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Flow Cytometric Analysis of Programmed Cell Death in Rose (*Rosa hybrida* cv. Dolce vita+) as Influenced by Physicochemical Treatments

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ABSTRACT

This study aimed to increase the vase life of cut rose flowers by improving the regulation of Programmed Cell Death (PCD). Experiments were carried out on cut rose (Rosa hybrida cv. Dolce vita+) flowers under either physical treatment of Static Magnetic Field (SMF: 15 and 25 mT) for 3 hours, or chemical treatments of silver nano particle (Nano-Ag: 5 and 10 ppm), 6-Benzyladenine (BA: 25 and 50 mg L^{-1}), 1% sucrose, and combinations of 5 and 10 ppm nano-Ag with 3 and 6% sucrose. Results showed that a 15 mT−SMF significantly increased vase life up to 25 days, compared to the controls and to all chemical treatments. Among the chemicals, 5 ppm Nano-Ag and 1% (w/v) sucrose increased vase life to 23 and 18 days, respectively. The smallest decline in fresh weight was observed in the 15 mT-SMF physical treatment. Markedly, the 15 mT-SMF treatment led to the least reduction in Chlorophyll (Chl) content . On the 17th day of the applied different treatments, both Water Uptake (WU) and Relative Fresh Weight (RFW) showed an inverse significant relationship with PCD in cut rose flowers, verifying the delayed PCD, which is favored in the market. As a whole, the most effective induced treatments (15 mT-SMF, 5 ppm nano-Ag, and 1% sucrose) are suggested to be promising for enhancing postharvest quality and prolonged vase life of cut rose flowers.

Keywords: Silver nanoparticle (Nano-Ag), 6-Benzyladenine (BA), Static Magnetic Field (SMF), Vase life.

INTRODUCTION

Rosa hybrida is a flowering plant of the *Rosa* genus. This genus is found in temperate regions of the northern hemisphere, including North America, Europe, Asia, and the Middle East. The largest variety of species is found in western China (Phillips and Rix, 1988). It is globally considered as one of the most significant ornamental plants, and its flowers are commercially sold as potted plants or cut flowers (Ross, 1991; Liao *et al*., 2000). Despite the significance of roses in the cosmetics industry as a provider of aromatic oils, volatile compounds (Ryu *et al*., 2020), and their medicinal benefits (Choi and Hwang, 2003; Yang *et al*., 2013), cut roses have a limited life span in vases (Lee *et al*., 2016).

Vase life can be affected by post-harvest factors such as temperature, humidity, water relations and conditioning (Gupta and Dubey, 2018). In addition to issues related to improper harvesting, handling, and storage of roses, harvested fresh-cut flowers have a

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short vase life due to limited water uptake, loss of water after cutting, low energy source, and susceptibility to ethylene (Fanourakis *et al*., 2013; Scariot *et al*., 2014; Khunmuang *et al*., 2019; Gun *et al*., 2023). Therefore, it is crucial to maintain the freshness and the quality of the flowers from harvesting until they reach the consumers. Although the vase life of cut flowers depends on the flower's type, conditions of the variety, and its growth, it can be widely influenced by postharvest treatment (Çelikel *et al*., 2011; Ramezanizadeh *et al*., 2012; Hosseinzadeh *et al*., 2014). Inadequate water uptake can be enhanced with proper wetting agent or surfactants (such as triton X-100, tween 20; Aros *et al*., 2016; El-Shoura and Arafa, 2017) while xylem blockage can be prevented with silver nano particles (Shuqin *et al*., 2019).

Applications of exogenous plant growth regulators are known to influence postharvest quality (Janowska and Andrzejak, 2023). In the natural environment, living things are exposed to abiotic stress induced by magnetic fields (MFs) due to the distribution of varied types of instruments and equipment, and SMF is an important environmental factor that can influence the growth and development of plants (Bhatnagar and Deb, 1977; De Souza *et al*., 2005, 2006). In *Allium cepa*, mitotic activity was increased under SMF at 0.06T (Hozayn *et al*., 2015). In a study on carnation cut flowers, it was stated that an Electromagnetic Field (EMF) with a flux density of 160 mT had a profound impact on prolonging the vase life of its cut bloom (Ayesha *et al*., 2023). However, the impact of non-ionizing radiation, such as the EMF, on the quality of cut flowers is still unknown. We did not find any noteworthy investigations about the effect of SMF on the vase life of roses. The life of a flower typically ends in senescence, culminating in a form of PCD (Rogers, 2013). In fact, PCD is a genetically regulated process of cell suicide that is central to the development, homeostasis, and integrity of multi cellular organisms (Ameisen, 2002). In plants, PCD is involved in a variety of situations, including responses to environmental stresses, the hypersensitive response to pathogen attack, plant senescence and fruit ripening (Pennell and Lamb, 1997; O'Brien *et al*., 1998).

Various methods have been employed for the detection of plants' PCD, one of those is FCM, which is utilized in numerous studies. This method is convenient, fast, and reliable (Doležel *et al*., 2007; Abedi *et al*., 2015; Tavan *et al*., 2015; Javadian *et al*., 2017; Sayadi *et al*., 2022; Mehravi *et al*., 2022; Rasekh and Karimzadeh, 2023; Khakshour *et al*., 2024). During cell death, the capability of the cell to scatter light alters as a result of morphological changes such as cell shrinkage, chromatin condensation, and nucleosomal fragmentation (Givan, 1992; Doležel *et al*., 2007). So, this event can be detected by FCM methods.

The current study was aimed to identify the most effective physicochemical treatments to reduce PCD, with the goal of increasing the vase life of cut rose flowers.

MATERIALS AND METHODS

Plant Material and Experimental Treatments

Fresh cut flowers of rose (*Rosa hybrida* cv. Dolce vita^{+}) were obtained from a local commercial greenhouse in Tehran, Iran. In tight bud stage, flowers were cut from the plants between 9:00 and 12:00 AM and re-cut to 50 cm in length. Detached flowers were immediately transported to the laboratory and placed in distilled water. All experiments were performed in a controlled environmental growth room (20±1ºC, 80± 10% RH, 12 hours photoperiod). The cut flowers were kept in a 1,000 mL-vessel containing 500 mL solution in 11 treatments (without control): T0= distilled water (control), T1= Nano-Ag (5 ppm), T2 = ano-Ag, (10 ppm), T3= BA (25 mg L^{-1}), T4= BA (50 mg L^{-1}), T5= Nano-Ag (5 ppm)×sucrose (3%), T6= Nano-Ag (5 ppm)×sucrose (6%),

T7= Nano-Ag (10 ppm)×sucrose (3%), T8= Nano-Ag (10 ppm)×sucrose (6%), T9= Static Magnetic Field (SMF: 15 mT), T10= SMF (25 mT) , and T11= Sucrose (1%, w/v). To exert different intensities of SMF, a magnetic field generator device consisting of two strong magnets (in repelling mode with the ability to adjust the distance) was used. The strength of the magnetic field was measured, using Teslameter (Leybold-Heraeus 51652, Germany). The cut flowers were placed between the different strength of magnet poles. It should also be noted that all methods were performed in accordance with relevant guidelines and regulations."

Measurement of Chlorophyll (Chl)

To determinate of leaf Chl content, leaf blades were sampled on days 1, 5, 10, and 17 during the vase life period. Chl content was evaluated according to Lichtenthaler (1987) by extracting in 80% (v/v) ethanol for 10 minutes at 75°C, with the process repeated until all pigments were extracted from the samples. Absorption was measured, using a UV/V Spectrophotometer (Scinco, UV S-2100, USA) at wavelengths of 700, 664, and 647 nm. Chl concentration was then calculated, using the following equation:

Chl a+b= 5.24 ($A_{664} - A_{700}$)+22.24 (A_{647}–A_{700})

Where, A_{700}, A_{664} and A_{647} were absorbances at the three wavelengths.

Measurement of Water Uptake (WU) and Relative Fresh Weight (RFW)

The weights of vases with and without cut stems were measured on day 0 and continued daily (on days 3, 5, 7, 10, 12, 14, 17, 20) during the vase life period. WU and RFW were then calculated, using the following formulae:

WU (g g^{-1} initial fresh weight-FW) = B_{n-1}–B_n/Initial FW ($A_0 - B_0$)

RFW (%)= $[(A_n–B_n)/(A_0–B_0)]\times100$

Where, A is used to denote the weight of the vase containing the cut stem, including the vase, solution, and stem (g). Meanwhile, B represents the weight of the vase without the cut stem, comprising the vase and solution only (g). B_{n-1} denotes the weight from the previous day (g), while A0 and B0 indicate the weights measured on day 0 (g). A_n and B_n represent the weights measured on day n, with n ranging from 1 onwards (Çelikel *et al*., 2011).

Flow Cytometric Analysis for PCD Measurements

Flow cytometric analysis was performed using a Partec PAS flow cytometer (PAS, Expandable by many light sources, Münster, Germany) on days 10, 18, and 25 of the vase life periods. On the 10^{th} day of the experimental protocol, the PCD% was determined in flowers treated with T3, T4, T5, T6, T7, and T8 treatments, which showed more effects on wilting compared to the control flowers (T0). Control flowers started wilting on day 18, when the PCD% was simultaneously measured in the treated flowers. On the 25^{th} day, the control flowers were completely wilted, when the PCD% was measured in flowers treated with T1, T2, T9, T10, and T11, which showed early symptoms of wilting. Samples were prepared according to Partec protocol by Cystain PI absolute Code No. 05-5022, Germany (Anonymous, 2014). Thirty mg of fresh uppermost leaf tissue was chopped without veins, using a sharp razor blade in a glass petri dish, containing 0.5 mL extraction buffer and 0.25 mL PVP. Fresh leaf tissue of an internal reference standard (Parsley, *Petroselinum crispum*, 2C DNA= 4.45 pg) was simultaneously chopped in a glass petri dish. After 60 seconds of incubation in extraction buffer, the isolated nuclei were filtered through a Partec (Partec, Münster, Germany) 30 µm green nylon mesh to remove cell debris. The nuclear suspension of each sample was then treated with 50 µg mL^{-1} RNase (Sigma-Aldrich Corporation, MO, USA) to prevent staining of double-stranded RNA, followed by staining with 50 µg mL^{-1} Propidium Iodide

(PI, Fluka). The relative fluorescence intensity of stained nuclei was measured on a linear scale, and typically, at least 5000 nuclei were analyzed per sample. According to previous studies (Darzynkiewicz *et al*., 1992; Dive *et al*., 1992; Weir, 2001; Riccardi and Nicoletti, 2006) cycling cells can be distinguished from dead cells with FCM, using fluorescent dye PI (for DNA staining) with PVP (1% w/v) in cell suspension. In the present experiments, PCD percentage was calculated, using the following equation:

PCD (%)= [Count (PCD)/Count (PCD+G1]×100

Statistical Analysis

The experiments were arranged as a Randomized Complete Block Design (RCBD) in three replicates. The data were analyzed, using ANOVA based on RCBD. The data underwent a normality test, using SAS (SAS Institute Inc, 2009). Mean comparisons were carried out, using Duncan's multiple range test in SPSS (v19.0; IBM SPSS Statistics, Chicago, IL, USA) statistical software. ANOVA, correlation, and polynomial regression analyses were also performed, using Minitab (Minitab® ver. 16.1.0, Minitab Ltd.) software. Gating region range was defined on FCM histograms, using Partec FloMax ver.2.4e (Partec, Münster, Germany) software.

RESULTS

To increase the vase life of the cut rose (*Rosa hybrida* cv. Dolce vita^{+}) flowers by assessing the PCD, 12 treatments including control, nine chemical, and two physical treatments were examined. On the 10th day, flowers treated by T3, T4, T5, T6, T7, and T8 wilted earlier than the control flowers (T0). Hence, these six treatments appeared to be ineffective. On the other hand, on the 25th day, flowers treated with other five treatments of T1, T2, T9, T10, and T11 showed early wilting symptoms, while the control flowers were completely wilted at this time. Thus, on the basis of flow cytometric analysis of PCD and of WU and RFW, the latter treatments performed to be the effective treatments, which will be discussed in more detail.

Flow Cytometric Analysis of PCD

The results of ANOVA on PCD% showed significant differences among treatments on days 10, 18 and 25 ($P< 0.01$; Table 1). On the 10th and 18th days, flowers treated with in-effective treatments (T3, T4, T5, T6, T7, T8) unexpectedly showed more remarkable PCD% (Figure 1) compared to the control flowers (T0), and resulted in more wilting. On the other hand, flowers treated with five effective treatments (T1, T2, T9, T10, T11) showed significantly ($P< 0.01$, Table 1) less PCD% compared to the controls on both experimental days of 18, and 25 (Figures 2, and 3). The cut rose flowers exposed to SMF-15 mT (T9) started wilting on day 25, reaching completely wilting after three days (day 28). Non-treated flowers (control) were wilted on day 18, but flowers treated by T9 remained alive and did not wilt. T9 treatment caused the least PCD% at all sampling times among all exposed chemical and physical treatments. The PCD% of flowers treated by T9 on days 18 and 25 were estimated as 22.64% and 23.19%, respectively (Figure 3). The flowers treated with 1% sucrose (T11) began wilting on day 18, showing slow senescence, and followed no clear changes during a week after (day 25, Figures 2, and 3). In fact, the cut flowers treated with T11 (1% sucrose) were more

Table 1. Mean Squares (MS) of the ANOVA for PCD% cut rose flowers sampled on days 10, 18, and 25. [a]

SOV	Df	MS	CV%
Day 10	6	2.910**	14.1
Day 18	11	1032.800**	2.6
Day 25	5	0.047**	2.4

[a] SOV: Source Of Variations, Df; Degree of freedom. **Significant difference at 1% probability level.

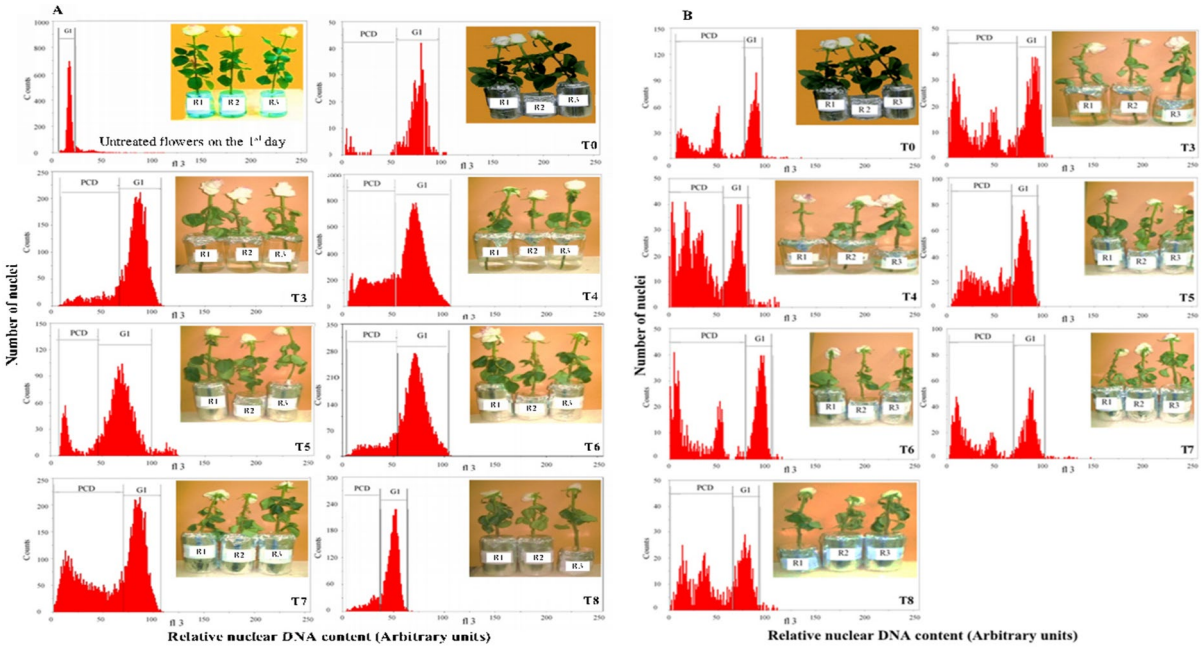

Figure 1. A: Control [T0, distilled water] and treated cut rose flowers with ineffective treatments, including: [BA (25 mg l^{-1})], T4 [BA (50 mg l^{-1})], T5 [Nano-Ag (5 ppm)×Sucrose (3%)], T6 [Nano-Ag (5 ppm)×Sucrose (6%)], T7 [Nano-Ag (10 ppm)×Sucrose (3%)], and T8 [Nano-Ag (10 ppm)×Sucrose (6%)] and related FCM histograms of PCD% on day 10 of harvesting time. **B:** Control [T0, distilled water] and treated cut rose flowers with ineffective treatments, including T3 [BA (25 mg l^{-1})], T4 [BA (50 mg l^{-1})], T5 [Nano-Ag (5 ppm)×Sucrose (3%)], T6 [Nano-Ag (5 ppm)×Sucrose (6%)], T7 [Nano-Ag (10 ppm)×Sucrose (3%)], and T8 [Nano-Ag (10 ppm)×Sucrose (6%)] and related FCM histograms of PCD% on day 18 of harvesting time.

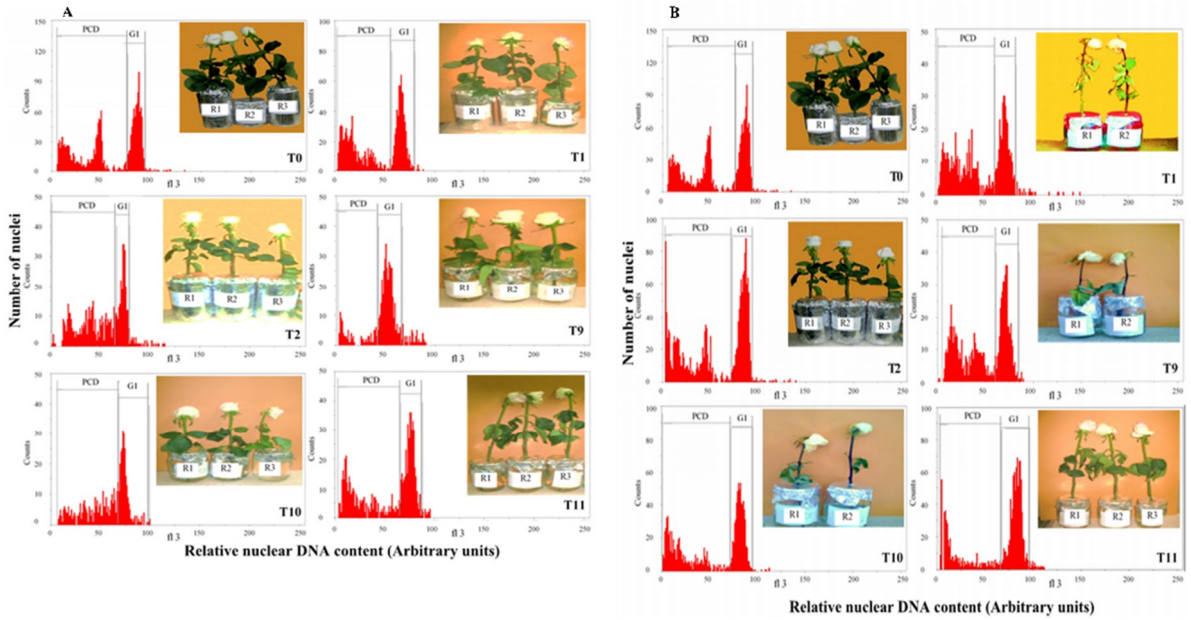

Figure 2. A: Control [T0, distilled water] and treated cut rose flowers with ineffective treatments, including: T1 [Nano-Ag (5 ppm)], T2 [Nano-Ag (10 ppm)], T9 [Static Magnetic Field (SMF; 15 mT)], T10 [SMF; 25 mT], T11 [Sucrose (1%, w/v)] and related FCM histograms of PCD% on day 18 of harvesting time. **B:** Control [T0, distilled water] and treated cut rose flowers with ineffective treatments including T1 [Nano-Ag (5 ppm)], T2 [Nano-Ag (10 ppm)], T9 [Static Magnetic Field (SMF; 15 mT)], T10 [SMF; 25 mT], T11 [Sucrose (1%, w/v)] and related FCM histograms of PCD% on day 25 of harvesting time.

rejuvenated compared to the control on day 18; roses were withering on the 25th day (Figure 2). On the other hand, based on data achieved from FCM analysis of PCD% for cut rose flowers treated with 1% sucrose, the PCD% on days 18 and 25 were estimated as 31.12% and 31.85%, respectively.

Morphological Traits

Three morphological traits including relative RFW, WU, and Chl content were studied in the current study. The result of ANOVA showed significant differences ($P< 0.01$; Table 2) between treatments for RFW and WU and between sampling times for all three traits. The changes of RFW during days 1-21 for effective treatments (T1, T2, T9, T10, T11) are shown in Figure 4. These treatments had a positive effect on increasing the vase life of roses and delaying the PCD. Since the 14th day, the flowers treated by effective treatments showed a slower rate of RFW loss compared to the control (Figure 4). Among effective treatments, T9, T10, and T11 treatments showed lower levels of RFW loss. T-test results showed no significant difference between these three treatments.

Figure 5 indicates the relative changes of WU during days 1-21 for the effective treatments (T1, T2, T9, T10, T11) on postharvest life. Both untreated control and treated flowers with T1, T2, T9, and T11 showed a declining trend in WU until the14th day, but since that time, the treated flowers absorbed more water compared to the controls (Figure 5). The *t*-test results between two physical induction treatment (T9 and T10) and also between two chemical treatments (T1 and T2) at a significance level of $P< 0.05$ showed that T9 and T2 treatments had higher water uptake

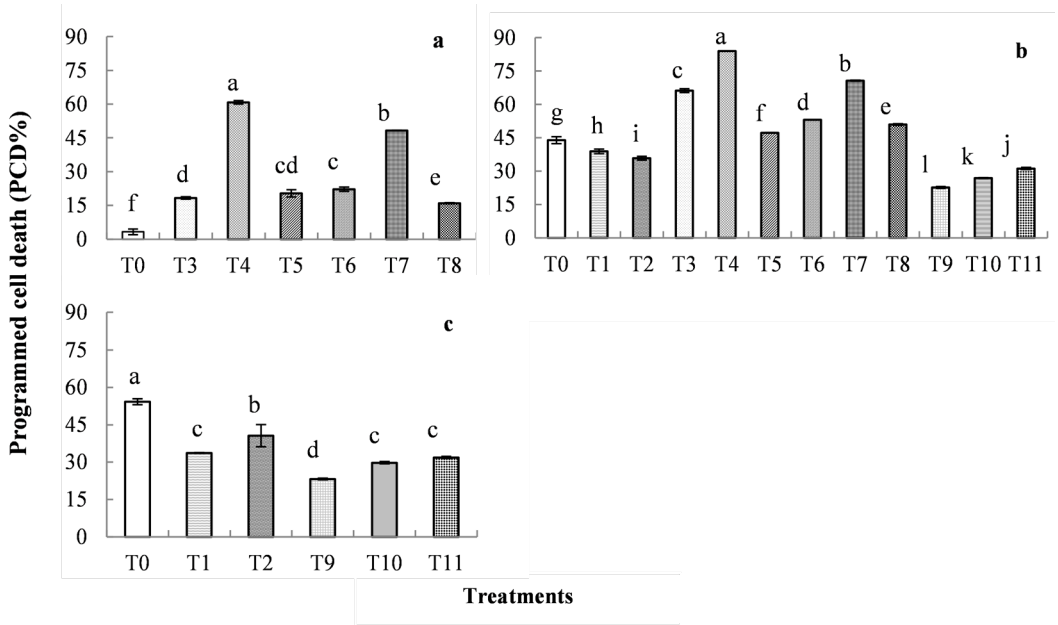

Figure 3. Programmed Cell Death (PCD%) of cut rose treated and control flowers on 10th day (a), 18th day (b), and 25th day (c). T0= Distilled water (control), T1= Nano-Ag (5 ppm), T2= Nano-Ag (10 ppm), T3= BA (25 mg l^{-1}), T4= BA (50 mg l^{-1}), T5= Nano-Ag (5 ppm)×Sucrose (3%), T6= Nano-Ag (5 ppm)×Sucrose (6%), T7 = Nano-Ag (10 ppm)×Sucrose (3%), T8= Nano-Ag (10 ppm)×Sucrose (6%), T9= Static Magnetic Field (SMF; 15 mT), T10= SMF; 25 mT, and T11= Sucrose (1%, w/v). Means with the same letter are not significantly different from each other ($P> 0.05$).

Table 2. Mean Squares (MS) of the ANOVA for Relative Fresh Weight (RFW), Water Uptake (WU) and Chlorophyll (Chl) treated cut rose flowers in different sampling times

SOV	Df	MS		Df	MS
		RFW	WU		Chl
Blocks	2	4.83**	1.9*	2	3.45**
Treatments (T)	11	1.66**	7.6**	11	0.56
Sampling Times (ST)	7	15.70**	9.5**	3	13.20**
T×ST	77	0.16	0.7*	33	0.92
Error	190	0.33	0.5	94	0.61

*, and ** Significant differences at 5 and 1% probability levels, respectively.

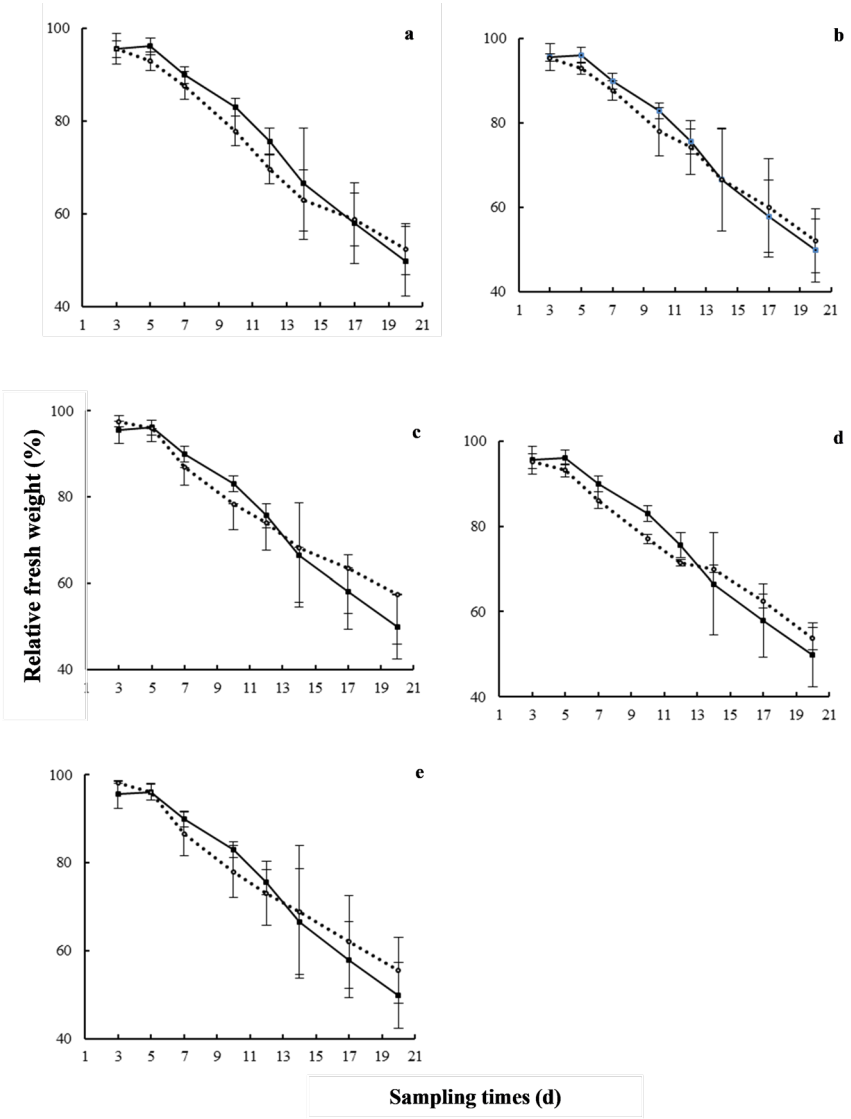

Figure 4. Changes of mean Relative Fresh Weight (RFW) of cut rose flowers treated with T1: (Nano-Ag (5 ppm), (a) T2: (Nano-Ag (10 ppm), (b), T9: (c), T10 (d), and T11: (e; solid lines) and the controls (dotted lines). Values are means±SE.

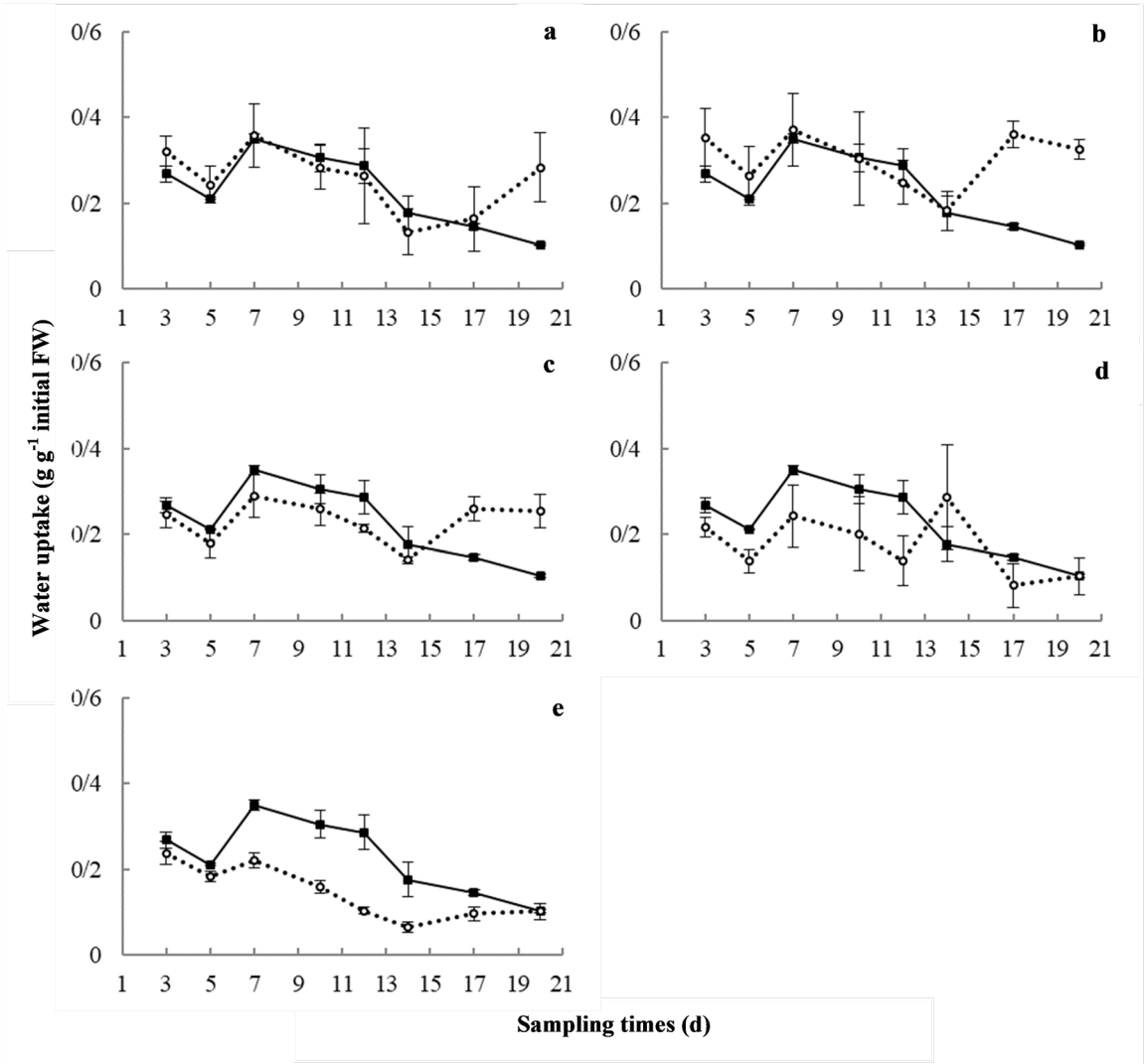

Figure 5. Changes of mean Water Uptake (g g^{-1} initial FW) of cut rose flowers treated with T1 (a), T2 (b), T9 (c), T10 (d), and T11 (e; dotted lines) and the controls (solid lines). Values are means±SE, but where bars are absent, the variation about the mean was less than the diameter of the symbol.

compared to T10 and T1, respectively. BA treatments (T3, T4) as well as combined treatments (T5, T6, T7, T8) displayed a downward trend in the amount of water absorption until the 14th day. After this day, the amount of water absorption increased. T6, T7, and T8 treatments showed less reduction in WU compared to other ineffective treatments. The changes of Chl content during days 1-18 for effective treatments (T1, T2, T9, T10, T11) are shown in Figure 6. Chl content had increased until day 4 in all treatments. T9 showed the least reduction in Chl amount.

Relationship between Morphological Traits and PCD%

The data of RFW, WU, and Chl were correlated with PCD%. Where significant correlations were identified, they were regressed upon PCD% on the 10th and 17th days of the experimental protocol. All morphological characteristics, except Chl, showed a remarkable relationship with PCD%. Hence, polynomial regression analysis between the PCD% and RFW of

rose cut flowers on the 10th day showed a significant linear regression (P< 0.01, Table 3, Figure 7-a). No significant correlation was identified between the PCD% and WU on the 10th day. On the 18th day, the PCD% had a significant correlation with RFW and WU (P< 0.01, Tables 3 and 4, Figure 7). There was no significant correlation among these traits on other days. The highly significant inverse linear relationship was identified between PCD% and RFW on day 10 (Figure 6-a). Significance inverse linear relationship was detected between PCD% and either RFW (Figure 7-b) or WU (Figure 7-c) on day 18.

Based on the study results, the treatments that had been more effective in increasing the vase life of flowers were Nano-Ag 10 ppm (T2), Static Magnetic Field 15 mT (T9), and Sucrose 1% (w/v) (T11). Nano-Ag treatments resulted in reduced wilting symptoms and delayed wilting, leading to increased vase life. Conversely, BA and Nano-Ag×sucrose treatments appeared ineffective, causing increased wilting symptoms and reduced vase life. In

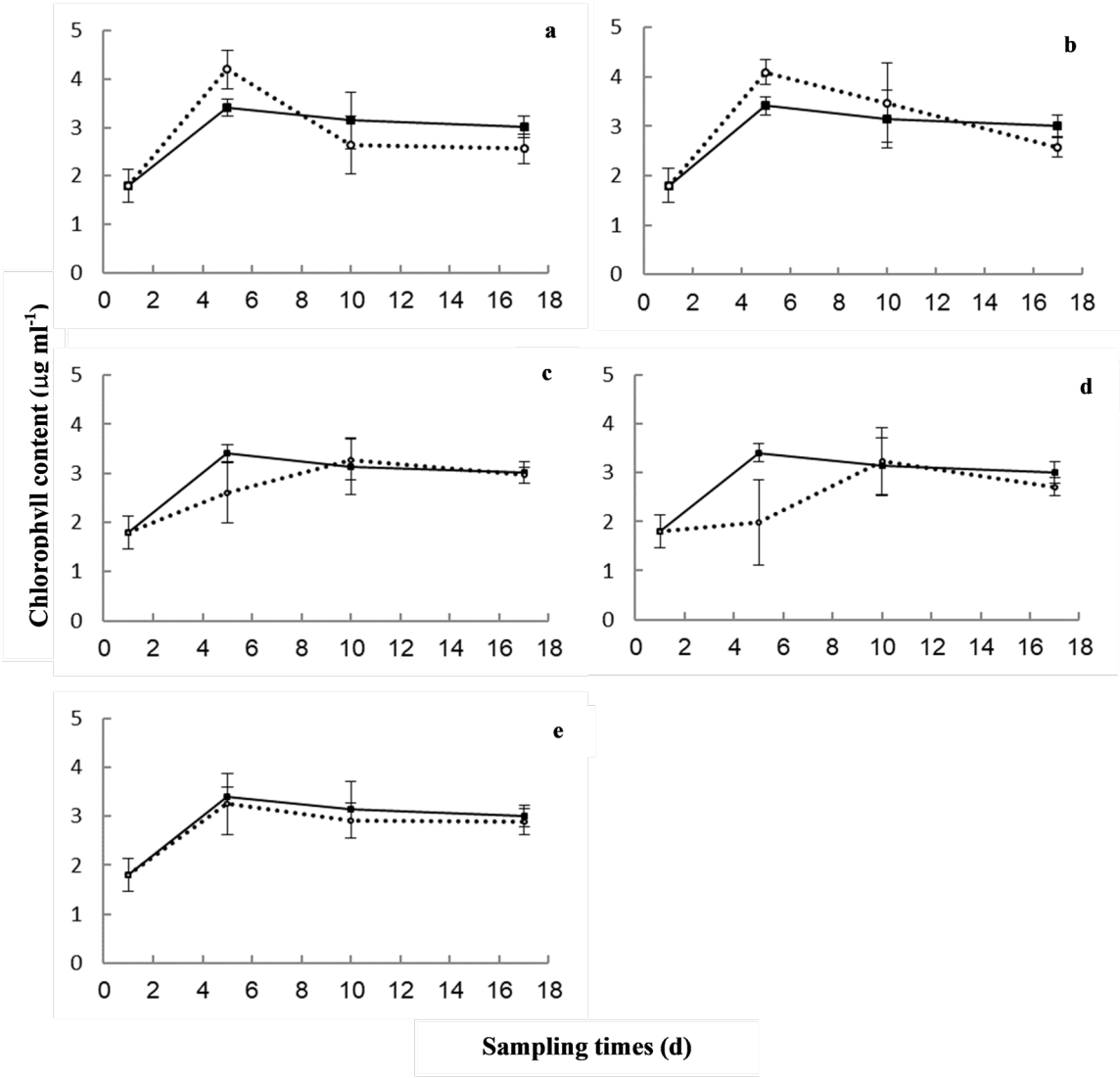

Figure 6. Changes of mean chlorophyll content (µg mL^{-1}) of cut rose flowers treated with T1 (a), T2 (b), T9 (c), T10 (d), and T11 (e; dotted lines) and the controls (solid lines). Values are means ± S.E.

Table 3. Polynomial regression analysis between PCD% and Relative Fresh Weight (RFW) of cut rose flowers at 10th day.

SOV	Df	MS
Regression	3	1261.9**
Linear	1	3466.2**
Quadratic	1	53.9
Cubic	1	265.7
Error	17	206.5

** Significant difference at 1% probability level.

Table 4. Polynomial regression analysis between the PCD% with Relative Fresh Weight (RFW) and Water Uptake (WU) cut rose flowers on 18th day for all treatments.

Parameters	SOV	Df	MS
RFW	Regression	3	1187.9**
	Linear	1	1884.9**
	Quadratic	1	447.4
	Cubic	1	1231.3
	Error	32	244.8
WU	Regression	3	944.3**
	Linear	1	1939.1**
	Quadratic	1	5.6
	Cubic	1	887.2
	Error	32	267.6

** Significant difference at 1% probability level.

summary, ineffective treatments (T3 to T8, T10) led to earlier wilting compared to the control (T0), indicating a shorter vase life. Conversely, effective treatments (T1, T2, T9, T11) resulted in reduced wilting symptoms and delayed wilting, significantly increasing the vase life of the treated flowers.

DISCUSSION

Applied chemical and physical treatments differently affected the cell viability (Table 1, Figure 3) and postharvest life of cut rose flowers. BA (T3 and T4) and Nano-Ag×sucrose (T5, T6, T7, and T8) treatments increase the effects of wilting and PCD% in flowers (Figures1, and 3). Therefore, these six treatments appeared to be ineffective. In the final stage of PCD, endonuclease attacks the connection between the nucleosomes and converts DNA into many small pieces about 18 bp. Staining with a DNA fluorochrome such as PI, which is capable of binding and labeling whole DNA, makes it possible to obtain a rapid and precise evaluation of cellular DNA content by FCM. These small DNA subpopulations appear as a sub-G1 or hypodiploid nuclei population, commonly known as the PCD peak (Darzynkiewicz *et al*., 1992; Dive *et al*., 1992; Weir, 2001; Riccardi and Nicoletti, 2006). Flowers treated with Nano-Ag (T1: 5 ppm and T2: 10 ppm), SMF (T9: 15 mT and T10: 25 mT), and sucrose 1% (T11) showed higher longevity and lower PCD% compared to T0 on days 18 and 25 (Figures 2 and 3), indicating effective treatments. Nano-Ag, with effective antibacterial activity can absorb and decompose ethylene (Hu and Fu, 2003). Many studies have shown the importance of Nano-Ag particles as an antibacterial agent (Alt *et al*., 2004; Son *et al*., 2004; Morones *et al*., 2005; Lok *et al*., 2007). Study of Liu *et al*. (2009) showed that Nano-Ag treatment inhibited bacterial

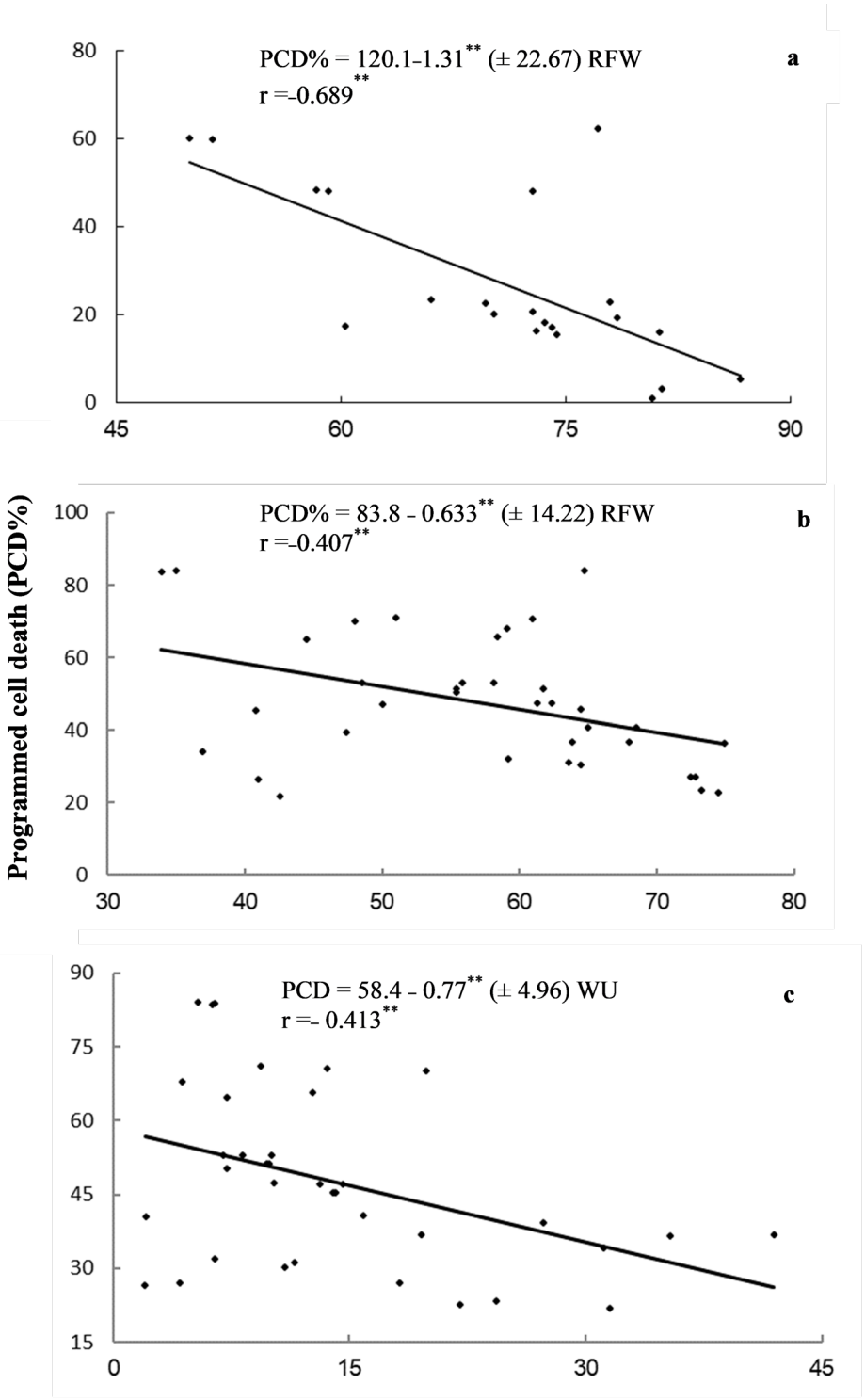

Figure 7. The relationship between the PCD% of treated cut rose flowers and either Relative Fresh Weight (RFW) on 10th day (a) and on 17th day (b), or Water Uptake (WU) on 17th day (c).

growth in the vase solution and at the cut stem ends of cut gerberas. In the current study, Nano-Ag (5 and 10 ppm)×sucrose (3 and 6%) treatments appeared to be ineffective. However, Lü *et al*. (2010) reported that the treatment with Nano-Ag 10

mg L^{-1}×5% sucrose solution for 24 hours, followed by holding samples in Nano-Ag 0.5 mg L^{-1}×2% sucrose solution, not only reduced and delayed vascular blockage caused by bacterial contaminations, but also inhibited stomatal conductance, such that the water balance in cut roses was significantly improved and the vase life of the cut rose flowers was prolonged by 11.8 days. These differences may be related to different treatment times. In this study, applications of Nano-Ag resulted in extending vase life, possibly due to its antibacterial effect. Sucrose can work as an energy source (Moalem-Beno *et al*., 1997) and osmotic regulator (Bieleski, 1993) thereby playing a role in flower opening and subsequent water balance regulation (Kuiper *et al*., 1995). Among effective treatments, flowers treated with T9 (SMF-15 mT, 3 hours) possessed the lowest rate of PCD (3.14, 22.64, and 23.19% on days 1, 18, and 25, respectively), followed by T10 (SMF-25 mT, 3 hours) and T11 (1% sucrose). These treatments appeared to be the most effective. In accordance with observations, the wilting process in cut rose flowers treated with T11 was slower than that in those exposed to other chemical treatments. In Ramezanizadeh *et al*. (2012) report, the physical treatment of 10 mT-SMF caused a remarkable decrease in PCD, resulting in the best vase life time of 14 d. MFs affect the synthesis of DNA and RNA as well as cellular proliferation. Additionally, MFs in both Extremely Low Frequency (ELF) and Radio Frequency (RF) activate the cellular stress response, a protective mechanism that induces the expression of stress response genes (Ruediger, 2009). Several studies have investigated the effects of MFs on plant antioxidant systems (Abdolmaleki *et al*., 2007). Various effective treatments such as silver thiosulfate, 8-hydroxyquinoline sulfate, and sucrose have been utilized by researchers (e.g. Liao *et al*., 2000; Ramezanizadeh *et al*., 2012; Hosseinzadeh *et al*., 2014) to examine their effects on PCD, aiming to increase the vase life of roses. The obtained results showed that these treatments significantly improved the flower quality and the vase life.

Effective treatments had a slower rate of fresh weight loss compared to the control from the 14^{th} day. Mean fresh weight loss was used for more accurately determining which treatments had the most or the least effect. Hence, three treatments of T9, T10, and T11 appeared to cause the least weight loss among all treatments examined (Figure 4): there was no significant difference among these three treatments. However, since T9 had a lower level of PCD, it was preferable to the other treatments. The flowers with the effective treatments absorbed more water compared to the control, indicating that they were more durable and absorbed more water. Amongst physical induction treatments, T9 and T2 amongst the chemical treatments were superior in terms of WU. Barbaz Esfahani *et al*. (2013) reported that flowers kept in preservation solution with 4% sucrose absorbed more solution than the flowers maintained only in distilled water. In Basiri *et al*. (2011) report, the most extended vase life cut flowers of carnation (*Dianthus caryophyllus* L.) was obtained from 5 ppm Nano-Ag combined with sucrose 6%. Furthermore, different levels of Nano-Ag concentrations had no significant effect on the RFW of cut carnations. Alimoradi *et al*. (2013) reported that the best treatment to enhance postharvest factors of cut *Alstroemeria* flowers was the exact 15 ppm Nano-Ag. This treatment could be proposed as additional substance for *Alstroemeria* postharvest quality increment. On the other hand, results of BarbazEsfahani *et al*. (2013) indicated that cut rose cv. Dolce vita^{+} flowers maintained in pure distilled water had more RFW reduction than those kept in 4% sucrose solution on the 11^{th} day, meaning that dH_2O-kept flowers were in more stress. Similar results were found in Mortazavi's (2006) study, using (0, 2%, 4%, 8%) sucrose in preservation solution had the greatest effect on increasing the vase life of Elona rose cultivar. The highly significant inverse linear relationship was identified

between PCD% and RFW on day 10 (Figure 7-a). A significant inverse linear relationship was detected between PCD% and either RFW (Figure 7-b), or WU (Figure 7-c) on day 17. Using chemical treatments, Ramezanizadeh *et al*. (2012) reported a significantly negative relationship for vase time (d) of cut rose (*Rosa hybrida* cv. Dolce vita^{+}) flowers with PCD%. In other words, flower vase time reduced significantly ($b=-0.312^{*}$) as PCD increased when cut flowers were chemically treated. No such relationship was detectable in their physical treatments. As conclusion, it can be stated that T11 [sucrose 1% (w/v)] and T9 (SMF; 15 mT) treatments caused the highest longevity among chemical and physical treatments examined; hence, they are suggested for extending the vase time of cut rose (cv. Dolce vita^{+}) flowers. The treatments had varying effects on the studied traits, including PCD percentage, RFW, WU, and Chl content. The study explores various chemical and physical treatments to enhance the vase life of cut roses. Results reveal that treatments with Nano-Ag at 10 ppm, SMF of 15 mT, and 1% sucrose were the most effective in prolonging vase life and reducing cellular death in cut roses. Conversely, treatments involving BA, combinations of Nano-Ag and sucrose, and a static magnetic field with 25 mT intensity were less effective in extending vase life. A combination of mechanisms including ethylene inhibition, antioxidant activity, enhanced water and nutrient uptake, likely contributes to the effectiveness of these treatments in delaying PCD and extending vase life. T1 and T2 treatments may work by inhibiting ethylene production or action, thus delaying plant senescence, while T9 and T11 treatments likely function through antioxidant mechanisms, protecting cells from oxidative damage. Additionally, these treatments may improve water uptake and prevent microbial growth in the vase solution, ensuring a longer vase life (Abdolmaleki *et al*. 2007; Khunmuang *et al*., 2019; Gun *et al*., 2023). The Chl content is crucial for increasing the vase life and improving the market quality of cut roses.

The findings of this study are consistent with other research, which has shown that treatments, such as silver nanoparticles, aid in preserving Chl content and enhancing water absorption (Jowkar *et al.,* 2013; Hassan *et al.,* 2014). Overall, the study demonstrates the potential of specific treatments in improving the vase life and the quality of cut roses, providing valuable insights for optimization of storage and marketing processes for these products.

CONCLUSIONS

This study investigated the methods to extend the vase life of cut roses by delaying Programmed Cell Death (PCD). Different treatments were applied, including chemicals like silver Nanoparticles (Nano-Ag) and Benzyladenine (BA), sucrose solution, and physical treatments involving Static Magnetic Field (SMF) at various intensities. Among the investigated treatments, SMF applied at 15 mT proved to be the most effective, with flowers exhibiting the lowest PCD levels and slowest decline in fresh weight throughout the experiment. A 1% sucrose, while not as effective as the SMF, also delayed wilting and maintained higher water uptake compared to the control group. Silver nanoparticles (Nano-Ag) showed some initial effectiveness in delaying wilting, but their overall impact was less significant compared to SMF and sucrose solution. The study suggests that applying 15 mT static magnetic field or a 1% sucrose solution can significantly improve the vase life of cut roses by delaying PCD.

ABBREVIATIONS

BA: 6-Benzyladenine, Chl: Chlorophyll, EMF: Electromagnetic Field; FCM: Flow Cytometry, FW: Fresh Weight, MFs: Magnetic Fields, PCD: Programmed Cell

Death, PI: Propidium Iodide, PVP: Polyvinylpyrrolidone, RCBD: Randomized Complete Block Design, RF: Radio frequency, RFW: Relative Fresh Weight, RH: Relative Humidity, SMF: Static Magnetic Field, WU: Water Uptake.

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آنالیز فلوسایتومتریک مرگ سلولی برنامه ریزی شده در گل رز (*Rosa hybrida* cv. Dolce vita+) تحت تأثیر تیمارهای فیزیکوشیمیایی

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چکیده

این مطالعه با هدف افزایش عمر گلدانی گل‌های رز شاخه بریده از طریق بهبود تنظیم مرگ برنامه‌ریزی شده سلولی (PCD) انجام گردید. آزمایش‌ها روی گل‌های رز شاخه بریده (*Rosa hybrida* cv. Dolce vita+) تحت تأثیر تیمارهای فیزیکی میدان مغناطیسی ایستا (SMF؛ ۱۵ و ۲۵ میلی تسلا) به مدت ۳ ساعت و تیمارهای فیزیکوشیمیایی نانو ذرات نقره (Nano-Ag؛ ۵ و ۱۰ پی‌پی‌ام)، ٦-بنزیل آدنین (BA؛ ۲۵ و ۵۰ میلی‌گرم در لیتر)، ۱ درصد ساکارز (وزن/حجم) و ترکیب‌های ۵ و ۱۰ پی‌پی‌ام نانو نقره با ۳ و ۶ درصد ساکارز انجام شد. نتایج نشان داد که SMF ۱۵ میلی تسلا به‌طور قابل‌توجهی عمر گلدانی را تا ۲۵ روز افزایش داد. از میان تیمارهای شیمیایی، نانو نقره ۵ پی‌پی‌ام و ۱ درصد ساکارز عمر گلدانی را به ترتیب تا ۲۳ و ۱۸ روز افزایش دادند. کمترین کاهش وزن تر در تیمار فیزیکیSMF ۱۵ میلی تسلا مشاهده گردید. این تیمار منجر به کمترین کاهش در محتوای کلروفیل (Chl)گردید. در روز هفدهم بعد از اعمال تیمارها، جذب آب (WU) و وزن تر نسبی (RFW) رابطه معکوس قابل‌ملاحظه‌ای با PCD در گل‌های رز شاخه بریده نشان دادند، که تأخیر در PCDرا تأیید می‌کند. به طور کلی، تیمارهای القایی SMF ۱۵ میلی تسلا، نانو نقره ۵ پی‌پی‌ام، و ۱ درصد ساکارز برای بهبود کیفیت پس از برداشت و افزایش عمر گلدانی گل‌های رز شاخه بریده پیشنهاد می‌شوند.