were sprayed with one of three treatments:(A) 1-Methylcyclopropene (gaseous release, 3.8 % 1-MCP, Rimi, LTD), N-(2-Chloro-4-pyridyl)-N'-phenylurea (CPPU; 240 μM with surfactant), or potassium nitrate (KNO_3; 100 mM with surfactant). Across independent experiments, all treatments significantly increased stomatal conductance, improved CO_2 assimilation, and reduced chilling-induced physiological impairment compared with untreated cold-stressed controls. No negative effects on PSII efficiency or chlorophyll content were observed.

These findings demonstrate that post-chilling foliar sprays with 1-MCP, CPPU, or KNO_3 effectively alleviate stress symptoms in mango by promoting stomatal reopening and photosynthetic recovery. By offering simple, practical treatments based on compounds already registered for agricultural use, this study highlights sustainable strategy to improve resilience of mango and other chilling-sensitive crops under increasingly variable climatic conditions.

1. Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruit crops globally, with an annual yield of approximately ~60 million tons (FAOstat, 2024). Mango cultivation has been mostly restricted to tropical and subtropical climatic areas. With climate change and global warming, mango flowering, and fruiting patterns have already undergone significant modifications (Asare-Nuamah et al., 2022; Normand *et al.*, 2015; Rajan, 2012), which induced a shift and expansion of mango growth areas to areas that were previously too cold for mango cultivation (Salunkhe et al., 2023). However, like other tropical and subtropical fruit trees, mango is particularly sensitive and susceptible to cold conditions (Allen and Ort, 2001; Feng and Cao, 2005).

Israel is one of the northernmost commercial mango cultivating countries, with over 3000 hectares producing ~70,000 tons annually, a third of which are exported. In temperate regions, such as the

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Mediterranean and other mango-growing areas, including India, the world's leading mango producer (Rajan, 2012), the abiotic stress of 'Cold Night-Bright Day' is common during winter. These night-chilling events are characterized by a temperature drop at night, followed by a high-irradiance clear day (Allen et al., 2000; Joshi et al., 2020; Nir et al., 1997). Exposure of trees to this stress leads to significant physiological damage that can result in symptoms such as leaf chlorosis or necrosis. In severe cases, branches and entire trees can die following even a short but severe cold stress, ultimately resulting in considerable yield loss. These agricultural challenges necessitate research focused on developing effective and economically viable solutions to mitigate night-chilling damage in mango trees. Several practices are used to prevent frost damage (but not for night-chill), as the use of giant fans or water sprinklers (Netafim, 2015; Noy and Gal-Paz, 2017). Alternatively shading nets are used for night-chilling stress damage (Chernoivanov et al., 2022; Farina et al., 2020; Wang et al., 2018). Besides the high costs of installing and maintaining, it was found that growing mangoes under nets, might lead to reduced yield (Mditshwa et al., 2019; Mohamed et al., 2023). As climate change intensifies, developing new strategies to mitigate the challenges posed by night chilling stress has become increasingly essential.

Night-chill of 'Cold Night-Bright Day' conditions, has been shown to disturb and induce various processes and signaling pathways (Agurla et al., 2018). One particularly intriguing affected physiological process is the disruption of stomatal function, preventing stomatal opening following night chilling which has been shown to occur across multiple plant species, including mango, due to night chill (Bauer et al., 2006; Bell et al., 1994; Bongi and Long, 2006; Flexas et al., 1999; Guo and Cao, 2015; Joshi et al., 2020; Liu et al., 2012b; Martin et al., 1981; Peoples and Koch, 1978; Van Heerden et al., 2004).

The stomate, which regulates the exchange of gases between the atmosphere and the interior of the leaf, includes two specialized guard cells, which increase and decrease in volume to control the size of the gap between them, thereby controlling the rate of transpiration and the diffusion of CO_2. For appropriate physical response to different environmental stimuli, stomatal guard cells sense and integrate various signals, mediating stomatal aperture size and stomatal conductance (Assmann and Jegla, 2016; Hetherington and Woodward, 2003; Kim et al., 2010; Kollist et al., 2014; Murata et al., 2015).

In prior research (Haque et al., 2024), night chilling followed by intense morning light was shown to disrupt stomatal reopening, leading to physiological damage manifested as leaf chlorosis and, in severe instances, necrosis. This disruption impairs gas exchange and overwhelms the photosynthetic apparatus with excess excitation energy. Application of fusicoccin (FC), a known activator of guard cell H^+-ATPase, has been demonstrated to effectively reopen stomata, restore gas exchange, and alleviate physiological stress symptoms. However, FC is costly and lacks regulatory approval for agricultural deployment, limiting its utility in commercial orchards. Moreover, the internal plant mechanisms impaired by night chill that lead to this stomatal malfunction remain unclear. In the current study, alternative foliar treatments that are already approved for agricultural use and act through distinct physiological pathways were investigated. These treatments were selected based on previous reports and preliminary screening for their potential to re-establish stomatal opening and mitigate night-chill-associated stress in mango trees.

A) Ethylene inhibitor 1-Methylcyclopropene (1-MCP)

Cold stress has been shown to elevate ethylene levels (Guye et al., 1987; Michaeli et al., 2002), which can mediate stomatal conductance (Azoulay-Shemer et al., 2023) and induce stomatal closure (Desikan et al., 2006). The working hypothesis was that using 1-MCP, a competitive inhibitor of ethylene receptors (Sisler and Serek, 2008), could help bypass the stomatal opening impairment caused by night chill.

B) Cytokinin N-(2-Chloro-4-pyridyl)-N′-phenyl urea (CPPU)

Cytokinins, such as CPPU, are known to inhibit ABA induce stomatal closure (Agurla et al., 2018; Bharath et al., 2021b), by reducing nitric oxide (NO) levels in guard cells (Xiao-Ping and Xi-Gui, 2006). Studies indicate that ABA concentrations increase in response to cold stress (Lang et al., 1994). Based on these findings, the working hypothesis was that applying CPPU could promote stomatal opening in mango leaves affected by night chill.

C) Inorganic salt potassium nitrate (KNO_3)

KNO_3 has been shown to enhance both leaf transpiration and photosynthetic activity (Borowski and Michałek, 2010) by mechanisms that increase stomatal conductance (Elhindi, 2016). This compound was included in the study to assess whether KNO_3 could stimulate stomatal opening and improve the physiological condition of night-chilled mango trees.

This study serves as a direct follow-up to our previously published work (Haque et al., 2024), which demonstrated the physiological benefit of restoring stomatal function after chilling using the non-agricultural compound fusicoccin. In this study, the previous findings were expanded by testing three agriculturally approved compounds—1-MCP, CPPU, and KNO_3—that act via distinct physiological pathways. This work advances our earlier results by offering practical, scalable solutions for mitigating chilling stress in mango trees through foliar treatments suitable for orchard use.

2. Materials and methods

2.1. Plant material and growth conditions

Two experimental systems distinguished by tree age were used. *Young trees* (1-year-old) were used for initial screening under controlled conditions (2.1.1), whereas *mature trees* (3-year-old) were grown in a portable orchard system to assess orchard-level applicability (2.1.2).

2.1.1. Young mango tree experimental-based system

Mango trees (*Mangifera indica* L., cv. 'Shelly') classified as young trees (~1-year-old), grafted onto polyembryonic clonal rootstock (13–1) were cultivated under controlled conditions designed to mimic orchard-like environments. The trees were maintained in 6-L containers filled with a commercial soil mix (Klasmann-Deilmann) and kept in a net-house or greenhouse at the Newe Ya'ar Research Center (Ministry of Agriculture, Israel; GPS 32.70808, 3517,937).). Seasonal adjustments included growth under a white 50-mesh net during spring to fall (April–December), providing high light intensity (~1400 μmol photons m^{-2} s^{-1}), with day/night temperatures maintained between 20 and 24 °C. During winter (January–March) trees were transferred to a heated greenhouse, exposed to PPFD of 900–1100 μmol photons m^{-2} s^{-1}, with day/night temperatures maintained between 15 and 20 °C. Daily irrigation and weekly fertilization with 7:3:7 NPK (Shefer, ICL) were applied using automated systems to ensure consistent plant care throughout the experiments.

2.1.2. Mature "portable mango orchard" experimental-based system

Mature 'Shelly' mango trees (3-year-old), grafted onto 13–1 clonal rootstock, were obtained from Zvieli Nursery in Moshavat Kinneret. Sixty trees were cultivated in 130-L containers filled with a growing medium from Tuf Marom Golan, consisting of one-third coarse peat and two-thirds tuff (0.8 mm) mixture. The portable orchard was established in Hukok, located in the sea of galilee southern-west region, Israel's natural mango-growing area. Trees were irrigated through a dedicated irrigation system using 20-mm blind pipes fitted with drippers (two drippers per tree, each with a flow rate of 4 L/h). The annual irrigation schedule followed the Israeli Growers Association recommendations.

Trees were fertilized with "Shefer" 7:3:7 NPK plus microelements (+3 +Fe). Plant protection measures against mango scale insects, thrips, and other pests were implemented throughout the growing season.

2.2. Experimental design for night-chilling events and morning foliar treatments

Foliar sprays were applied in both experimental systems (young trees, Section 2.1.1, and mature trees, Section 2.1.2) after night chilling to address stomatal impairment that manifests during post-chilling light exposure.

Timing of foliar spray application. Due to the sporadic and localized nature of night-chilling conditions, preemptive foliar treatments are not always feasible or cost-effective. Post-chill applications, on the other hand, provide a practical alternative by directly targeting the physiological dysfunction after it occurs. In this study, foliar treatments were administered in the early morning following cold nights, coinciding with the timing onset of stomatal malfunction and transition to high irradiance.

Previous work in mango demonstrated that stomatal reopening is strongly inhibited following a night-chilling event, and that most physiological injury occurs during the morning when irradiance rises (Haque et al., 2024). Similarly, Allen et al. (2000) showed that an overnight chill (5–7 °C) in mango results in delayed photosynthetic inhibition that becomes evident during midday due to stomatal limitation. Comparative studies in other subtropical fruit trees further support this timing: Joshi et al. (2020) reported that overnight frost (≈ −2 to −3 °C for ~10 h) followed by high light caused strong inhibition of CO_2 assimilation in avocado, while Feng and Cao (2005) demonstrated in tropical tree seedlings that night chilling combined with subsequent high irradiance induces severe photoinhibition and oxidative stress.

Together, these findings highlight that early morning post-chill represents the most critical window for intervention. Our choice of timing was therefore guided by literature and by our own earlier work, ensuring that treatments directly address the physiological bottleneck associated with stomatal reopening after chilling nights.

Spray solution preparation

All spray solutions were freshly prepared on the day of application. For all treatments, deionized double-distilled water (DDW) containing 0.025 % (v/v) Triton X-100 was used as the carrier solution.

- **Mock control:** DDW + 0.025 % Triton X-100.
- **CPPU:** 240 μM CPPU (N-(2-Chloro-4-pyridyl)-N'-phenylurea; Gulliver, Almandine Corporation S.A.) prepared in DDW + 0.025 % Triton X-100.
- **KNO_3:** 100 mM KNO_3 prepared in DDW + 0.025 % Triton X-100.
- **1-MCP:** Applied as a commercial formulation (Hervista, 3.8 % 1-Methylcyclopropene, Rimi LTD), released in gaseous form according to manufacturer instructions.

2.2.1. Young tree system

Trees were moved from a greenhouse or net-house at dusk to a temperature-controlled dark room at 18–22 °C. The trees were randomly divided into two groups for distinct treatments: (i) 'Control', where trees remained in the dark at ambient temperature (18–22 °C) throughout the night, and (ii) 'Cold', where trees were exposed to severe night-chilling events (4 °C for 12 h). The chilling treatments were carried out within a controlled temperature chamber (Inomak), applying gradual cooling programs (described in detail in Haque et al., 2024). At the end of the night and before direct sunlight exposure, all trees were removed from the chamber to the darkened room, where they acclimated to room temperature alongside 'Control' trees.

After acclimation to room temperature, 'Control' (18–22 °C) and 'Cold' (4 °C for 12 h) exposed trees were randomly assigned to one of the following foliar treatments: A) mock (0.025 % Triton X-100), B) treatment with CPPU 240 μM + 0.025 % Triton X-100, C) potassium nitrate (KNO_3 100 mM, + 0.025 % Triton X-100), or D) 1-MCP (Hervista (, 3.8 % 1-Methylcyclopropene, Rimi LTD). Foliar sprays were applied to the entire canopy of each tree, thoroughly covering both adaxial and abaxial leaf surfaces using an electric/pressure backpack sprayer. The spray volume per tree was adjusted to achieve full coverage, typically ~250–300 mL for young potted trees and was identical across treatments. A minimum of three biological replicates per treatment ($N \geq 3$ trees) were used, as indicated in the figure legends. After spraying, trees were kept in the shade for 30 min until leaves dried and then returned to the net house or greenhouse.

2.2.2. Mature "portable orchard" experimental-based system

Mango trees were lifted onto a closed truck and transported from their growing site at Hukok to a temperature-controlled dark chamber in Tzemach at dusk. The trees were divided into two groups for distinct treatments: (i) 'Control', where trees remained at room temperature (18–22 °C) in the dark throughout the night, and (ii) 'Cold', where trees were exposed to a severe night-chilling event (4 °C 12 h). The chilling treatments were conducted using a gradual cooling program in the temperature chamber of 'Tzemach packinghouse'. At the end of the cold night and before sunrise, all trees were removed from the chamber and transferred with the 'Control' group outdoors. Next, the trees were randomly assigned to one of the following post-chill treatments: (i) Control spray (water + 0.025 % Triton X-100), or (ii) CPPU spray (10 mg L^{-1} + 0.025 % Triton X-100). Sprays were applied to the full canopy until runoff using an electric backpack sprayer. The total volume per tree ranged from 5 to 8 L, reflecting differences in canopy size among individual trees; otherwise, all treatments were applied identically. Each treatment included six biological replicates ($N = 6$ trees).

2.3. Measurements of gas exchange and chlorophyll-fluorescence

Stomatal behavior was assessed using gas exchange and chlorophyll fluorescence, robust and widely accepted proxies for stomatal function and photosynthetic performance under stress (Busch et al., 2024; Ceciliato et al., 2019; Long and Bernacchi, 2003)

Gas exchange and chlorophyll *a* fluorescence measurements were performed using a LI-6800 portable photosynthesis system (LI-COR Biosciences, USA) equipped with a Multiphase Flash™ Fluorometer, utilizing a measured leaf area of 2 cm^2. Measurements were conducted under controlled conditions for consistency on *fully expanded mature leaves*. To minimize variability, leaves were selected according to the following criteria: (i) located on the mid-canopy, (ii) fully expanded but not senescent (no visible yellowing or damage), (iii) sun-exposed, and (iv) similar developmental stage across trees. For each tree, three to five leaves fulfilling these criteria were randomly selected from comparable canopy positions, and measurements were repeated on at least three biological replicates (trees) per treatment, resulting in 9–15 leaves per treatment. This sampling strategy ensured that all treatments were assessed on physiologically comparable leaves and reduced variability caused by non-experimental factors. Gas exchange and chlorophyll fluorescence were measured after ~3–5 h of exposure to natural light. A reference CO_2 concentration of 400 ppm was set for gas exchange measurements while maintaining ambient relative humidity and temperature levels. The light intensity of 1000 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) was provided by blue/red light-emitting diodes (10 % blue and 90 % red). The LI-6800 chamber's airflow was set at 500 μmol s^{-1} with a boundary layer of around 3 mol m^{-2} s^{-1}. Following the attainment of leaf steady-state, gas exchange measurements for net CO_2 assimilation rate (*A*), stomatal conductance (*gs*), and internal CO_2 (*Ci*), the steady-state chlorophyll fluorescence signal (*Fs*) was logged. A saturating light pulse of 8000 μmol m^{-2} s^{-1} was employed to establish maximum light-adapted fluorescence (*Fm'*). The operating efficiency of photosystem II (PSII) under light conditions, calculated as $\Phi PSII = (Fm\text{-}Ft)/Fm'$, where *Fm* represents the maximum fluorescence

signal from dark-adapted material when all PSII centers are closed, *Ft* is the steady-state fluorescence yield in light, and *Fm'* stands for the maximum fluorescence signal from light-adapted material when all PSII centers are closed. To calculate maximum PSII efficiency (*Fv/Fm*), variable fluorescence (*Fv*) was divided by maximum fluorescence (*Fm*) during complete darkness. For maximum fluorescence (*Fm*) during complete darkness, at the end of gas exchange measurements, the same leaves were dark adapted for 1 h and measured ~7 h after the end of night-chill.

2.4. Statistical analysis

One-way ANOVA was used to analyze the data, followed by Tukey's multiple comparison tests. Results are reported as mean ± standard error of the mean (SEM) and *P*-values. GraphPad Prism version 9.5 (GraphPad Software, San Diego, CA, USA, www.graphpad.com) was used for the analysis.

3. Results

3.1. Mango leaves produce ethylene in response to a night-chilling event

To test whether ethylene biosynthesis is induced in mango leaves in response to night chilling, ethylene levels were quantified using gas chromatography from leaves of young mango trees that were exposed to a night-chilling event or kept under ambient growth conditions. While there were no detectable levels of ethylene produced in un-stressed control plant leaves, significant levels of ethylene were detected in leaves of trees that were subjected to a night-chill which was followed by exposure to light (Fig. S1).

Building on these results, three distinct foliar treatments, 1-MCP, CPPU, and KNO_3, were evaluated for their ability to restore physiological functions impaired by night chilling. The different treatments were tested on "Shelly" mango trees grafted onto 13–1 rootstocks following exposure to a severe night chilling event. Representative experiments showcasing the impacts of these three compounds are presented.

3.2. The ethylene inhibitor 1-MCP restores stomatal conductance, and photosynthetic carbon assimilation in mango trees challenged by night chill

Young mango trees were subjected to either 1) "Cold": severe night-chilling event or 2) "Control": non-stressed ambient temperatures. The next day, just before sunrise, the leaves of cold-stressed trees were sprayed with 1-MCP. Fully developed leaves were analyzed for gas exchange ~3 h after trees were exposed to natural sunlight. The results revealed a ~47 % decrease in leaf stomatal conductance in night-chill stressed trees compared to non-stressed controls (g_s; Cold 0.025 ± 0.005 vs. Control 0.047 ± 0.006 mol m^{-2} s^{-1}, $P = 0.05$, Fig. 1A). Cold-night-stressed mango trees treated with 1-MCP exhibited stomatal conductance values that were indistinguishable from non-stressed control trees (Fig. 1A). Furthermore, leaves of untreated cold-stressed mango trees showed a reduction by 42 % in net CO_2 assimilation rates

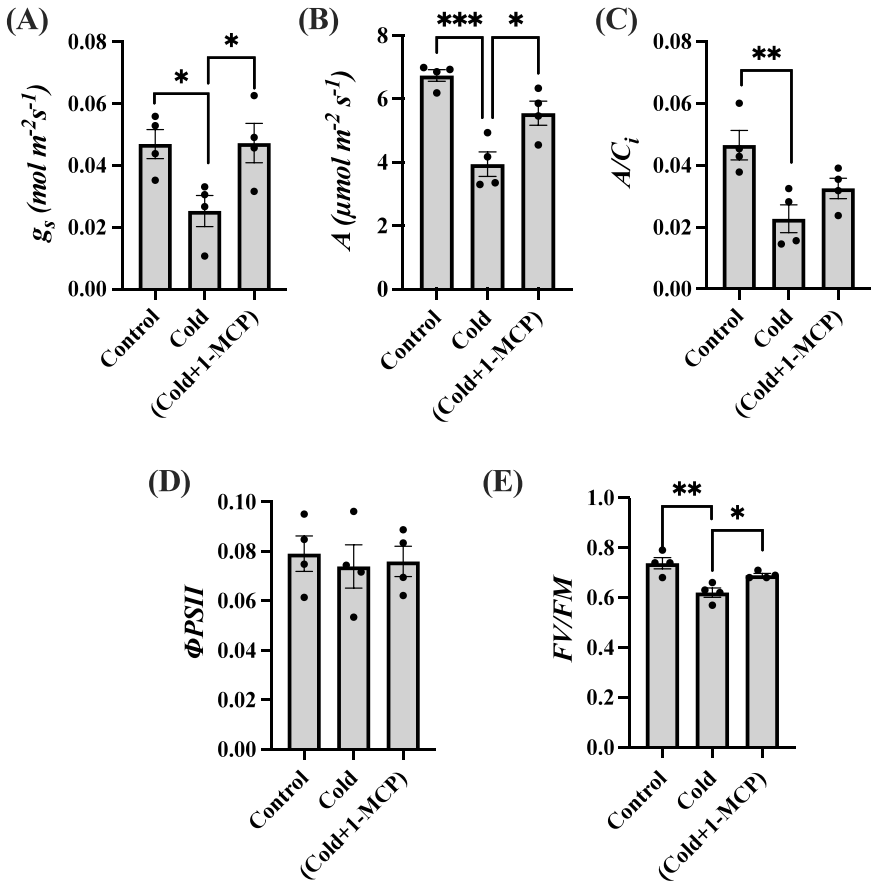

Fig. 1. Foliar application of 1-MCP following a severe night-chilling event prevents inhibition of leaf gas exchange and protects mango leaf photosynthetic capabilities from malfunction.

Gas exchange and chlorophyll fluorescence-based parameters measurements show **(A)** stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}) **(B)** net CO_2 assimilation rates (*A*; µmol CO_2 m^{-2} s^{-1}), **(C)** intercellular CO_2 (*Ci*; µmol CO_2 mol air^{-1}), **(D)** actual efficiency of PSII (*ΦPSII*), and **(E)** maximal efficiency of PSII (*Fv/Fm*). Statistical analysis was conducted using one-way ANOVA, followed by Tukey's multiple comparison test. Bars represent means ± SEM, where each treatment comprised four trees, where five leaves were measured from each tree. The corresponding significant *P*-values are indicated above bars. Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S2, respectively.

compared to the control non-stressed trees (*A*; Cold 3.9 ± 0.4 vs. Control 6.7 ± 0.2 μmol m^{-2} s^{-1}, $P < 0.001$, Fig. 1B). Conversely, net CO_2 assimilation rates of 1-MCP treated cold-stressed mango trees were significantly higher compared to those detected in mango leaves of untreated cold-stressed trees (*A*; Cold+1-MCP 5.5 ± 0.4 vs. Cold 3.9 ± 0.4 μmol m^{-2} s^{-1}, $P = 0.02$, Fig. 1B). The computed ratio of internal CO_2 within the leaf to its net CO_2 assimilation rate (*A/Ci*) revealed a statistically significant reduction in leaves of cold-night-stressed mango trees when compared to the control non-stressed trees (*A/Ci*; Cold 0.02 ± 0.004 vs. Control 0.05 ± 0.004 μmol CO_2 m^{-2} s^{-1} per ppm, $P = 0.01$, Fig. 1C). *A/Ci* of 1-MCP treated cold-stressed mango trees found to be between *A/Ci* levels detected in mango leaves of untreated cold-stressed trees and control trees (*A/Ci*; Cold+1-MCP 0.03 ± 0.003 μmol CO_2 m^{-2} s^{-1} per ppm, Fig. 1C). In addition, the overall efficiency of the photosystem II reaction center in the light, showed no statistically significant differences among treatments (Fig. 1D). Notably, 1-MCP treatment of cold-stressed trees significantly reduced the photoinhibitory effect, characterized by a decline in *Fv/Fm*, as observed in the night-chill-stressed trees (*Fv/Fm*; Cold+1-MCP 0.7 ± 0.007 vs. Cold 0.6 ± 0.02, $P = 0.05$, Fig. 1E). Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S2, respectively.

3.3. Foliar spray of CPPU protects mango tree leaves from night-chilling physiological damage

Young mango trees were exposed to severe night-chilling events or

Table 1

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Treatment	tree	A	Ci	gsw	PhiPS2	ETR	FV/FM	A/Ci
Control	1	6.988	163	0.0529	0.0951	40.1	0.68	0.042871
Control	2	6.927	183	0.0559	0.0615	25.93	0.74	0.037852
Control	3	6.854	151	0.0438	0.0749	31.56	0.79	0.045391
Control	4	6.188	103	0.0352	0.0849	35.78	0.74	0.060078
Cold	1	3.303	227	0.0331	0.0962	40.53	0.63	0.014551
Cold	2	4.177	148	0.0267	0.0717	30.22	0.57	0.028223
Cold	3	4.938	152	0.0306	0.0745	31.4	0.66	0.032487
Cold	4	3.358	215	0.0107	0.0535	22.55	0.62	0.015619
Cold+1-MCP	1	4.548	143	0.0316	0.0835	35.2	0.68	0.031804
Cold+1-MCP	2	5.461	230	0.0626	0.0888	37.44	0.68	0.023743
Cold+1-MCP	3	6.335	162	0.0491	0.0695	29.31	0.71	0.039105
Cold+1-MCP	4	5.861	165	0.0457	0.0622	26.22	0.69	0.035521
Note: The data presented in each row is the avarage of 3–5 meauerments from different leaves for each tree measuered (*N* = 6 trees).								
Treatment	tree	A	Ci	gsw	PhiPS2	FV/FM	A/Ci	
Control	1	4.556312	216.8844	0.042622	0.14	0.71	0.021008	
Control	2	5.373727	192.0274	0.044972	0.13	0.69	0.027984	
Control	3	3.41894	179.4868	0.027368	0.1	0.72	0.019048	
Cold	1	2.436443	147.1561	0.017818	0.09	0.66	0.016557	
Cold	2	2.784144	75.26961	0.016401	0.04	0.69	0.036989	
Cold	3	2.361106		0.007077	0.1	0.71	0.022275	
Cold+CPPU	1	3.864101	184.7633	0.030394	0.24	0.66	0.020914	
Cold+CPPU	2	3.614635	217.4769	0.033666	0.24	0.75	0.016621	
Cold+CPPU	3	4.216788	201.0506	0.037339	0.22	0.72	0.020974	
Note: The data presented in each row is the avarage of 3–5 meauerments from different leaves for each tree measuered (*N* = 3 trees).								
Treatment	tree	A	Ci	gsw	PhiPS2	ETR	A/Ci	FV/FM
Control	1	5.1	254.042	0.064	0.119	50.353	0.020075	0.77
Control	2	3.5	195.53	0.031	0.104	44.029	0.0179	0.75
Control	3	4.8	209.163	0.049	0.123	51.699	0.022949	0.7
Control	4	6.6	232.889	0.076	0.145	61.112	0.02834	0.73
Cold	1	2.6	203.882	0.023	0.166	70.082	0.012752	0.7
Cold	2	2.3	232.893	0.022	0.145	60.935	0.009876	0.73
Cold	3	1.8	263.708	0.023	0.118	49.792	0.006826	0.71
Cold	4	0.7	330.746	0.018	0.091	38.333	0.002116	0.72
Cold+KNO3	1	4.6	194.252	0.041	0.171	72.181	0.023681	0.72
Cold+KNO3	2	6.9	239.196	0.085	0.163	68.808	0.028847	0.73
Cold+KNO3	3	4.3	253.131	0.052	0.129	54.313	0.016987	0.73
Cold+KNO3	4	3.3	240.467	0.037	0.137	57.863	0.013723	0.75
Note: The data presented in each row is the avarage of 3–5 meauerments from different leaves for each tree measuered (*N* = 4 trees).								
Treatment	tree	A	Ci	gsw	PhiPS2	FV/FM	A/Ci	
Cold	1	−0.02225	393.0635	0.022683	0.056097	0.822308	−5.7E-05	
Cold	2	1.253714	353.8157	0.038582	0.051492	0.832417	0.003543	
Cold	3	1.788389	329.4093	0.039584	0.055956	0.855249	0.005429	
Cold	4	1.592773	307.8248	0.029197	0.071453	0.847198	0.005174	
Cold	5	2.088519	312.6657	0.044939	0.09469	0.794101	0.00668	
Cold	6	0.228387	390.548	0.026938	0.062511	0.726095	0.000585	
Control	1	5.276908	298.5382	0.088027	0.064967	0.85548	0.017676	
Control	2	4.12579	311.2344	0.083544	0.027274	0.883622	0.013256	
Control	3	4.126478	304.5028	0.077605	0.068604	0.844316	0.013552	
Control	4	4.81543	295.3991	0.083918	0.050674	0.863374	0.016301	
Control	5	5.786599	274.6463	0.083206	0.123056	0.818763	0.021069	
Control	6	7.539486	275.8548	0.106448	0.044412	0.853961	0.027331	
Cold+CPPU	1	2.65673	325.5174	0.060469	0.074315	0.875731	0.008162	
Cold+CPPU	2	5.688559	258.9531	0.069502	0.061359		0.021968	
Cold+CPPU	3	4.355963	252.0114	0.051725	0.070604	0.850174	0.017285	
Cold+CPPU	4	5.684706	273.9495	0.079118	0.098957	0.820282	0.020751	
Cold+CPPU	5	3.199127	301.6293	0.060195	0.053763	0.689916	0.010606	
Cold+CPPU	6	4.630717	275.3758	0.064962	0.056732	0.802372	0.016816	

Note: The data presented in each row is the avarage of 3–5 meauerments from different leaves for each tree measuered (*N* = 6 trees).

were kept under ambient conditions. The subsequent morning, cold-stressed trees received mock or CPPU treatments (10 ppm) with 0.025 % Triton X-100 as a surfactant. A severe night-chilling event led to a pronounced decline in stomatal opening (g_s; Cold 0.01 ± 0.003 vs. Control 0.04 ± 0.005 mol m^{-2} s^{-1}, $P = 0.01$, Fig. 2A) and a significant reduction in net CO_2 assimilation rates (A; Cold 2.5 ± 0.1 vs. Control 4.5 ± 0.6 µmol m^{-2} s^{-1}, $P = 0.02$, Fig. 2B). Treating the cold-stressed trees with CPPU led to a significant increased stomatal conductance (g_s; Cold+CPPU 0.03 ± 0.002 vs. Cold 0.01 ± 0.003 mol m^{-2} s^{-1}, $P = 0.03$, Fig. 2A) and significantly improved net CO_2 assimilation rates (A; Cold+CPPU 3.9 ± 0.2 vs. Cold 2.5 ± 0.1 µmol m^{-2} s^{-1}, $P = 0.07$, Fig. 2B) compared to cold-stressed trees. The computed ratio of internal CO_2 within the leaf to its net CO_2 assimilation rate (A/Ci) showed similar levels between the different treatments (Fig. 2C). On the other hand, the efficiency of the photosystem II reaction center in the light, found to reduce in cold-stress trees slightly ($\Phi PSII$: Cold 0.07 ± 0.02 vs. Control 0.12 ± 0.01, $P = 0.1$, Fig. 2D). Notably, $\Phi PSII$ increased significantly in cold-stressed CPPU treated trees, when compared to cold-stressed trees ($\Phi PSII$; Cold+CPPU 0.23 ± 0.01 vs. Cold 0.07 ± 0.02, $P < 0.01$, Fig. 2D), and found to be even higher than the control ($\Phi PSII$; Cold+CPPU 0.23 ± 0.01 vs. Control 0.12 ± 0.01, $P < 0.01$, Fig. 2D). Analysis of Fv/Fm showed similar ratios in all three treatments (Fv/Fm; ~0.7, Fig. 2E). Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S3, respectively.

3.4. Foliar spray with KNO_3 after night-chilling event protects leaf stomatal conductance, and photosynthetic carbon assimilation from malfunction

KNO_3 effects on plant gas exchange and chlorophyll fluorescence parameters were analyzed in young mango trees. Trees were exposed to severe night chill. Our results show that extreme chilling night stress impairs stomatal opening during the following day (g_s: Cold 0.02 ± 0.001 vs. Control 0.05 ± 0.01 mmol m^{-2} s^{-1}, $P = 0.06$, Fig. 3A), leading to substantial reductions in net CO_2 assimilation rates (A; Cold 1.9 ± 0.4 vs. Control 5 ± 0.6 µmol m^{-2} s^{-1}, $P = 0.02$, Fig. 3B). KNO_3 treatment on cold-stressed trees demonstrated a positive impact on gas exchange (g_s) and as a consequence on leaf carbon fixation (A), surpassing cold-stressed trees effects (g_s; Cold+KNO_3 0.05 ± 0.01 vs. Cold 0.02 ± 0.001 mmol m^{-2} s^{-1}, $P = 0.06$, Fig. 3A), (A; Cold+KNO_3 4.8 ± 0.8 vs. Cold 1.9 ± 0.4 µmol m^{-2} s^{-1}, $P = 0.03$, Fig. 3B). Interestingly, when the computed ratio of internal CO_2 within the leaf to its net CO_2 assimilation rate was calculated (A/Ci), a significant reduction in A/Ci levels in leaves of cold-stressed mango plants when compared to control trees was observed (A/Ci; Cold 0.008 ± 0.002 vs. Control 0.02 ± 0.002 µmol CO_2 m^{-2} s^{-1} per ppm, $P = 0.01$, Fig. 3C). Fascinatingly, A/Ci levels recovered and reached similar levels as in the control non-stressed mango plant leaves in KNO_3 treated cold-stressed mango plants (A/Ci; Cold+KNO_3 0.02 ± 0.003 vs. Control 0.02 ± 0.002 µmol CO_2 m^{-2} s^{-1} per ppm, $P = 0.9$, Fig. 3C). Chlorophyll a-based fluorescence PAM analysis

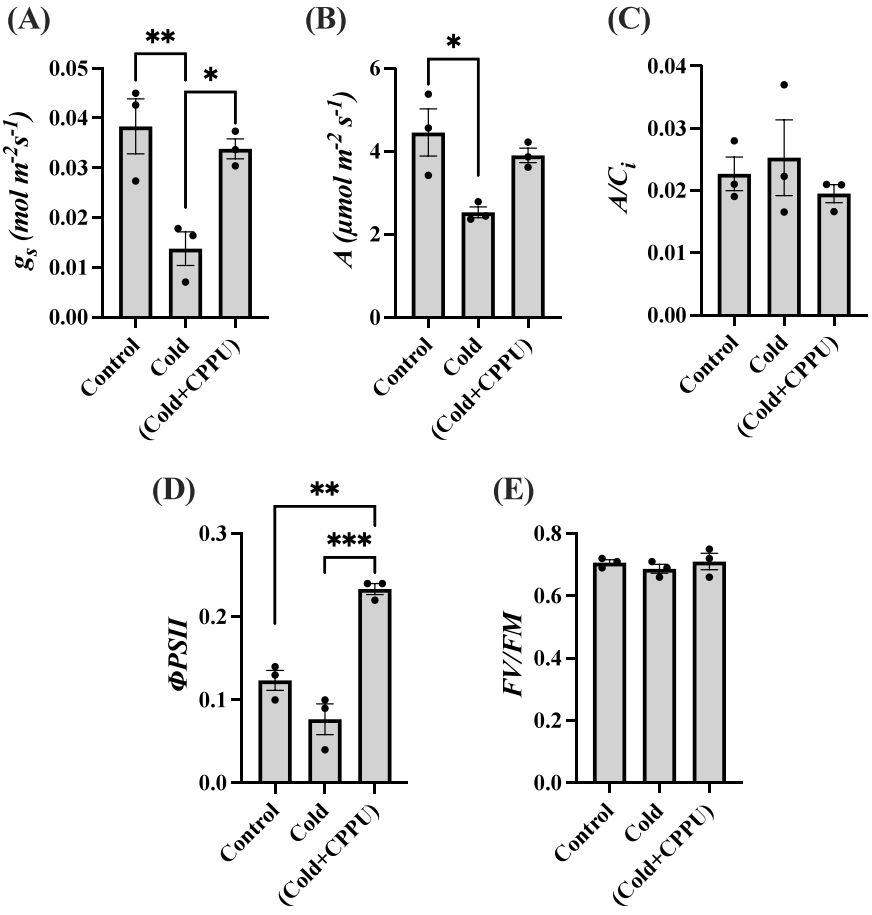

Fig. 2. Foliar treatment with CPPU after a severe night-chilling event results in full recovery of leaf CO_2 assimilation (A) and stomatal function (g_s), together with protection from damage to photosystem II.
Gas exchange and chlorophyll fluorescence-based parameters measurements show **(A)** stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}) **(B)** net CO_2 assimilation rates (A; µmol CO_2 m^{-2} s^{-1}), **(C)** ratio of internal CO_2 within the leaf to its net CO_2 assimilation rate (A/Ci), **(D)** actual efficiency of PSII ($\Phi PSII$), and **(E)** maximal efficiency of PSII (Fv/Fm). Statistical analysis was conducted using one-way ANOVA, followed by Tukey's multiple comparison test. Bars represent means ± SEM, where each treatment comprised four trees, where five leaves were measured from each tree. The corresponding P-value is indicated above bars. Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S3, respectively.

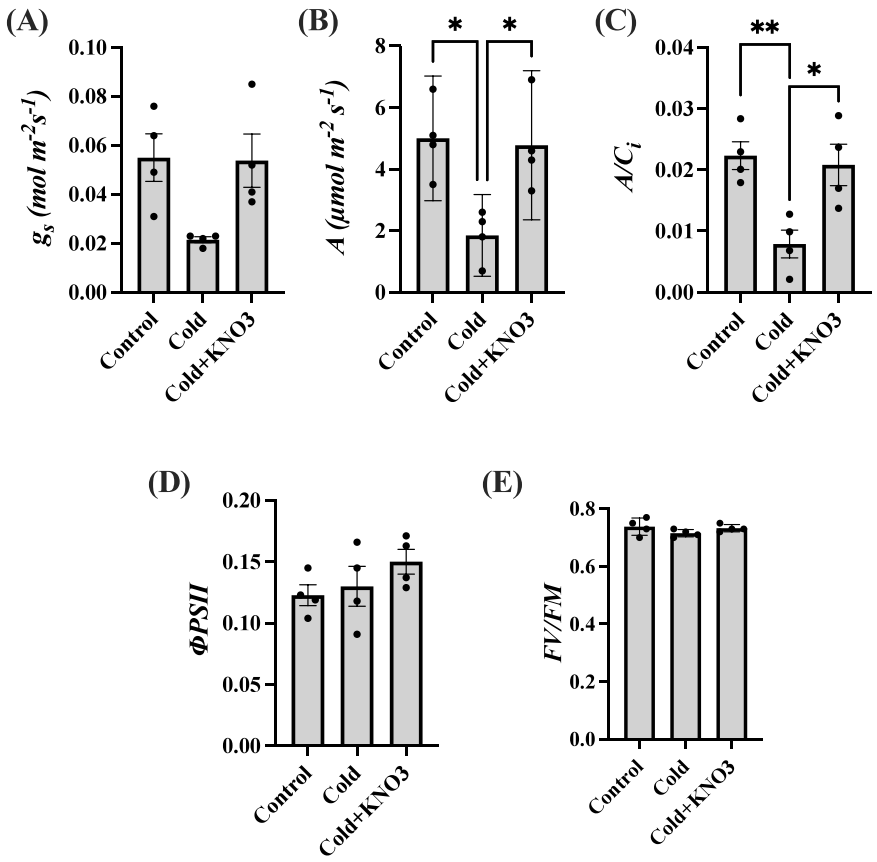

Fig. 3. Following a severe night-chilling event, foliar application of KNO_3 protect gas exchange and CO_2 assimilation rates from declining. Gas exchange and chlorophyll fluorescence-based parameters measurements show (A) stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}) (B) net CO_2 assimilation rates (*A*; µmol CO_2 m^{-2} s^{-1}), (C) ratio of internal CO_2 within the leaf to its net CO_2 assimilation rate (*A/Ci*), (D) actual efficiency of PSII (*ΦPSII*), and (E) maximal efficiency of PSII (*Fv/Fm*). Statistical analysis was conducted using one-way ANOVA, followed by Tukey's multiple comparison test. Bars represent means ± SEM, where each treatment comprised four trees, where five leaves were measured from each tree. The corresponding *P*-value is indicated above bars. Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S4, respectively.

of *ΦPSII* and *Fv/Fm* showed similar levels between all three treatments (i.e., Cold/Control/ Cold+KNO_3) (Fig. 3D&E). Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S4, respectively.

3.5. *Efficient protection of adult mango trees by CPPU treatment from night-chill physiological damage*

Our study, which tested the competence of 1-MCP, CPPU, and KNO_3 to bypass the impaired stomatal opening of mango leaves after night chill, revealed significant performance of all three substances. With the need for an innovative agricultural practice to protect mango trees from physiological night-chill damages, CPPU foliar treatment performances was further tested on mature 3-year-old mango trees using our "portable orchard system" (as described in the method section and illustrated in Fig. S5).

Mature, 3-year-old 'Shelly' mango trees were grown in 130-L containers in Hukok (Fig. S5A). To simulate a night chilling event, the portable mango orchard system was transported using a close truck (Fig. S5B) to a temperature-controlled dark chamber in Tzemach Packing House at dusk (Fig. S5C). The trees were divided into two groups for distinct treatments: (i) 'Control', where trees remained at room temperature in the dark throughout the night, and (ii) 'Cold', where trees were exposed to severe night-chilling event, using a gradual cooling program in the temperature chambers (Fig. S5C). At the end of the cold night and before sunrise, all trees were removed from the chamber and transferred with the 'Control' group outdoors (Fig. S5D). Trees were randomly assigned ($n = 6$ trees to each treatment) and treated by foliar spray, using an electric backpack sprayer, to one of the following post-cold treatments: (i) Control spray, where trees were sprayed with water containing 0.025 % Triton X-100, or (ii) CPPU spray, where trees were treated with a solution of 240 µM CPPU dissolved in water with 0.025 % Triton X-100 (Fig S5E). Gas exchange measurements analyses, conducted following 5 h of exposure to natural light show that a severe night-chilling event in mature mango trees led to a pronounced decline in stomatal opening (g_s; Cold 0.03 ± 0.003 vs. Control 0.09 ± 0.004 mol m^{-2} s^{-1}, $P < 0.0001$, Fig. 4A) and a significant reduction in net CO_2 assimilation rates (*A;* Cold 1.2 ± 0.4 vs. Control 5.3 ± 0.5 µmol m^{-2} s^{-1}, $P < 0.0001$, Fig. 4B). Treating the cold-stressed trees with CPPU led to a significant increased stomatal conductance (g_s; Cold+CPPU 0.06 ± 0.004 vs. Cold 0.03 ± 0.004 mol m^{-2} s^{-1}, $P = 0.0001$, Fig. 4A) and significantly improved net CO_2 assimilation rates (*A;* Cold+CPPU 4.4 ± 0.5 vs. Cold 1.2 ± 0.4 µmol m^{-2} s^{-1}, $P = 0.0006$, Fig. 4B) compared to cold-stressed trees. These results are similar to those found using the young mango tree experimental-based system (Fig. 2), further supporting the significant protection of CPPU foliar spray from night-chill-induced physiological damage.

The computed ratio of internal CO_2 within the leaf to its net CO_2 assimilation rate (*A/Ci*) revealed a statistically significant reduction in leaves of cold-night-stressed mango trees when compared to the control non-stressed trees (*A/Ci*; Cold 0.004± 0.001 vs. Control 0.02 ± 0.002 µmol CO_2 m^{-2} s^{-1} per ppm, $P = 0.0002$, Fig. 4C). *A/Ci* of CPPU treated cold-stressed mature mango trees found to be significantly higher than *A/Ci* detected in mango leaves of untreated cold-stressed trees (*A/Ci*; Cold+CPPU 0.02 ± 0.002 vs. Cold 0.004 ± 0.001 µmol CO_2 m^{-2} s^{-1} per ppm $P = 0.001$, Fig. 4C), and similar to those in control trees (*A/Ci*; Control 0.02 ± 0.002 µmol CO_2 m^{-2} s^{-1} per ppm, Fig. 4C). The overall

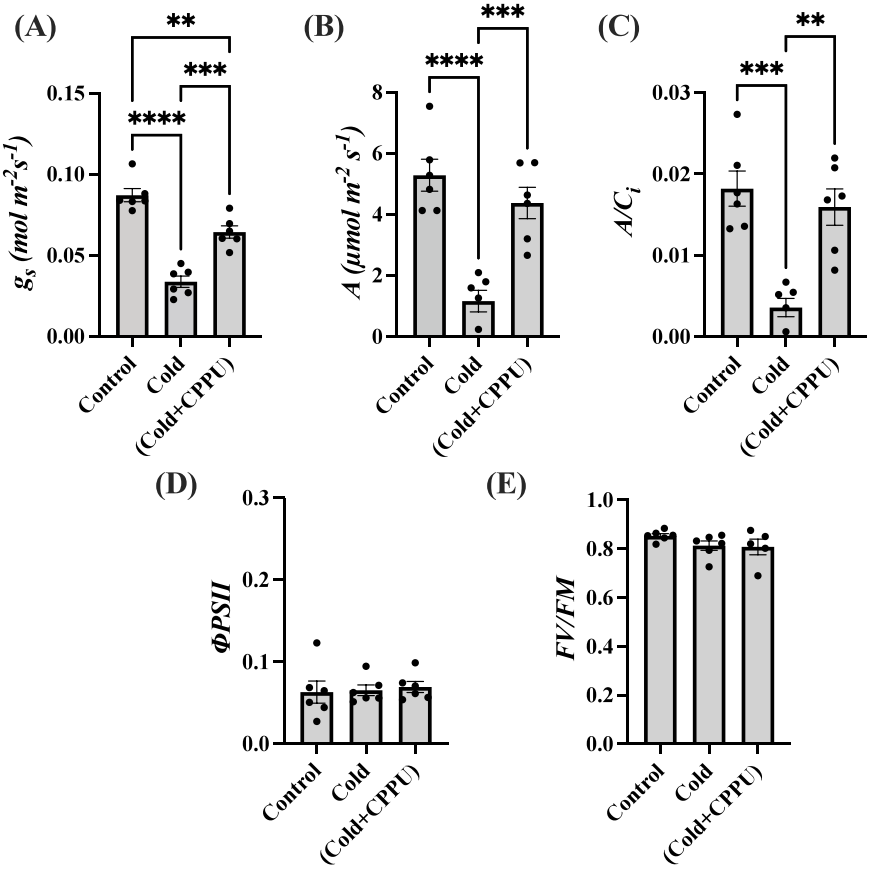

Fig. 4. Foliar application of CPPU provides physiological protection to mature mango orchards exposed to night-chilling event. Foliar application of CPPU was tested on a portable mango orchard experimental system as described in the method section and illustrated in Fig S5. Gas exchange and chlorophyll fluorescence-based parameters measurements show **(A)** stomatal conductance (g_s; mmol H_2O m^{-2} s^{-1}) (B) net CO_2 assimilation rates (A; µmol CO_2 m^{-2} s^{-1}), **(C)** ratio of internal CO_2 within the leaf to its net CO_2 assimilation rate (A/Ci), **(D)** actual efficiency of PSII ($\Phi PSII$), and **(E)** maximal efficiency of PSII (Fv/Fm). Statistical analysis was conducted using one-way ANOVA, followed by Tukey's multiple comparison test. Bars represent means ± SEM, where each treatment comprised four trees, where five leaves were measured from each tree. The corresponding P-value is indicated above bars. Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S6, respectively.

efficiency of the photosystem II reaction center in the light ($\Phi PSII$; ~0.6 Fig. 4D), nor ratios of Fv/Fm (~0.8 Fig. 4E) showed statistically significant differences among treatments. Data and 95 % confidence intervals (Tukey) are shown in Table 1 and Fig. S6, respectively.

4. Discussion

Extreme weather conditions, including heat/cold waves, have become more common with climate change. The sensitivity of mango to night chills raised the need for research and development of new agricultural practices for protecting mango trees from this abiotic stress physiological damage. Our prior work demonstrated that mango trees subjected to cold night–bright day conditions benefit from post-chilling application of fusicoccin (FC), which facilitates stomatal reopening, enhances leaf-level gas exchange, and reduces the photodamage associated with chilling-induced stress (Haque et al., 2024). In the current study, using a controlled experimental-based system mimicking a night-chilling event, additional compounds were tested that could alleviate night-chill-induced impaired stomatal conductance, are simple to apply in orchards, and are cost-effective for growers. Foliar applications of 1-MCP, CPPU, and KNO_3 were each found to improve stomatal behavior and enhancing carbon assimilation in mango leaves subjected to night-chill stress, thereby preserving photosynthetic function and alleviating chilling-related physiological impairment. Furthermore, foliar application of mature trees with CPPU in a "portable orchard system" showed significant protection against night-chill-induced physiological damage. These findings provide valuable insights for managing cold stress in mango trees and display a practical solution for enhancing crop resilience in changing climatic conditions. The signaling pathways involved in cold-stress-impaired stomatal conductance regulation and how these pathways can be bypassed through foliar application of 1-MCP, CPPU, or KNO_3, and the practical significance of these findings in the context of climate change are discussed.

4.1. *The role of ethylene* in cold stress response

Cold stress is known to interfere with multiple plant signaling networks, although the precise mechanisms that hinder stomatal opening in mango following night chilling remain poorly understood. Chilling temperatures disrupt plant water status and activate hormonal responses, including elevated synthesis of phytohormones that promote stomatal closure (Acharya and Assmann, 2009; Agurla et al., 2018; Shi et al., 2015; Zhang et al., 2022a). In the current study, a significant rise in ethylene production was observed in mango leaves subjected to night-chilling stress (Fig. S1). Ethylene accumulation in response to cold has also been documented in other cold-sensitive species (Huang et al., 2023), and this effect appears to intensify when plants are later exposed to light, a phenomenon linked to light-activated ethylene biosynthetic pathways (Harkey et al., 2019).

In addition, cold-induced oxidative stress, characterized by elevated ROS levels (Hariyadi and Parkin, 1993; Joshi et al., 2020; Lukatkin, 2002; Prasad et al., 1994), has been shown to enhance ethylene

biosynthesis (Kato et al., 2000; Ke and Sun, 2004; Weckx and Van Poucke, 1989). These findings support a possible regulatory link between ROS and ethylene in cold-stressed mango foliage. Ethylene is broadly involved in modulating stomatal conductance (Dodd, 2003; Pospíšilová, 2003; Tanaka et al., 2005, 2006; Wilkinson and Davies, 2009), although its impact on aperture dynamics is complex—promoting either opening or closure depending on species and context (Desikan et al., 2006; Iqbal et al., 2011; Levitt et al., 1987).

In our system, mango trees exposed to 'Cold Night–Bright Day' conditions showed elevated ethylene and reduced stomatal aperture (Fig. S1, Fig. 1). Treatment with 1-MCP, an inhibitor of ethylene perception, reversed the stomatal closure observed following night chilling. This implies that ethylene plays a contributing role in limiting stomatal aperture under cold stress conditions that persist into the photoperiod following the chilling night. Ethylene-responsive pathways in guard cells include ROS production (Desikan et al., 2006; Watkins et al., 2014), as well as signaling through H_2S and NO (Liu et al., 2012a). Ethylene signaling also exhibits crosstalk with other phytohormones—including auxins, cytokinins, brassinosteroids, jasmonates, salicylic acid, and ABA—integrating environmental cues into the stomatal response (Acharya and Assmann, 2009; Daszkowska-Golec and Szarejko, 2013; Liu et al., 2012a; Zhang et al., 2022a).

This study showed that foliar application of 1-MCP following night chill significantly increases stomatal conductance and net CO_2 assimilation rate and minimizes photoinhibition (Fig. 1A, B, and E, respectively). These results indicate that inhibition of ethylene reception in cold-stressed mango plants can alleviate physiological damage. As 1-MCP is widely used as a pre-harvest/post-harvest treatment in different crops, in particular for the improvement of fruit quality (Q. Vilhena et al., 2023; Vilhena et al., 2022; Watkins et al., 2010), its use to protect trees from night chill events in the orchards should be evaluated.

4.2. The role of cytokinin in cold stress response

Abscisic acid (ABA) is a sesquiterpene phytohormone that is involved in various biotic and abiotic stress responses and induces stomatal closure (Bharath et al., 2021a) via a well-studied signaling pathway (Hsu et al., 2021). Plants under cold stress conditions respond by promoting ABA biosynthesis and ABA signal transduction (Huang et al., 2017; Lang et al., 1994). As the phytohormone ethylene, ABA can interact with other hormones that mediate stomatal conductance regulation. Cytokinin is a major phytohormone in the plant and is involved in various pathways. Concerning stomata, few studies have suggested that cytokinin is involved in stomatal closure via the regulation of ROS homeostasis within the guard cells. Accumulated body of evidence indicates that cytokinin acts as an antagonist of ABA during environmental stress responses (Guan et al., 2014; Peleg and Blumwald, 2011), which results in the induction of stomatal opening (Tanaka et al., 2006) and increased transpiration (Badenoch-Jones et al., 2006) by the reduction of guard cell NO levels (Xiao-Ping and Xi-Gui, 2006). It is important to note that cytokinin has a diverse effect on stomatal conductance regulation, which was found to be species-dependent (Blackman and Davies, 1983) and dependent on the abundance and cross-talk between different hormones (Novak et al., 2013).

Foliar application with CPPU following a severe night-chilling event had a significant recovery effect on leaf gas exchange, leaf stomatal conductance (g_s) and net CO_2 assimilation (A). Notably, foliar application of CPPU following night-chill found to increase the actual efficiency of PSII ($\Phi PSII$) (Fig. 2D). These results are in line with previous reports, which showed that cytokinins promote the synthesis of photosynthetic enzymes and electron transport components (Feierabend and de Boer, 1978), to induce photorespiration (Rivero et al., 2009), stimulate g_s, and can protect the decline of A following abiotic stress responses (Bradford, 1983; Rivero et al., 2009). CPPU is widely used during preharvest and postharvest, particularly for improving fruit production and crop-specific traits (Aremu et al., 2020). The result of our study and others further supports the involvement of cytokinins in the regulation of photosynthetic capacity and the applicative use of CPPU against night-chill physiological damage.

4.3. The role of potassium (K^+) in cold stress response

Prior studies emphasized potassium nitrate (KNO_3) as a promoter of heightened transpiration and photosynthesis. Foliar application of KNO_3 was found to induce increases in relative chlorophyll content, enhance stomatal conductance, photosynthetic rate, carboxylation efficiency, and photosynthetic levels of drought-stressed plants (Ávila et al., 2022; Elhindi, 2016). Similarly, KNO_3 was found to protect leaf stomatal conductance, and photosynthetic carbon assimilation from malfunction following night-chilling event.

Cold stress causes loss of membrane integrity and compartmentation, leakage of solutes, ROS production (Einset et al., 2007; Suzuki and Mittler, 2005), frequently results in lipid peroxidation (Wang et al., 2023), as well as $K+$ leakage from cells by activating $K+$ efflux channels (Demidchik, 2018), which results in stomatal closure (Hosy et al., 2003; Schroeder, 2003). Furthermore, the plasma membrane $H+$-ATPase was found to be disturbed when Na+ replaces $K+$ in plant cells (Fricker and Willmer, 1990). Furthermore, with chill stress disruption of lipid fluidity (Kasamo et al., 1992), the activity of various membrane-bound enzymes are negatively affected, including $H+$-ATPase (Kasamo et al., 2000). The plasma membrane H^+-ATPase (De Michelis et al., 2014) is a key regulator in guard cells' stomatal conductance regulation. Activation of $H+$-ATPase found to induce stomatal opening (Kinoshita and Shimazaki, 2001) and to overcome stomatal closure that is caused by different environmental factors (Hashimoto et al., 2006; She et al., 2010), including cold stress (Haque et al., 2024). For a detailed discussion on the possible mechanism/s involved in night chill via the guard cells $H+$-ATPase, see (Haque et al., 2024).

5. Conclusions

For appropriate physiological response to different environmental stimuli, stomatal guard cells sense and integrate various contradictory signals from the internal and external environment (Hetherington and Woodward 2003; Vavasseur and Raghavendra 2005). The events in guard cells are fine-tuned to achieve an appropriate physical response and survive under the prevailing stress conditions. The results of this study demonstrate the complexity and interaction between different cold-stress-induced signals in regulating stomatal conductance. The effect of such cross-talk on the molecular response of guard cells and the question of which pathway/s that regulate stomatal conductance within the guard cells are affected by night chill have yet to be assessed.

This research provides potential horticultural treatments to alleviate chilling stress in mango, which currently lacks cost-effective strategies to combat tree physiological chilling damage. Large commercial experiments should attempt to explore their contribution to yield in the orchard. Beyond mango, other tropical and subtropical species are also sensitive to cold stress, including avocado (Joshi et al., 2020), lychee (Zhang et al., 2022b), coffee (Bauer et al., 2006; Guo and Cao, 2015), and cacao (Joly and Hahn, 1991). Climate change, coupled with projections of increased extreme weather events and the expansion of tropical and subtropical crop-growing regions into previously unsuitable colder areas (Salunkhe et al., 2023), is expected to drive demand for agricultural solutions of this nature.

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CRediT authorship contribution statement

Md Intesaful Haque: Writing – review & editing, Project administration, Investigation, Formal analysis. **Or Shapira:** Investigation, Formal analysis, Conceptualization. **Ziv Attia:** Investigation, Formal analysis. **Shay Tsaidi:** Project administration, Investigation. **Marc Perel:** Methodology. **Yuval Cohen:** Writing – review & editing, Conceptualization. **Dana Charuvi:** Writing – review & editing, Conceptualization. **Tamar Azoulay-Shemer:** Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2025.114448.

Data availability

Data will be made available on request.

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