Postharvest pulsing promotes flower opening and improves vase characteristics by regulating hydration and ethylene status in cut roses

Nisar Hussain[ab*], Hafiz Ishtiaq Ahmad[c], Adeel Shahid[d], Sabir Aziz[e], Sultan Mehmood[f]

[a] Beijing Key Laboratory of Development and Quality Control of Ornamental Crops, College of Horticulture, China Agricultural University, Beijing, 100193, China; [b] Department of Production Technology and Quality Control, Kunming 24Hua Modern Agricultural Technology Co., Ltd, Kunming, 650305, China; [c] College of Landscape and Horticulture, Yunnan Agricultural University, Kunming, 650201, China; [d] Department of Agriculture, Government College University, Lahore, 54000, Pakistan; [e] Department of Horticulture, Bahauddin Zakariya University, Multan, 60800, Pakistan; [f] Horticultural Research Institute, National Agricultural Research Center, Islamabad, 45500, Pakistan;
[*]Corresponding author: Nisar Hussain

Abstract- Rose is a top-cut flower in production, but due to improper flower opening, it faces heavy losses in postharvest handling. This study explores postharvest pulsing effects on the physiological changes that lead to flower opening and vase quality characteristics. Cut roses were treated with Sucrose (SUC), α-aminoisobutyric acid (AIB), and 8-hydro-quinoline citrate (8- HQC) under different combinations: CON; control, SUC; Sucrose, SUCA; SUC plus AIB, SUCH; SUC plus 8-HQC, AH; AIB plus 8-HQC, and SUCAH; SUCA Plus 8-HQC. We examined the respiratory rate, stomatal conductance, and hydraulic conductance of flower stems, SUCAH, SUCA, SUCH, and AH showed positive feedback, while ethylene production was lowest compared to CON. The flower opening percentages and speed were highest with more water uptake and flower diameter, and less microbial growth in SUCAH, followed by SUCA, SUCH, and AH than CON. Compared to CON, ethylene biosynthesis genes (RhACS2, RhACO1), and senescence-associated gene (RhSAG12) transcription were lowest, and the vase characteristics significantly improved in SUCAH, followed by AH, SUCH, and SUCA. Our results indicated that postharvest pulsing by SUCAH substantially affected the water and ethylene status, which led to flower opening and improved vase quality characteristics.

Index Terms- cut rose, ethylene, flower opening, vase life, sucrose, α-aminoisobutyric acid, 8-hydroquinoline citrate

I. Introduction

Flower opening is a complex physiological process that determines the market value of cut flowers. Rose (*Rosa hybrida* L.), is one of the leading cut flowers supplied by long-distance transportation. After harvest, robust ethylene production and water cut disturb proper flower opening and diminish the ornamental period (Hussain et al., 2024). Flower opening is mainly attributable to cell expansion via the actions of phytohormones, water status, and carbohydrates (Horibe and Yamada, 2017; Sun et al., 2021). Ethylene production was measured in roses, and found that short-lived varieties reached the ethylene peak earlier than long-lived varieties (Xue et al., 2008). Applying ethylene to roses results in repressed petal cell expansion by directly inhibiting the aquaporin proteins, while ethylene inhibitor 1-methylcyclopropene (1-MCP) application resulted in more curved petals with larger cell size (Ma et al., 2008). The α-aminoisobutyric acid (AIB) is an analog of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene. Exogenous application of AIB repressed ethylene biosynthesis from ACC by repressing ACC oxidase activity (Satoh and Esashi, 1980). Some studies report that AIB application extends the vase life in cut carnation and Lilium (Onozaki et al., 1998; Shimamura et al., 1997). However, the role of AIB in ethylene-regulated flower opening largely remains unknown.

The water status is also very important in flower opening and the vase of cut flowers. For flower opening, elongation is an essential movement that is driven by water uptake or osmotic adjustment by the petals. During postharvest, cut flowers generally undergo water deficit stress, which disturbs flower opening quality and restricts their vase life extension. Air embolism is a disorder produced due to dehydration, which leads to abnormal opening of flowers and early senescence and subsequently disturbs the economic value of cut flowers (Luo et al., 2013). Aquaporins are transmembrane functional proteins that ease water transmission transversely in membranes and perform vital roles in cell expansion (Chaumont and Tyerman, 2014). The PIPs are inhibited by exogenous ethylene treatment in rose 'Samantha'. The expression profile of RhPIP2;1 and RhTIP1 were lower, which correlates with reduced petal size and results in an abnormal flower opening process (Ma et al., 2008; Xue et al., 2009).

In flowering plants, carbohydrates play an important role during the flower opening process. Carbohydrates are commonly supplemented for an improvement of water status and as a source of energy for respiration by petals; hence, they are used to lengthen the ornamental value of cut roses. Sucrose usage changes the hormonal equilibrium of several floral tissues, speed's petal elongation, and delays senescence in cut lilies (Arrom and Munné-Bosch, 2012). During petal growth, carbohydrate concentrations increase promptly in the vacuole, which promotes osmotic pressure, enables water transportation inward to cells and leads to cell expansion (Yamada et al., 2009). In rose, during flower opening at stage 3, sucrose relative contents were higher than stage 1 and stage 5 contents. The RhSUC2-silenced plants had smaller flower diameter and petal

area, which advocates sucrose may help in proper cell expansion by maintaining turgor pressure. Whereas, reduced sucrose contents at stages 5 and 6 let the ethylene production, promote petal shedding and reduce the flower vase life (Horibe and Yamada, 2017; Liang et al., 2020). However, there is no such study available that comprehensively covers ethylene, water, and carbohydrate functionality together in cut roses for proper flower opening and vase life.

This study explored the postharvest pulsing effects of ethylene inhibitor, carbohydrate, and water absorbent germicide on the physiological changes that led to flower opening and improved vase quality. We found that the respiratory rate, stomatal conductance, and hydraulic conductance of flower stems showed positive feedback while ethylene production was lower on the application of SUCAH followed by AH, SUCH, and SUCA. Our results indicated that pulsing by SUCAH significantly affects the ethylene biosynthesis genes and senescence associated gene transcription, leading to flower opening and improving vase quality.

II. MATERIALS AND METHODS

2.1. Plant materials and treatments

Rosa hybrida 'Samantha' plantlets were grown in the greenhouse under a 16 h light/8 h dark photoperiod at (22 ±1) °C with a relative humidity of 60%. The flower opening stages for expression pattern analysis were defined as previously described (Chen et al., 2023). Flowers were taken at stage 2 and after harvesting instantly placed in deionized water (DW) and re-cutted under treatment solution. Cut roses were treated with Sucrose (SUC), α-aminoisobutyric acid (AIB), and 8-hydroquinoline citrate (8- HQC) under different combinations, as mentioned in Table 1. Freshly cut flowers were put in those as mentioned above postharvest pulsing solutions for 4 hours. Then, they were shifted to the cold room at 5 °C overnight and transferred to room temperature for 48 hours (as shipping to the end customer). Then, the flowers were moved to a vase solution (Long Life SL 1ml. L-1) in a uniformly controlled room environment at 25 °C and 50 ± 5% relative humidity under 12 h light/dark conditions.

2.2. Respiration rate

The respiration rate (mg CO2 $kg^{-1}FW\ h^{-1}$) of the flowers was detected by recording the carbon dioxide concentration on a flame-ionized detector using a gas chromatograph (GC2014, Shimadzu, Japan). The chromatograph was fitted with a stainless-steel column (1.8 m) packed with WG100 KA1144. Helium was used as a carrier gas, and the temperature of the column, injector, and detector was 50 °C.

2.3. Stomatal conductance

The stomatal conductance of flower petals was measured in the above-mentioned uniformly controlled room environment using an AP4 porometer (SC-1; Decagon Devices, USA). Before the measurements were taken, the porometer was stabilized in the room environment. The analysis was executed under the light condition during day time after vase holding. One decorative petal was randomly chosen and put in a small cuvette of sensor head. The stomatal conductance or humidification was noted at 1.5 min after the sensor head was clipped. The data were taken from five randomly harvested cut flower petals for each treatment, and the average of five readings was taken as the stomatal conductance expressed as mmol $H_2O\ m^{-2}\ s^{-1}$.

2.4. Ethylene production rate

The ethylene production rate (μL C2H4 kg−1FW h−1) of cut flowers was measured by enclosing three flowers in an airtight chamber of polyethylene. As carrier gas nitrogen was used. After 2 h of ethylene collection, samples were collected by syringe, and a flame-ionized detector was used to analyse ethylene on a chromatograph for gas (GC8A, Shimadzu, Japan). The temperatures of the column were 220 °C, while for injector and detector were 100 °C.

2.5. Hydraulic Conductance

The hydraulic conductance (Kh) was determined according to Gilman and Steponkus's method (1972). A stem of 20 cm in length comprising the peduncle and leaf bud. Cutting and fitting the stems into silicone tubes were done underwater to prevent air entrance into the vessels. Further, the stem was flushed and degassed with 0.1 mol KCl in micro-filtrated water at a pressure of 0.01 MPa. The same solution was forced through the stems at 0.01 MPa, for 10 min and water was collected in glass tubes. Hydraulic conductance (kg m s–1 MPa–1) was determined by measuring the amount of water passed through the stem segments (water flux) divided by the pressure gradient (Darlington and Dixon, 1991).

Table 1. Postharvest Pulse treatment and their detail.

Treatment	Detail
CON	Distill water
SUC	Sucrose (SUC) 50 gm L^{-1}
SUCA	SUC 50 gm L^{-1}, α-aminoisobutyric acid (AIB) 100 μM
SUCH	SUC 50 gm L^{-1}, 8-hydro-quinoline citrate (8- HQC) 200 mg L^{-1}
AH	AIB 100 μM, 8- HQC 200 mg L^{-1}
SUCAH	SUC 50 gm L^{-1}, AIB 100 μM, 8- HQC 200 mg L^{-1}

2.6. Flower opening (%) and Days to flower bud opening

Flower opening percentage is the ratio of fully opened normal flowers to unopened (or abnormal opened) flowers. Whereas, days to flower bud opening were determined as the number of days from vase holding to the day when the flower fully opened.

2.7. RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted from rose petals (stage 5) using the hot borate method as previously described (Hussain et al., 2024). According to the manufacturer's instructions, cDNA was synthesized from 1 μg total RNA using M-MLV reverse transcriptase (Promega). Quantitative RT-PCR (qRT-PCR) was performed in an Applied Biosystems StepOnePlus TM Real-Time PCR system using a KAPA SYBR FAST Universal qRT-PCR kit (Kapa Biosystems). The RhACT1 was used as an internal control. The primers used for qRT-PCR are listed in Table 2.

Table 2. List of primers used for qRT-PCR analysis.

Gene	Forward primer	Reverse primer
RhACS2	GCGAACAGGGGTACAACTTC	GGGTTTGAGGGGTTGGTAAT
RhACO1	CGTTCTACAACCCAGGCAAT	TTGAGGCCTGCATAGAGCTT
RhSAG12	AGCGGAGAAGCCTTTCAGTC	CAGCATGGTTCAGGCTGGTA
RhAPX1	TTGTGCTCTACTCGTGCCAG	TGACGGTTGGGTAGCACTTC
RhACT1	GTTCCCAGGAATCGCTGATA	ATCCTCCGATCCAAACACTG

2.8. Solution uptake

Solution uptake was recorded on the 5th day from the volume losses of the vase solutions. Another vessel with distilled water was used as a control to check water evaporation. The volume of solution uptake (mL stem−1 day−1) was calculated as the average volume loss of three vessels by subtracting the volume of evaporated water and dividing by the total number of cut flower stems in respective pulsing treatment.

2.9. Anti-Bacterial Activity

The antibacterial activity of the treatments was assessed at the base of the cut stems. On the fourth day of vase holding, total microbial contamination was assessed by employing the swab method. The samples were taken from the stem base (2 cm), and diluted with 10 mL sterile normal saline (0.9% NaCl). Then, 1 mL of the aliquots (diluted sample) was dispensed for colony growth on the agar plate, and incubated at 37°C for 48 h. After incubation, the bacterial count was determined by counting the number of colonies forming units per liter ($CFU.L^{-1}$) on the plate.

2.10. Measurement of Fresh Weight and Flower diameter

At the time of harvesting, we chose uniform-sized flowers concerning length, stem girth, and flower size and randomly divided them for treatment application, so considered the initial FW uniform. The final fresh weight was measured on the 6th day when all flowers were opened and some started senescence symptoms. The flower diameter was determined by measuring the perpendicular and widest diameter of the flower by using a digital vernier caliper.

2.11 Senescence incidence % and vase life

Flowers were assessed daily for visual symptoms such as petal abscission (PA); petal wilting (PW); bluing (BL); and petal desiccation (DP), during the vase life evaluation period. Vase Life (VL) was determined as the number of days from vase holding day to the day when petals showed at least one of the following senescence symptoms; wilting of petals (50% petal turgor loss), petal abscission (3 petal drop), bluing (50% blue petals), bent peduncle (50% necks bent), leaf yellowing (more than 50% of leaf area affected) or flower stem lost 10% of its fresh weight.

2.12. Statistical analysis

The data were mean ±SD from at least five biological replicates for statistical analysis. Statistical analyses were performed using Student's t-test with GraphPad Prism 8 (GraphPad Software Inc. San Diego, CA. USA). Statistically significant differences are indicated with $P < 0.05$ (∗) and $P < 0.01$ (∗∗).

III. RESULTS

Exogenous pulsing alters the physiological behaviours of energy, water, and ethylene

In ornamental, the flower's opening speed is closely associated with their opening quality and ornamental period. After harvest, flower water and food supply disconnected causing robust ethylene production and higher respiration rate which disturbs proper flower opening and diminishes the ornamental period. This study explores the postharvest pulsing effects of sucrose (SUC), α-aminoisobutyric acid (AIB), and 8-hydroquinoline citrate (8-HQC) under different combinations as mentioned in Table 1. We examined the respiration rate and stomatal conductance compared to CON in all pulsing treatments. The application of SUCAH significantly lowers respiration rate and stomatal conductance, followed by AH, SUCH, and SUCA (Fig. 1A, 1B). The AIB application represses ethylene production, as lowest in SUCAH, followed by AH, SUCA, and SUCH (Fig. 1C). In the case of hydraulic conductance of flower stems, we found SUCAH, SUCH, SUCA, and AH showed positive feedback. The postharvest pulsing results showed positive effects of ethylene repressor, carbohydrate, and water absorbent germicide on the physiological changes, which are helpful for potential vase value.

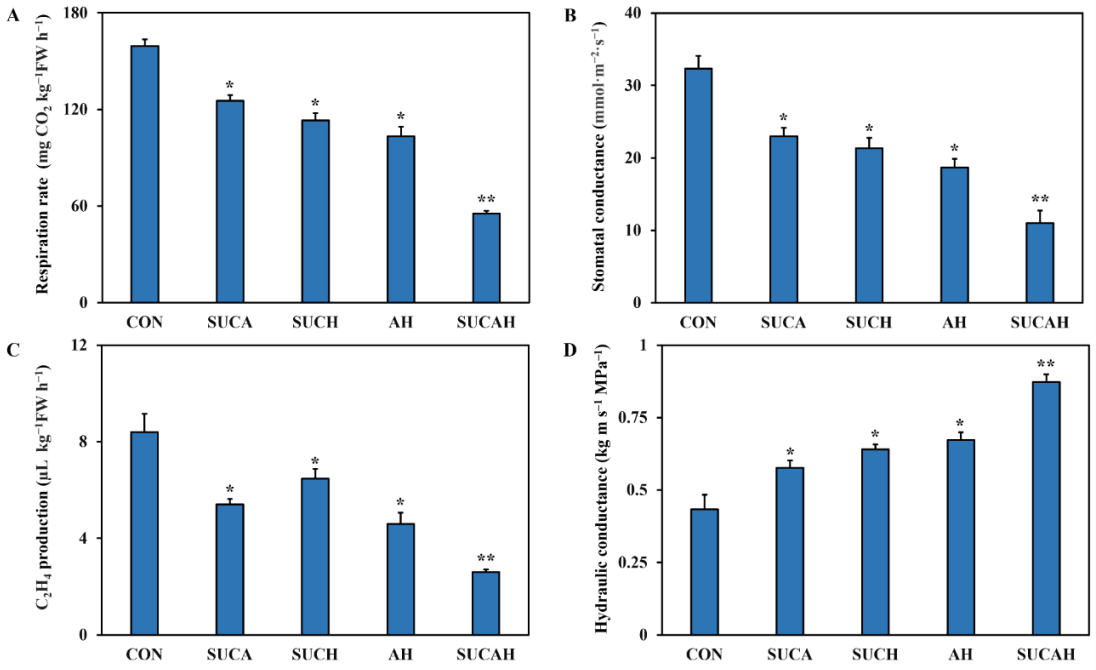

Figure 1. Exogenous pulsing alters the physiological behavior of energy, water, and ethylene in cut rose (A) Respiration rate (B) Stomatal conductance (C) Ethylene production (D) Hydraulic conductance. Results are the means of five biological replicates from different plants with SE. Asterisks indicate statistically significant differences, as determined by Student's t-test (*P < 0.1, **P < 0.01).

Exogenous pulsing controls flower opening

Then, we determined the functional role of postharvest pulsing in the flower opening process and found that flower opening percentages were better in SUCAH, followed by SUCH, AH, and SUCA (Fig. 2A). Further we examined the days to flower opening, and saw that all postharvest pulsing improved flower opening, whereas all three elements SUCAH comprehensively led flower opening promptly and in almost all flowers (Fig. 2B). These results indicated that postharvest pulsing plays an important role in flower opening.

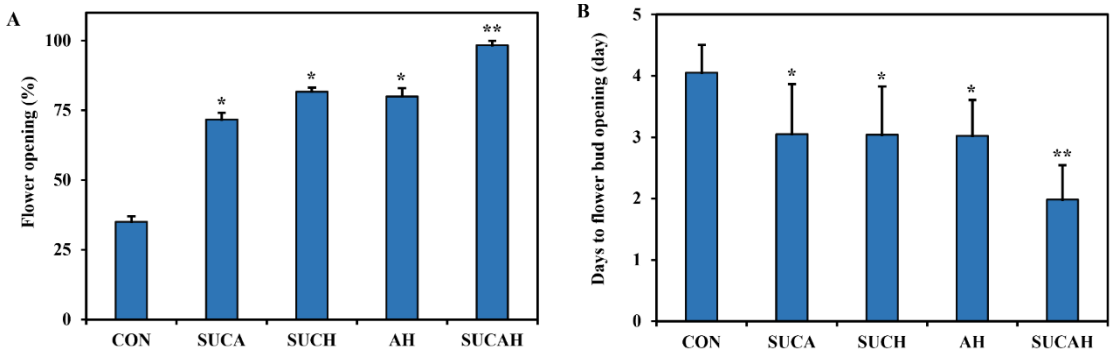

Figure 2. Exogenous pulsing controls flower opening of cut rose (A) Flower opening (%) (B) Days to flower bud opening (day). Results are the means of five biological replicates from different plants with SE. Asterisks indicate statistically significant differences, as determined by Student's t-test (*P < 0.1, **P < 0.01).

Exogenous pulsing regulates ethylene and senescence-associated genes

We saw that pulsing affects ethylene production, to see pulsing effects on ethylene's change at the molecular level we determined the transcription level of ethylene biosynthesis genes, senescence-associated genes SAG12, and ascorbate peroxidase (APX1) at stage 5 (Figure 3). We saw that compared to the control pulsing treatments, significantly lowered the transcription of the ethylene biosynthesis genes RhACS2 and RhACO1 and the senescence-associated genes RhSAG12. The AIB-containing treatments strongly repressed ethylene genes as compared to CON or SUCH. Further, we determined the transcription of an

antioxidant enzyme ascorbate peroxidase transcription, that saves flowers from internal damage. We saw that pusling with SUCAH strongly promoted RhAPX1 transcription/activity followed by AH, SUCA, and SUCH (Figure 3).

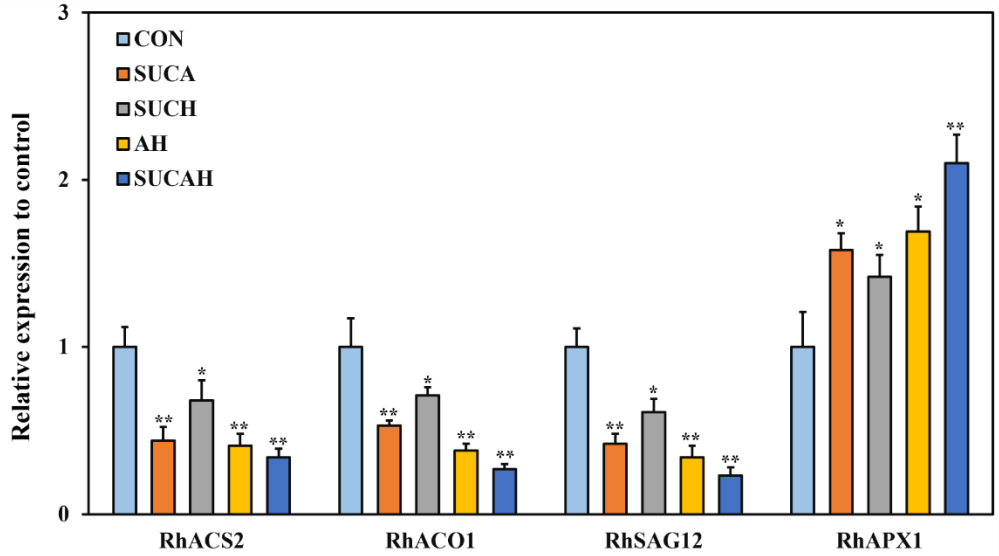

Figure 3. Exogenous pulsing regulates ethylene and senescence-associated genes in cut rose. qRT-PCR analysis of *Rosa hybrida* 1-aminocyclopropane-1-carboxylic acid synthase 2 (RhACS2), 1-aminocyclopropane-1-carboxylic acid oxidase 1 (RhACO1), senescence-associated genes 12 (RhSAG12), and ascorbate peroxidase 1 (RhAPX1) from outermost petals. Results are the means of five biological replicates from different plants with SE. Asterisks indicate statistically significant differences, as determined by Student's t-test (*P < 0.1, **P < 0.01).

Exogenous pulsing promotes water uptake and flower size by inhibiting microbial growth

Then, we measured the solution uptake, microbial growth, fresh weight, and flower diameter (Figure 4). We found that treatments having 8-HQC had more solution uptake (Fig. 4A) and strongly inhibited microbial growth (Fig. 4B). whereas, SUC and AIB applications also promoted solution uptake (Fig. 4A), but could not control microbial growth (Fig. 4B). Furthermore, we examined the flower's fresh weight and diameter. We found that flowers having pulsing treatment showed more fresh weight and longer flower diameter (Fig. 4C, 4D).

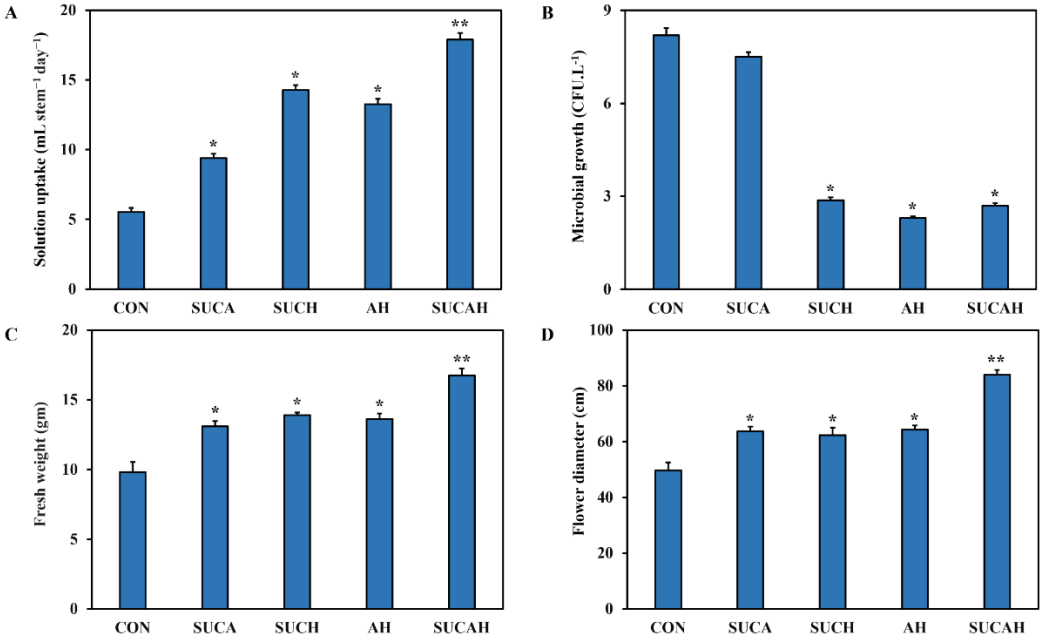

Figure 4. Exogenous pulsing promotes water uptake and flower size by inhibiting microbial growth in cut rose (**A**) solution uptake (**B**) microbial growth (**C**) fresh weight and **(D)** flower diameter. Results are the means of five biological replicates from different plants with SE. Asterisks indicate statistically significant differences, as determined by Student's t-test (*P < 0.1, **P < 0.01).

Exogenous pulsing alters senescence symptoms and vase life

Finally, we study the response of pulsing on senescence symptoms and vase life. We noticed that the control had maximum senescence-associated incidence, higher petal abscission, desiccation of petals, bluing, and wilting (Fig. 5A), and resulted in short vase life (Fig. 5B). whereas the pulsing treatments showed less senescence-associated incidence (Fig. 5A), SUCAH had significant performance and resulted in longer vase life, followed by AH, SUCH, and SUCA (Fig. 5A, 5B).

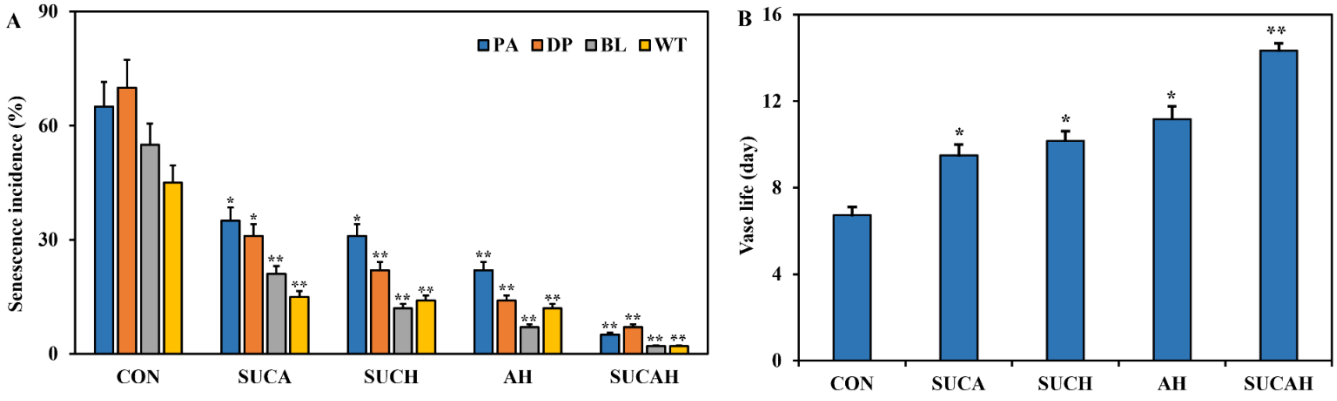

Figure 5. Exogenous pulsing alters senescence symptoms and vase life in cut rose (**A**) petal abscission (PA), desiccation of petals (DP), bluing (BL), and wilting (WT) (**B**) vase life (day). Results are the means of five biological replicates from different plants with SE. Asterisks indicate statistically significant differences, as determined by Student's t-test (*P < 0.1, **P < 0.01).

IV. DISCUSSION

Rose is one of the leading cut flowers whose ornamental value mainly depends on the petals. After harvest, water is disconnected, robust ethylene production and being supplied by long-distance transportation disturb proper flower opening and diminish the ornamental period (Chen et al., 2020). Flower opening is mainly attributable to cell expansion via the actions of phytohormones, water status, and carbohydrates (Horibe and Yamada, 2017; Sun et al., 2021). We examined the respiration rate and stomatal conductance compared to CON in all pulsing treatments. The application of SUCAH significantly lowers respiration rate and stomatal conductance, followed by AH, SUCH, and SUCA (Fig. 1A, 1B). The ethylene production promotes respiration rates (Wills et al., 1998). The AIB application represses ethylene production, as highest in SUCAH, followed by AH, SUCA, and SUCH (Fig. 1C). The α-aminoisobutyric acid (AIB) is an analog of 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene. Exogenous application of AIB repressed ethylene biosynthesis from ACC by repressing ACC oxidase activity (Satoh and Esashi, 1980). orna

In the case of hydraulic conductance of flower stems, we found SUCA, SUCH, AH, and SUCAH showed positive feedback. In rose, ethylene repressed petal cell expansion by directly inhibiting the aquaporin proteins (Ma et al., 2008; Hussain et al., 2024). Carbohydrates are commonly supplemented for an improvement of water status and as a source of energy for respiration by petals (Arrom and Munné-Bosch, 2012). During the petal growth, carbohydrate concentrations increase promptly in the vacuole, which promotes osmotic pressure, which enables water transportation inward to cells and leads to cell expansion (Yamada et al., 2009). The postharvest treatment of carbohydrate, STS with 8- HQC resulted in high hydraulic conductance in cut roses 'Red Calypso' (Bushen and Bekele 2014).

During postharvest, cut flowers generally undergo water deficit stress, resulted in air embolism is a disorder which leads to abnormal opening of flowers (Luo et al., 2013). we determined the functional role of postharvest pulsing in the flower opening process and found that flower opening percentages were better in SUCAH, followed by SUCH, AH, and SUCA (Fig. 2A). These results correlate with Ma et al., 2008 and Xue et al., 2009 that aquaporins PIPs are inhibited by ethylene in rose, which correlates with reduced petal size and results in an abnormal flower opening process (Chen et al., 2023). Further, we examined the time to flower opening, and saw that all postharvest pulsings improve flower opening time, whereas all three elements (SUCAH) comprehensively lead to flower opening promptly and in almost all flowers (Fig. 2B). Carbohydrate enables cell expansion and lead to flower opening (Yamada et al., 2009). The RhSUC2-silenced plants have a smaller flower diameter and reduced petal area, which suggests sucrose helped in proper cell expansion by maintaining turgor pressure (Liang et al., 2020).

In cut roses, mRNA transcripts of RhACS2 and RhACO1 were up-regulated by ethylene and correlated with flowers vase (In et al., 2017). We saw that pulsing affects ethylene production, to see pulsing effects on ethylene's change at the molecular level we determined the transcription level of ethylene biosynthesis genes, RhSAG12 (Figure 3). We saw that compared to the control pulsing treatment significantly lowered the transcription of the ethylene biosynthesis genes RhACS2, RhACO1, and RhSAG12. The AIB-containing treatments strongly repressed ethylene genes as compared to CON or SUCH. The study aligned with Ha et al., 2018 that AIB exogenous application reduced the expression of RhACS2 and RhACO1 and delayed the ethylene-induced process of flower senescence. When cut flowers are exposed to temperature or water stress it causes the production of reactive

oxygen species. The APX is involved in the ascorbate-glutathione cycle and uses ascorbate as the electron donor that scavenges ROS and saves flowers from internal damage. Postharvest treatment of ascorbate can thus be imperative for the quality retention of horticultural crops (Imahori 2014; Bashir et al., 2015). We determined the transcription of RhAPX1 and saw that pulsing with SUCAH strongly promoted RhAPX1 transcription/activity followed by AH, SUCA, and SUCH (Figure 3). The exogenous application of ascorbate saves from water deficiency, protects cell membrane lipids bilayers from damage, and delays senescence (Chen et al., 2021). Another study found that the ascorbate application promotes APX activity and reduces SOD activity. Further, it increased flower diameter and preserved high water potential resulting in longer vase life (Jin et al., 2006).

Then, we measured the solution uptake, microbial growth, fresh weight, and flower diameter (Figure 4). We found that treatments having 8- HQC had more solution uptake (Fig. 4A) and strongly inhibited microbial growth (Fig. 4B). whereas, SUC and AIB applications also promoted solution uptake (Fig. 4A), but could not control microbial growth (Fig. 4B). Furthermore, we examined the flower's fresh weight and diameter. We found that flowers having pulsing treatment showed more fresh weight and longer flower diameter (Fig. 4C, 4D). The postharvest treatment of carbohydrate, STS with 8- HQC resulted in larger flower diameters with high relative contents in cut roses (Bushen and Bekele 2014). In Hydrangea, 8-HQS improved water uptake by inhibiting microbes and delayed relative fresh weight loss (Kazaz et al., 2020).

The vase of cut flowers is governed by ethylene production and water status with carbohydrates supply. Ethylene induces abnormal flower opening, wilting, bluing, leaf yellowing, petal abscission, and senescence (Macnish et al., 2010). We observed that the CON had maximum senescence-associated incidence, higher petal abscission, desiccation of petals, bluing, and wilting (Fig. 5A), and resulted in short vase life (Fig. 5B). Whereas, the pulsing treatments showed less senescence-associated incidence (Fig. 5A), SUCAH had significant performance and resulted in longer vase life, followed by AH, SUCH, and SUCA (Fig. 5A, 5B). In miniature roses, ethylene-induced laccase expressions involved in abscission cause early flower senescence (Ahmadi et al., 2009). Another study demonstrates that ethylene inhibits the aquaporin proteins that cause water shortage and lead to early wilting of leaves and petals, whereas variety with long vase life had higher water status (Nergi and Ahmadi, 2014).

It was found in the cut orchid that ethylene-induced color fading while recovered by 1-MCP. The rapid ethylene production promotes peroxidase activity, which causes anthocyanin degradation and results in color bluing (Khunmuang et al., 2019). In rose, during flower opening at stage 3, sucrose relative contents were high compared with stage 1 and stage 5 contents. The reduced sucrose contents at stages 5 and 6 let the ethylene production, promote petal shedding and reduce the flower vase life (Horibe and Yamada, 2017; Liang et al., 2020), whereas pulsing with sucrose and ethylene inhibitors prolong vase life in Lilium (Krause et al., 2021). Some studies report that AIB application extends the vase life in cut carnation and Lilium (Onozaki et al., 1998; Shimamura et al., 1997). These results are consistent with previous studies that exogenous treatment of ethylene inhibitors combined with sucrose interrupted the senescence process in cut flowers (Nergi and Ahmadi, 2014; Ha et al., 2018). Ethylene production was measured in rose, and found that short-lived varieties reached the ethylene peak earlier than long-lived varieties (Xue et al., 2008). Since AIB inhibited ethylene biosynthesis, it prolonged vase life by delaying flower senescence.

Overall, the results and discussion support and signify the role of carbohydrate, water-absorbent germicide, and ethylene inhibitors each one important in his physiological perspective. Compared to CON, the physiological and vase characteristics significantly improved in SUCAH followed by AH, SUCH, and SUCA maximum.

V. CONCLUSION

In summary, our results advocate that postharvest pulsing induces a positive effect on the respiratory rate, stomatal conductance, and hydraulic conductance of flowers. The water uptake, fresh weight, and flower diameter were higher, and microbial growth was lowest in SUCAH followed by SUCH and AH which led to higher flower opening percentages, and early flower opening compared to CON. Further, ethylene production and biosynthesis genes, and senescence associated gene transcription were lowest in SUCAH followed by AH, SUCA, and SUCH related to CON. Compared to CON, the vase characteristics significantly improved in SUCAH followed by AH, SUCH, and SUCA. Our results signified postharvest pulsing by SUCAH (Sucrose+AIB+8-HQC) affects hydration and ethylene status, leading to proper flower opening and improving vase quality characteristics. This study laid an important foundation for understanding the physiological process of flower opening and postharvest handling of cut flowers. It would help maximize cut flowers' ornamental and economic value.

AUTHORS CONTRIBUTIONS

NH and SA conceptualized the research, NH and HIA conducted experiments, and NH, SM, AS, and SA wrote and revised the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCES

[1] Ahmadi N., Mibus H., & Serek M. (2009). Characterization of ethylene-induced organ abscission in F1 breeding lines of miniature roses (*Rosa hybrida* L.). *Postharvest Biology and Technology*, 52(3), 260-266. https://doi.org/10.1016/j.postharvbio.2008.12.010

[2] Arrom, L., & Munné-Bosch, S. (2012). Sucrose accelerates flower opening and delays senescence through a hormonal effect in cut lily flowers. *Plant Science*, 188, 41-47. https://doi.org/10.1016/j.plantsci.2012.02.012

[3] Bashir, M., Qadri, R. W. K., Khan, I., Asif, M., Jahangir, M. M., Basra, S. M.A., Ashraf, U., & Hussain, N. (2015). Natural plant extracts induced-alterations in growth, physiology and quality of turf grass (*Cynodon dactylon*) under summer stress. *Pakistan Journal of Science*, 67(3), 446-449. https://doi.org/10.57041/pjs.v67i3.618

[4] Bushen E. M., & Abebie B. (2014). Pulsing of Carbohydrates, biocides, and ethylene action inhibitor on vase life of cut rose (*Rosa hybrida* L.) flowers. *Journal of Agricultural Science and Technology*, 2(2), 71-83.

[5] Chaumont, F., & Tyerman, S. D. (2014). Aquaporins: highly regulated channels controlling plant water relations. *Plant Physiology*, 164(4), 1600–1618. https://doi.org/10.1104/pp.113.233791

[6] Chen, C.,Galon, Y.,Ishka, M.R.,Malihi, S.,Shimanovsky, V.,Twito, S.,Rath, A.,Vatamaniuk, O.K., & Miller, G. (2021). ASCORBATE PEROXIDASE6 delays the onset of age-dependent leaf senescence, *Plant Physiology*, 185(2), 441–456. https://doi.org/10.1093/plphys/kiaa031

[7] Chen, C.,Hussain, N.,Wang, Y.,Li, M.,Liu, L.,Qin, M.,Ma, N.,Gao, J., & Sun, X. (2020). An Ethylene-inhibited NF-YC Transcription Factor RhNF-YC9 Regulates Petal Expansion in Rose. *Horticultural Plant Journal*, 6(6), 419-427. https://doi.org/10.1016/j.hpj.2020.11.007

[8] Chen, C., Hussain, N., Ma, Y., Zuo, L., Jiang, Y., Sun, X., & Gao, J. (2023). The ARF2-MYB6 module mediates auxin-regulated petal expansion in rose. *Journal of Experimental Botany*, 74(15), 4489–4502. https://doi.org/10.1093/jxb/erad173

[9] Darlington, A. B., & Dixon, M. A. (1991). The hydraulic architecture of roses (*Rosa hybrida*). *Canadian Journal of Botany*, 69(4), 702-710. https://doi.org/10.1139/b91-095

[10] Gilman, K. F., & Steponkus, P. L. (1972). Vascular blockage in cut roses. *Journal of the American Society for Horticultural Science*, 97(5), 662–667. https://doi.org/10.21273/JASHS.97.5.662

[11] Ha, S. T. T., & In, B. C. (2022). Combined Nano Silver, α-Aminoisobutyric Acid, and 1-Methylcyclopropene Treatment Delays the Senescence of Cut Roses with Different Ethylene Sensitivities. *Horticulturae*, 8(6), 482. https://doi.org/10.3390/horticulturae8060482

[12] Ha, S. T. T., Lim, J. H., & In, B. C. (2018). Extension of the vase life of cut roses by both improving water relations and repressing ethylene responses. *Horticultural Science and Technology*, 37(1), 65-77. https://doi.org/10.12972/kjhst.20190007

[13] Horibe, T., & Yamada, K. (2017). Petal Growth Physiology of Cut Rose Flowers: Progress and Future Prospects. *Journal of Horticultural Research*, 25(1), 5-18. https://doi.org/10.1515/johr-2017-0001

[14] Hussain, N., Shahid, A., Jia, Y., Nabi, H. G., & Ahmad, M. (2024). The RhMYB24/bHLH71 complex coordinates flower opening by mediating auxin and ethylene crosstalk in *Rosa hybrida*. *Advances in Agriculture, Horticulture and Entomology*, 2024(4), AAHE-213. doi:10.37722/AAHAE.2024401

[15] Imahori Y. (2014). Role of Ascorbate Peroxidase in Postharvest Treatments of Horticultural Crops. *Oxidative Damage to Plants*, 4, 25-45. https://doi.org/10.1016/B978-0-12-799963-0.00014-9

[16] In, B. C., Ha, S. T. T., Lee, Y. S., & Lim, J. H. (2017). Relationships between the longevity, water relations, ethylene sensitivity, and gene expression of cut roses. *Postharvest Biology and Technology*, 131, 74–83. https://doi.org/10.1016/j.postharvbio.2017.05.003

[17] Jin, J., Shan, N., Ma, N., Bai, J., Gao, J. (2006). Regulation of ascorbate peroxidase at the transcript level is involved in tolerance to postharvest water deficit stress in the cut rose (*Rosa hybrida* L.) cv. Samantha, *Postharvest Biology and Technology*, 40(3), 236-243. https://doi.org/10.1016/j.postharvbio.2006.01.014

[18] Kazaz, S., Kiliç, T., Doğan, E., & Sekmen, Ş. (2020). Vase life extension of cut hydrangea (*Hydrangea macrophylla*) flowers. *The Journal of Horticultural Science and Biotechnology*, 95, 325–330. https://doi.org/10.1080/14620316.2019.1676660

[19] Khunmuang, S., Kanlayanarat, S., Wongs-Aree, C., Meir, S., Philosoph-Hadas, S., Oren-Shamir, M., Ovadia, R., & Buanong, M. (2019). Ethylene Induces a Rapid Degradation of Petal Anthocyanins in Cut Vanda 'Sansai Blue' Orchid Flowers. *Frontiers in Plant Science*, v.10, 1004, 2019. https://doi.org/10.3389/fpls.2019.01004

[20] Krause, M. R., Santos, M. N. S., Moreira, K. F., Tolentino, M. M., & Mapeli, A. M. (2021). Extension of the vase life of *Lilium pumilum* cut flowers by pulsing solution containing sucrose, citric acid and silver thiosulfate. *Ornamental Horticulture*, 27(3), 344-350. https://doi.org/10.1590/2447-536X.v27i3.2330

[21] Liang, Y., Jiang, C., Liu, Y., Gao, Y., Lu, J., Aiwaili, P., Fei, Z., Jiang, C. Z., Hong, B., Ma, C., & Gao, J. (2020). Auxin Regulates Sucrose Transport to Repress Petal Abscission in Rose (*Rosa hybrida*). *The Plant Cell*, 32(11), 3485–3499. https://doi.org/10.1105/tpc.19.00695

[22] Luo, J., Ma, N., Pei, H., Chen, J., Li, J., & Gao, J. (2013). A DELLA gene, RhGAI1, is a direct target of EIN3 and mediates ethylene-regulated rose petal cell expansion via repressing the expression of RhCesA2. *Journal of Experimental Botany*, 64(16), 5075–5084. https://doi.org/10.1093/jxb/ert296

[23] Ma, N., Xue, J., Li, Y., Liu, X., Dai, F., Jia, W., Luo, Y., & Gao, J. (2008). Rh-PIP2;1, a rose aquaporin gene, is involved in ethylene-regulated petal expansion. *Plant Physiology*, 148(2), 894–907. https://doi.org/10.1104/pp.108.120154

[24] Macnish, A. J., Leonard, R.T., Borda, A.M., & Nell, T.A. (2010). Genotypic variation in the postharvest performance and ethylene sensitivity of cut rose flowers. *HortScience*, 45(5), 790-796. https://doi.org/10.21273/HORTSCI.45.5.790

[25] Nergi, M. A. D., & Ahmadi, N. (2014). Effects of 1-MCP and ethylene on postharvest quality and expression of senescence-associated genes in cut rose cv. Sparkle. *Scientia Horticulturae*, 166, 78-83. https://doi.org/10.1016/j.scienta.2013.12.015

[26] Onozaki, T., Ikeda, H., & Yamaguchi, T. (1997). Effect of calcium nitrate addition to α-aminoisobutyric acid (AIB) on the prolongation of the vase life of cut carnation flowers. *Journal of the Japanese Society for Horticultural Science*, 67(2), 198–203. https://doi.org/10.2503/jjshs.67.198

[27] Satoh, S., & Esashi, Y. (1980). α-Aminoisobutyric acid: A probable competitive inhibitor of conversion of 1-aminocyclopropane-1-carboxylic acid to ethylene. Plant and Cell Physiology. 21(6), 939–949. https://doi.org/10.1093/oxfordjournals.pcp.a076082

[28] Shimamura, M., Ito, A., Suto, K., Okabayashi, H., & Ichimura, K. (1997). Effects of α-aminoisobutyric acid and sucrose on the vase life of hybrid *Limonium*. *Postharvest Biology and Technology*, 12(3), 247-253. https://doi.org/10.1016/S0925-5214(97)00062-8

[29] Sun, X., Qin, M., Yu, Q., Huang, Z., Xiao, Y., Li, Y., Ma, N., & Gao, J. (2021). Molecular understanding of postharvest flower opening and senescence. *Molecular horticulture*, 7(2021). https://doi.org/10.1186/s43897-021-00015-8

[30] Van Ieperen, W., VanMeeteren, U., & VanGelder, H. (2000). Fluid ionic composition influences hydraulic conductance of xylem conduits. *Journal Of Experimental Botany*, 51(345), 769–776. https://doi.org/10.1093/jexbot/51.345.769

[31] Xue, J., Yang, F., & Gao, J. (2009). Isolation of Rh-TIP1;1, an aquaporin gene and its expression in rose flowers in response to ethylene and water deficit. *Postharvest Biology and Technology*, 51(3), 407–413. https://doi.org/10.1016/j.postharvbio.2008.08.011

[32] Xue, J., Li, Y., Tan, H., Yang, F., Ma, N., & Gao, J. (2008). Expression of ethylene biosynthetic and receptor genes in rose floral tissues during ethylene-enhanced flower opening. *Journal Of Experimental Botany*, 59(8), 2161–2169. https://doi.org/10.1093/jxb/ern078

[33] Yamada, K., Norikoshi, R., Suzuki, K., Imanishi, H., & Ichimura, K. (2009). Determination of subcellular concentrations of soluble carbohydrates in rose petals during opening by nonaqueous fractionation method combined with infiltration-centrifugation method. *Planta*, 230(6), 1115–1127. https://doi.org/10.1007/s00425-009-1011-6

Correspondence Author – Nisar Hussain