

Flow cytometric analysis of programmed cell death in rose (*Rosa hybrida* cv. Dolce vita+) as influenced by physico-chemical 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 there markable delayed PCD which is favored 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), Sucrose, Vase life.

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.

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 (Philips and Rix, 1988). It is

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35 globally considered as one of the most significant ornamental plants, and its flowers are
36 commercially sold as potted plants or cut flowers (Ross, 1991; Liao *et al.*, 2000). Despite the
37 significance of roses in the cosmetics industry as a provider of aromatic oils, volatile
38 compounds (Ryu *et al.*, 2020), and their medicinal benefits (Choi and Hwang, 2003; Yang *et*
39 *al.*, 2013), cut roses have a limited life span in vases (Lee *et al.*, 2016).

40 Vase life can be affected by post-harvest factors such as temperature, humidity, water
41 relations and conditioning (Gupta and Dubey, 2018). In addition to issues related to improper
42 harvesting, handling, and storage of roses, harvested fresh-cut flowers have a short vase life
43 due to limited water uptake, loss of water after cutting, low energy source, and susceptibility
44 to ethylene (Fanourakis *et al.*, 2013; Scariot *et al.*, 2014; Khunmuang *et al.*, 2019; Gun *et al.*,
45 2023). Therefore, it is crucial to maintain the freshness and the quality of the flowers from
46 harvesting until they reach the consumers. Although the vase life of cut flowers depends on the
47 flower's type, conditions of the variety and its growth, it can be widely influenced by
48 postharvest treatment (Çelikel *et al.*, 2011; Ramezanizadehet *al.*, 2012; Hosseinzadeh *et al.*,
49 2014). Inadequate water uptake can be enhanced with the proper wetting agent or surfactants
50 (such as triton X-100, tween 20; Aros *et al.*, 2016; El-Shoura and Arafa, 2017) while xylem
51 blockage can be prevented with silver nano particles (Shuqin *et al.*, 2019). Applications of
52 exogenous plant growth regulators are known to influence postharvest quality (Janowska and
53 Andrzejak, 2023). In the natural environment, living things are exposed to abiotic stress
54 induced by MFs due to the distribution of varied types of instruments and equipment and SMF
55 is an important environmental factor that can influence the growth and development of plants
56 (Bhatnagar and Deb, 1977; De Souza *et al.*, 2005, 2006). In *Allium cepa*, mitotic activity was
57 increased under SMF at 0.06T (Hozayn *et al.*, 2015). In a study on carnation cut flowers, it was
58 stated that an Electromagnetic Field (EMF) with a flux density of 160 mT has a profound
59 impact on prolonging the vase life of its cut bloom (Ayesha *et al.*, 2023). However, the impact
60 of non-ionizing radiation, such as the EMF, on the quality of cut flowers is still unknown. We
61 did not find any noteworthy investigations about the effect of SMF on the vase life of roses.
62 The life of a flower typically ends in senescence, culminating in a form of PCD (Rogers, 2013).
63 In fact, PCD is a genetically regulated process of cell suicide that is central to the development,
64 homeostasis, and integrity of multi cellular organisms (Ameisen, 2002). In plants, PCD is
65 involved in a variety of situations, including responses to environmental stresses, the
66 hypersensitive response to pathogen attack, plant senescence and fruit ripening (Pennell and
67 Lamb, 1997; O'Brien *et al.*, 1998). Various methods have been employed for the detection of
68 plants' PCD, one of those is FCM, which is utilized in numerous studies. This method is

69 convenient, fast, and reliable (Doležel *et al.*, 2007; Abedi *et al.*, 2015; Tavan *et al.*, 2015;
70 Javadian *et al.*, 2017; Sayadi *et al.*, 2022; Mehravi *et al.*, 2022; Rasekh and Karimzadeh, 2023;
71 Khakshour *et al.*, 2024). During cell death, the capability of the cell to scatter light alters as a
72 result of morphological changes such as cell shrinkage, chromatin condensation, and
73 nucleosomal fragmentation (Givan, 1992; Doležel *et al.*, 2007). So, this event can be detected
74 by FCM methods. The current study was aimed to identify the most effective physico-chemical
75 treatments to reduce PCD, with the goal of increasing the vase life of cut rose flowers.

76

77 **MATERIALS AND METHODS**

78 **Plant Material and Experimental Treatments**

79 Fresh cut flowers of rose (*Rosa hybrida* cv. Dolce vita⁺) were obtained from a local
80 commercial greenhouse in Tehran, Iran. In tight bud stage, flowers were cut from the plants
81 between 9:00 and 12:00 AM and re-cut to 50 cm in length. Detached flowers were immediately
82 transported to the laboratory and placed in distilled water. All experiments were performed in
83 a controlled environmental growth room (20 ± 1°C, 80 ± 10% RH, 12 hours photoperiod). The
84 cut flowers were kept in a 1,000 ml-vessel containing 500 ml solution in 11 treatments (without
85 control): T0 = distilled water (control), T1 = Nano-Ag (5 ppm), T2 = Nano-Ag (10 ppm),
86 T3 = BA (25 mg L⁻¹), T4 = BA (50 mg L⁻¹), T5 = Nano-Ag (5 ppm) × sucrose (3%),
87 T6 = Nano-Ag (5 ppm) × sucrose (6%), T7 = Nano-Ag (10 ppm) × sucrose (3%), T8 = Nano-
88 Ag (10 ppm) × sucrose (6%), T9 = Static magnetic field (SMF; 15 mT), T10 = SMF; 25 mT,
89 and T11 = Sucrose (1%, w/v). To exert different intensities of SMF, a magnetic field generator
90 device consisting of two strong magnets (in repelling mode with the ability to adjust the
91 distance) was used. The strength of the magnetic field was measured, using Teslameter
92 (Leybold-Heraeus 51652, Germany). The cut flowers were placed between the different
93 strength of magnet poles. It should also be noted that all methods were performed in accordance
94 with relevant guidelines and regulations."

95

96 **Measurement of Chlorophyll (Chl)**

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

103 Chl a+b = 5.24 (A664 – A700) + 22.24 (A647 – A700)

104 Where, A700, A664 and A647 were Absorbance at the three wavelengths.

105

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

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

110 $WU (g\ g^{-1}\ \text{initial fresh weight-FW}) = B_{n-1} - B_n / \text{Initial FW} (A_0 - B_0)$

111 $RFW (\%) = [(A_n - B_n) / (A_0 - B_0)] \times 100$

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

117

118 **Flow Cytometric Analysis for PCD Measurements**

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

137 at least 5000 nuclei were analyzed per sample. According to previous studies (Darzynkiewicz
138 *et al.*, 1992; Dive *et al.*, 1992; Weir, 2001; Riccardi and Nicoletti, 2006) cycling cells can be
139 distinguished from dead cells with FCM, using fluorescent dye PI (for DNA staining) with
140 PVP (1% w/v) in cell suspension. In the present experiments, PCD percentage was calculated,
141 using the following equation:

$$142 \text{ PCD (\%)} = [\text{Count (PCD)} / \text{Count (PCD+G1)}] \times 100$$

143

144 **Statistical Analysis**

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

153

154 **RESULTS**

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

164

165 **Flow Cytometric Analysis of PCD**

166 The results of ANOVA on PCD% showed significant differences among treatments on days
167 10, 18 and 25 ($P < 0.01$; Table 1). On the 10th and 18th day, flowers treated with in effective
168 treatments (T3, T4, T5, T6, T7, T8) unexpectedly showed more remarkable PCD% (Figure 1)
169 compared to control flowers (T0), resulted in more wilting. On the other hand, flowers treated
170 with five effective treatments (T1, T2, T9, T10, T11) showed significantly ($P < 0.01$, Table 1)
171 less PCD% compared to the controls on both experimental days of 18, and 25 (Figures 2, and

172 3). The cut rose flowers exposed to SMF-15 Mt (T9) started wilting on day 25, reaching
173 completely wilting after three days (day 28). Non-treated flowers (control) were wilted on day
174 18, but flowers treated by T9 remained alive and did not wilt. T9 treatment caused the least
175 PCD% at all sampling times among all exposed chemical and physical treatments. The PCD%
176 of flowers treated by T9 on days 18 and 25 was estimated as 22.64 and 23.19%, respectively
177 (Figure 3). The flowers treated with 1% sucrose (T11) began wilting on day 18, showing slow
178 senescence, followed no clear changes during a week after (day 25, Figures 2, and 3). In fact,
179 the cut flowers treated with T11 (1% sucrose) were more rejuvenated compared to the control
180 on day 18; roses were withering on the 25th day (Figure 2). On the other hand, based on data
181 achieved from FCM analysis of PCD% for cut rose flowers treated with 1% sucrose, the PCD%
182 on days 18 and 25 were estimated as 31.12 and 31.85%, respectively.

183

184 **Morphological traits**

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

194 In the present study, Figure 5 indicates the relative changes of WU during days 1-21 for the
195 effective treatment (T1, T2, T9, T10, T11) on postharvest life. Both untreated control and
196 treated flowers with T1, T2, T9, and T11 showed a declining trend in WU until the 14th day, but
197 since that time, the treated flowers absorbed more water compared to the controls (Figure 5).
198 The *t*-test results between two physical induction treatment (T9 and T10) and also between two
199 chemical treatments (T1 and T2) at a significance level of $P < 0.05$ showed that T9 and T2
200 treatments had higher water uptake compared to T10 and T1, respectively. BA treatments (T3,
201 T4) as well as combined treatments (T5, T6, T7, T8) displayed a downward trend in the amount
202 of water absorption until the 14th day. After this day, the amount of water absorption increased.
203 T6, T7, and T8 treatments showed less reduction in WU compared to other ineffective
204 treatments. The changes of Chl content during days 1-18 for effective treatments (T1, T2, T9,

205 T10, T11) are shown in Figure 6. Chl content had increased until day 4 in all treatments. T9
206 showed the least reduction in Chl amount.

207

208 **Relationship between morphological traits and PCD%**

209 The data of RFW, WU, and Chl were correlated with PCD%, where significant correlations
210 were identified, they were regressed upon PCD% on the 10th and 17th days of the experimental
211 protocol. All morphological characteristics except Chl showed a remarkable relationship with
212 PCD%. Hence, polynomial regression analysis between the PCD% and RFW of rose cut
213 flowers on the 10th day showed a significant linear regression ($P < 0.01$, Table 3, Figure 7-a).
214 No significant correlation was identified between the PCD% and WU on the 10th day. On the
215 18th day, the PCD% had a significant correlation with RFW and WU ($P < 0.01$, Tables 3, and
216 4, Figure 7). There was no significant correlation among these traits on other days. The highly
217 significant inverse linear relationship was identified between PCD% and RFW on day 10
218 (Figure 6-a). Significance inverse linear relationship was detected between PCD% and either
219 RFW (Figure 7-b) or WU (Figure 7-c) on day 18.

220 Based on the study results, the treatments that have been more effective in increasing the
221 vase life of flowers were Nano-Ag 10 ppm (T2), Static Magnetic Field 15 mT (T9), and Sucrose
222 1% (w/v) (T11). Nano-Ag treatments resulted in reduced wilting symptoms and delayed
223 wilting, leading to increased vase life. Conversely, BA and Nano-Ag×sucrose treatments
224 appeared ineffective, causing increased wilting symptoms and reduced vase life. In summary,
225 ineffective treatments (T3 to T8, T10) led to earlier wilting compared to the control (T0),
226 indicating a shorter vase life. Conversely, effective treatments (T1, T2, T9, T11) resulted in
227 reduced wilting symptoms and delayed wilting, significantly increasing the vase life of treated
228 flowers.

229

230 **DISCUSSION**

231 Applied chemical and physical treatments differently affected the cell viability (Table 1,
232 Figure 3) and postharvest life of cut rose flowers. BA (T3 and T4) and Nano-Ag×sucrose (T5,
233 T6, T7, and T8) treatments increase the effects of wilting and PCD% in flowers (Figures 1, and
234 3). Therefore, these six treatments appeared to be ineffective treatments. In the final stage of
235 PCD, endonuclease attacks the connection between the nucleosomes and converts DNA into
236 many small pieces about 18 bp. Staining with a DNA fluorochrome such as PI, which is capable
237 of binding and labeling whole DNA, makes it possible to obtain a rapid and precise evaluation
238 of cellular DNA content by FCM. These small DNA subpopulations appear as a sub-G1 or

239 hypodiploid nuclei population, commonly known as the PCD peak (Darzynkiewicz *et al.*, 1992;
240 Dive *et al.*, 1992; Weir, 2001; Riccardi and Nicoletti, 2006). Flowers treated with Nano-Ag
241 (T1: 5 ppm and T2: 10 ppm), SMF (T9: 15 mT and T10: 25 mT), and sucrose 1% (T11) showed
242 higher longevity and lower PCD% compared to T0 on days 18 and 25 (Figures 2, and 3),
243 indicating effective treatments. Nano-Ag, with effective antibacterial activity can absorb and
244 decompose ethylene (Hu and Fu, 2003). Many studies have shown the importance of Nano-Ag
245 particles as an antibacterial agent (Alt *et al.*, 2004; Son *et al.*, 2004; Morones *et al.*, 2005; Lok
246 *et al.*, 2007). Study of Liu *et al.* (2009) showed that Nano-Ag treatment inhibited bacterial
247 growth in the vase solution and at the cut stem ends of cut gerberas. In the current study, Nano-
248 Ag (5 and 10 ppm)×sucrose (3 and 6%) treatments appeared to be ineffective. However, Lü *et*
249 *al.* (2010) reported that the treatment with Nano-Ag 10 mg L⁻¹×5% sucrose solution for 24
250 hours, followed by holding samples in Nano-Ag 0.5 mg L⁻¹×2% sucrose solution, not only
251 reduced and delayed vascular blockage caused by bacterial contaminations, but also inhibited
252 stomatal conductance so that the water balance in cut roses was significantly improved and the
253 vase life of the cut rose flowers was prolonged by 11.8 days. These differences may be related
254 to different treatment times. In this study, applications of Nano-Ag resulted in extending vase
255 life, possibly due to its antibacterial effect. Sucrose can work as an energy source (Moalem-
256 Beno *et al.*, 1997) and osmotic regulator (Bialeski, 1993) thereby playing a role in flower
257 opening and subsequent water balance regulation (Kuiper *et al.*, 1995). Among effective
258 treatments, flowers treated with T9 (SMF-15 mT, 3 hours) possessed the lowest rate of PCD
259 (3.14, 22.64, and 23.19% on days 1, 18, and 25, respectively), followed by T10 (SMF-25 mT,
260 3 hours) and T11 (1% sucrose). These treatments appeared to be the most effective. In
261 accordance with observations, the wilting process in cut rose flowers treated with T11 was
262 slower than that in those exposed to other chemical treatments. In Ramezanizadeh *et al.* (2012)
263 report, the physical treatment of 10 mT-SMF caused a remarkable decrease in PCD, resulting
264 in the best vase life time of 14 d. MFs affect the synthesis of DNA and RNA as well as cellular
265 proliferation. Additionally, MFs in both Extremely Low Frequency (ELF) and Radio
266 Frequency (RF) activate the cellular stress response, a protective mechanism that induces the
267 expression of stress response genes (Ruediger, 2009). Several studies have investigated the
268 effects of MFs on plant antioxidant systems (Abdolmaleki *et al.*, 2007). Various effective
269 treatments such as silver thiosulfate, 8-hydroxyquinoline sulfate, and sucrose have been
270 utilized by researchers (e.g. Liao *et al.*, 2000; Ramezanizadeh *et al.*, 2012; Hosseinzadeh *et al.*,
271 2014) to examine their effects on PCD, aiming to increase the vase life of roses. The obtained
272 results showed that these treatments significantly improved the flower quality and the vase life.

273 Effective treatments had a slower rate of fresh weight loss compared to control from the 14th
274 day. Mean fresh weight loss was used for more accurately determining which treatments had
275 the most or the least effect. Hence, three treatments of T9, T10, and T11, appeared to cause the
276 least weight loss among all treatments examined (Figure 4); there was no significant difference
277 among these three treatments. However, since T9 had a lower level of PCD, it was preferable
278 to other treatments. The flowers treated with effective treatments absorbed more water
279 compared to control which indicates that they are more durable and absorbed more water
280 compared to control. T9 treatment amongst physical induction treatments, and T2 treatment
281 amongst chemical treatments, were superior in the terms of WU. BarbazEsfahani *et al.* (2013)
282 reported that flowers kept in preservation solution with 4% sucrose had more solution absorbed
283 than the flowers maintained only in pure distilled water. In Basiri *et al.* (2011) report, the most
284 extended vase life cut flowers of carnation (*Dianthus caryophyllus* L.) was obtained from 5
285 ppm Nano-Ag combined with sucrose 6%. Furthermore, different levels of Nano-Ag
286 concentrations had no significant effect on the RFW of cut carnations. Alimoradi *et al.* (2013)
287 reported that the best treatment to enhance postharvest factors of cut *Alstroemeria* flowers was
288 the exact 15 ppm Nano-Ag. This treatment could be proposed as additional substance for
289 *Alstroemeria* postharvest quality increment. On the other hand, results of BarbazEsfahani *et al.*
290 (2013) indicated that cut rose cv. Dolce vita⁺ flowers maintained in pure distilled water had
291 more RFW reduction than those kept in 4% sucrose solution on the 11th day, meaning that
292 dH₂O-kept flowers were in more stress. Similar results were found in Mortazavi's (2006) study,
293 using (0, 2, 4, 8%) sucrose in preservation solution had the greatest effect on increasing of vase
294 life of Elona rose cultivar. The highly significant inverse linear relationship was identified
295 between PCD% and RFW on day 10 (Figure 7-a). A significant inverse linear relationship was
296 detected between PCD% and either RFW (Figure 7-b), or WU (Figure 7-c) on day 17. Using
297 chemical treatments, Ramezanizadeh *et al.* (2012) reported a significantly negative relationship
298 of vase time (d) of cut rose (*Rosa hybrida* cv. Dolce vita⁺) flowers with PCD%. In other words,
299 flower vase time reduced significantly ($b = -0.312^*$) as PCD increased when cut flowers were
300 chemically treated. No such relationship was detectable in their physical treatments. As
301 conclusion, it can be stated that T11 [sucrose 1% (w/v)] and T9 (SMF; 15 Mt) treatments
302 caused the highest longevity among chemical and physical treatments examined; hence they
303 are suggested for extending the vase time of cut rose (cv. Dolce vita⁺) flowers. The treatments
304 had varying effects on the studied traits, including PCD percentage, RFW, WU, and Chl
305 content. The study explores various chemical and physical treatments to enhance the vase life
306 of cut roses. Results reveal that treatments with Nano-Ag at 10 ppm, SMF of 15 mT, and 1%

307 sucrose were the most effective in prolonging vase life and reducing cellular death in cut roses.
308 Conversely, treatments involving BA, combinations of Nano-Ag and sucrose, and a static
309 magnetic field with 25 mT intensity were less effective in extending vase life. A combination
310 of mechanisms including ethylene inhibition, antioxidant activity, enhanced water and nutrient
311 uptake, likely contributes to the effectiveness of these treatments in delaying PCD and
312 extending vase life. T1 and T2 treatments may work by inhibiting ethylene production or
313 action, thus delaying plant senescence, while T9, and T11 treatments likely function through
314 antioxidant mechanisms, protecting cells from oxidative damage. Additionally, these
315 treatments may improve water uptake and prevent microbial growth in the vase solution,
316 ensuring a longer vase life (Abdolmaleki *et al.* 2007; Khunmuang *et al.*, 2019; Gun *et al.*,
317 2023). The Chl content is crucial for increasing the vase life and improving the market quality
318 of cut roses. The findings of this study are consistent with other research, which has shown that
319 treatments such as silver nanoparticles aid in preserving Chl content and enhancing water
320 absorption (Jowkar *et al.*, 2013; Hassan *et al.*, 2014). Overall, the study demonstrates the
321 potential of specific treatments in improving the vase life and the quality of cut roses, providing
322 valuable insights for the optimization of storage and marketing processes for these products.

323

324 CONCLUSIONS

325 **This study investigates methods to extend** the vase life of cut roses by delaying Programmed
326 Cell Death (PCD). Different treatments were applied, including chemicals like silver
327 Nanoparticles (Nano-Ag) and Benzyladenine (BA), sucrose solution, and physical treatments
328 involving Static Magnetic Field (SMF) at various intensities. Among the investigated
329 treatments, a Static Magnetic Field (SMF) applied at 15 mT proved to be the most effective,
330 with flowers exhibiting the lowest PCD levels and slowest decline in fresh weight throughout
331 the experiment. A 1% sucrose, while not as effective as the SMF treatment, also delayed wilting
332 and maintained higher water uptake compared to the control group. Silver nanoparticles (Nano-
333 Ag) showed some initial effectiveness in delaying wilting, but their overall impact was less
334 significant compared to SMF and sucrose solution. Overall, the study suggests that applying a
335 15 mT static magnetic field or a 1% sucrose solution can significantly improve the vase life of
336 cut roses by delaying PCD.

337

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343
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506 **Table 1.** Mean Squares (MS) of the ANOVA for PCD% cut rose flowers sampled on days 10, 18, and
 507 25.

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

508 **Significant difference at 1% probability
 509 level.
 510

511 **Table 2.** Mean Squares (MS) of the ANOVA for Relative Fresh Weight (RFW), Water Uptake (WU) and
 512 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

513 *, and ** Significant differences at 5 and 1% probability levels, respectively.
 514

515 **Table 3.** Polynomial regression analysis between PCD% and Relative Fresh Weight (RFW) of cut
 516 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

517 ** Significant difference at 1%
 518 probability level.
 519

520 **Table 4.** Polynomial regression analysis between the PCD% with Relative Fresh Weight (RFW) and
 521 Water Uptake (WU) cut rose flowers at 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

522 ** Significant difference at 1% probability level.

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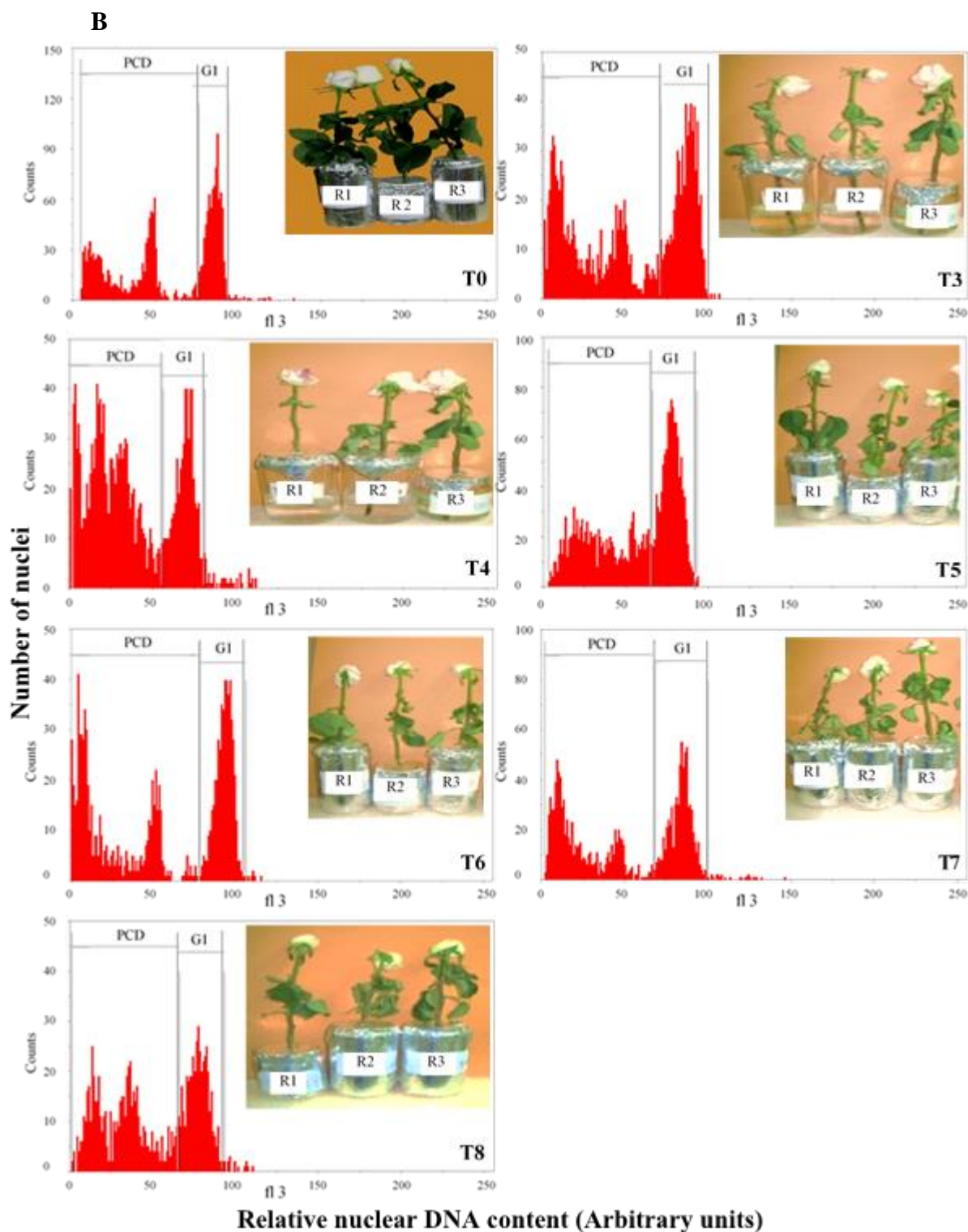
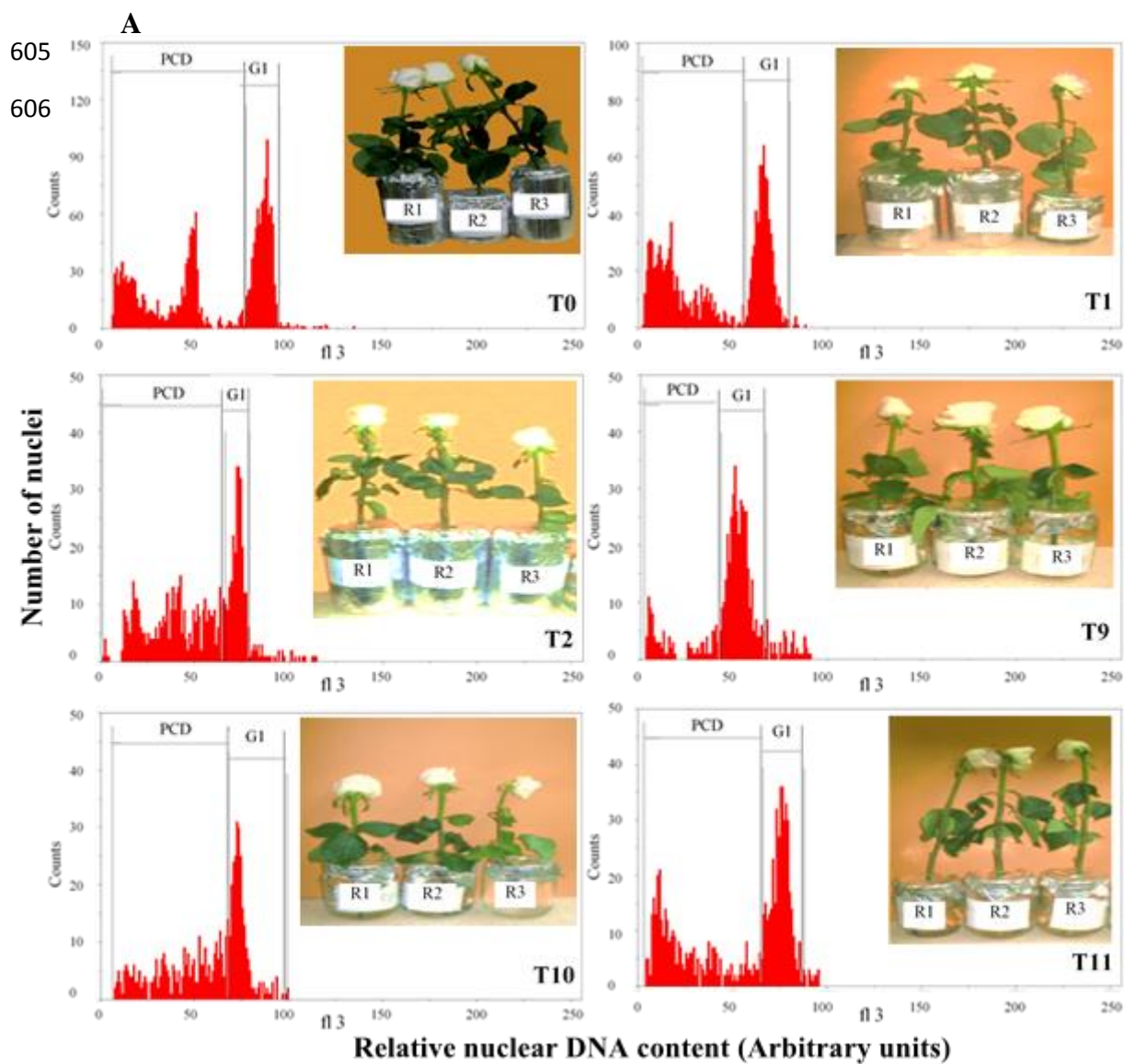


Figure 1. A: Control [T0, distilled water] and treated cut rose flowers with ineffective treatments, including: T3 [BA (25 mg l⁻¹)], T4 [BA (50 mg l⁻¹)], T5 [Nano-Ag (5 ppm)×Sucrose (3%)], T6 [Nano-Ag (5 ppm)×Sucrose (6%)], T7 [Nano-Ag (10 ppm)×Sucrose (3%)], and T8 [Nano-Ag (10 ppm)×Sucrose (6%)] and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



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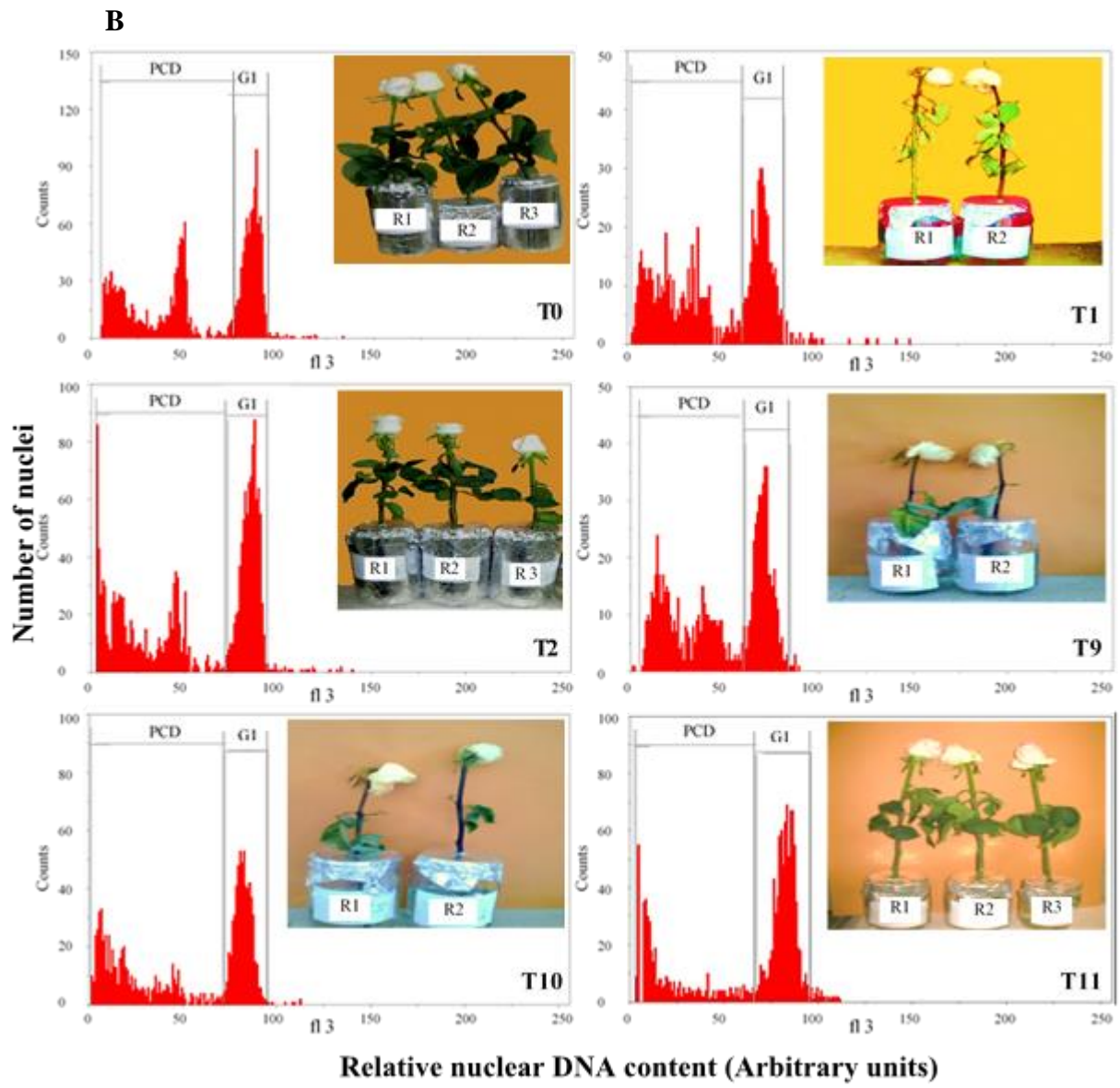
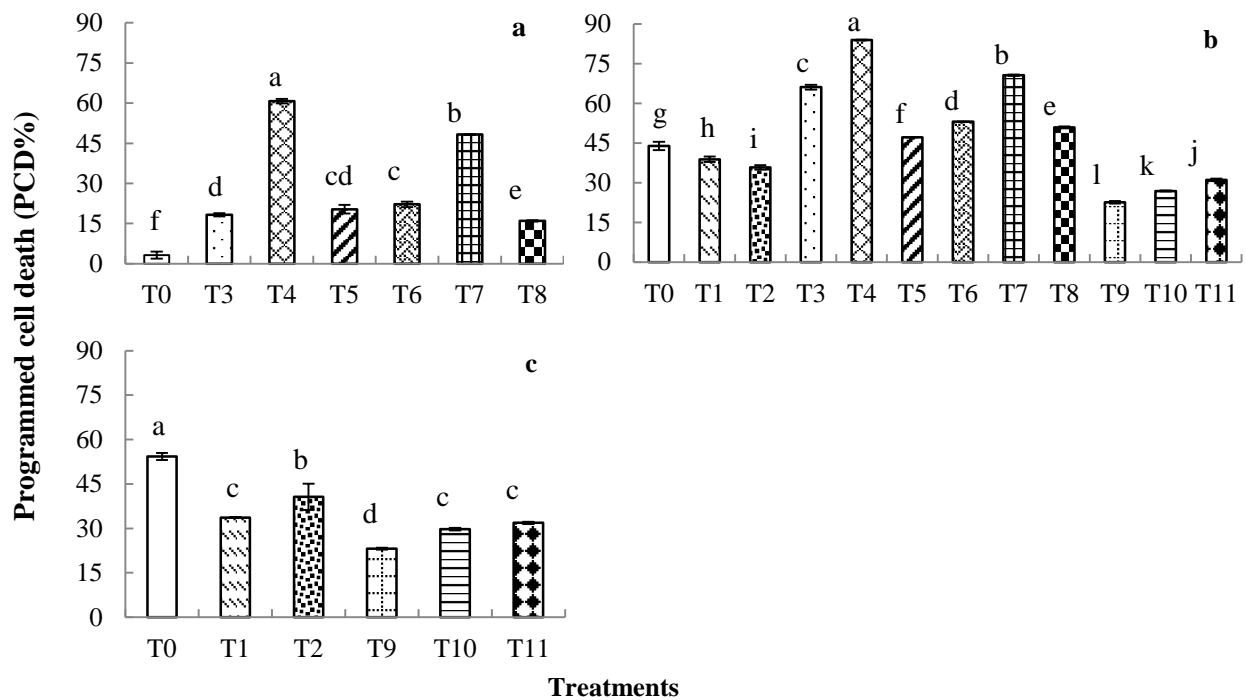
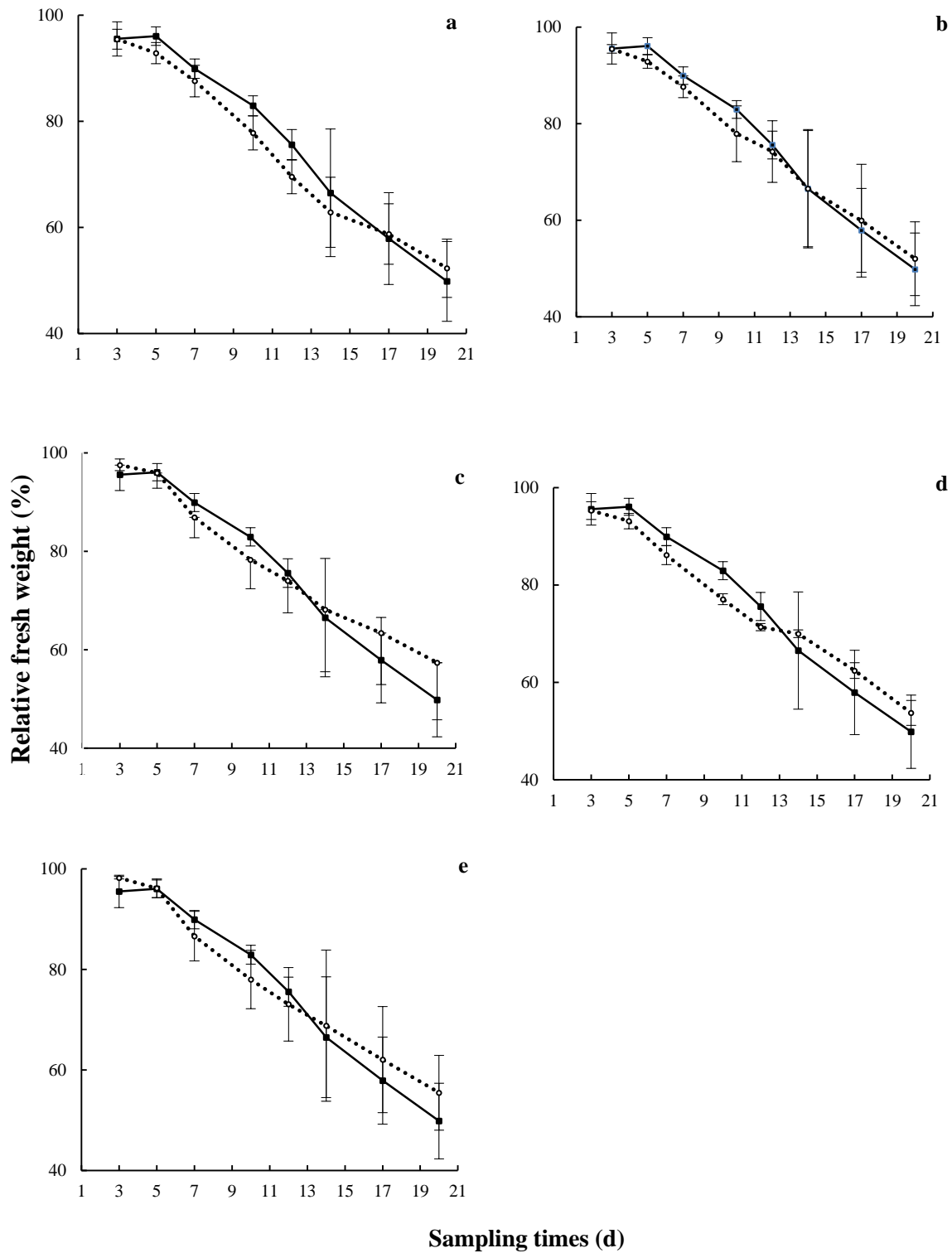


Figure 2. A: Control [T0, distilled water] and treated cut rose flowers with ineffective treatments, including: T1 [Nano-Ag (5 ppm)], T2 [Nano-Ag (10 ppm)], T9 [Static Magnetic Field (SMF; 15 mT)], T10 [SMF; 25 mT], T11 [Sucrose (1%, w/v)] and related FCM histograms of PCD% on day 18 of harvesting time.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

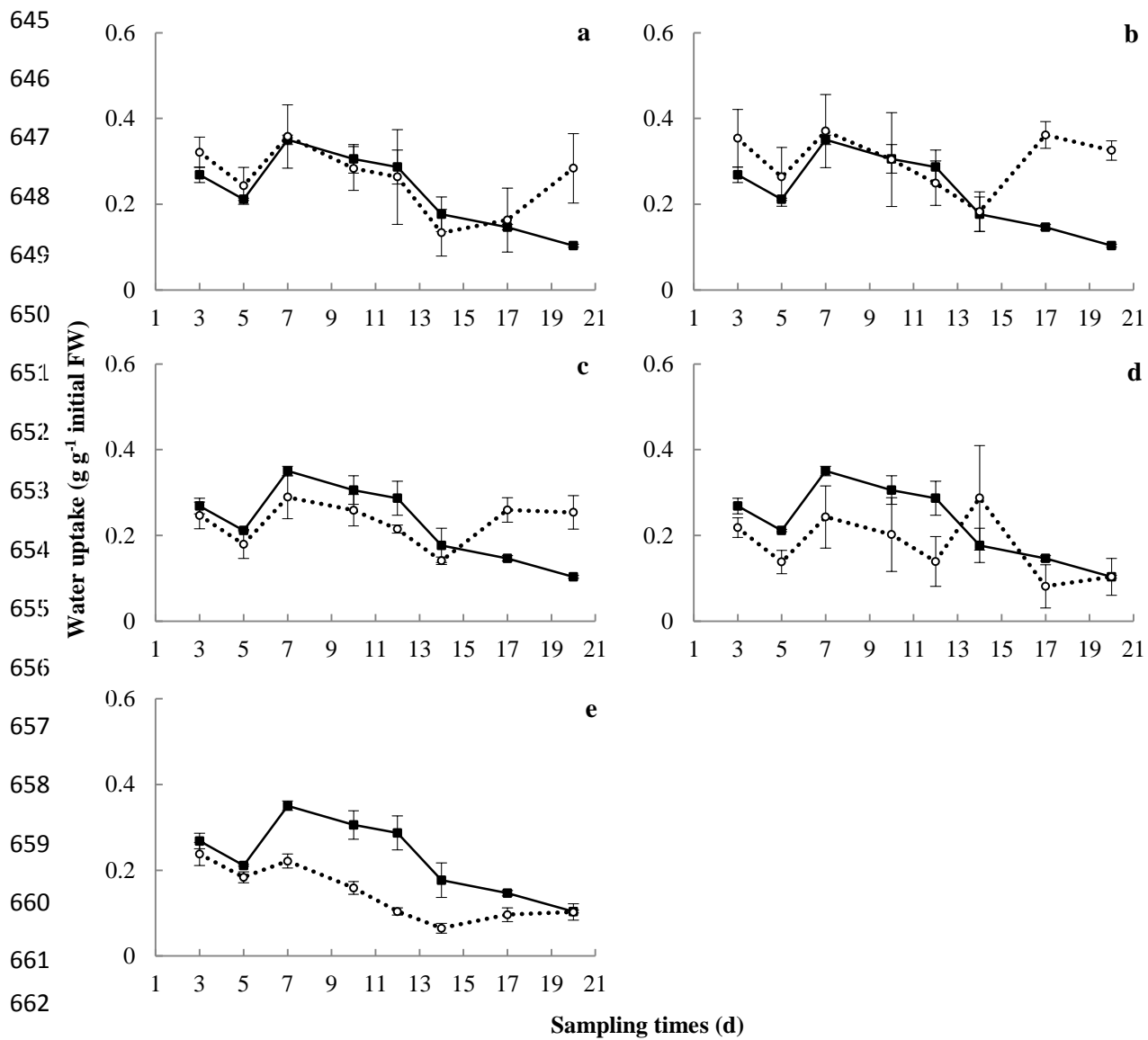


634 **Figure 3.** Programmed Cell Death (PCD%) of cut rose treated and control flowers on 10th day (a), 18th day (b),
 635 and 25th day (c). T0= distilled water (control), T1= Nano-Ag (5 ppm), T2= Nano-Ag (10 ppm), T3= BA (25 mg
 636 l⁻¹), T4= BA (50 mg l⁻¹), T5= Nano-Ag (5 ppm)×Sucrose (3%), T6= Nano-Ag (5 ppm)×Sucrose (6%),
 637 T7 = Nano-Ag (10 ppm)×Sucrose (3%), T8= Nano-Ag (10 ppm)×Sucrose (6%), T9= Static Magnetic Field (SMF;
 638 15 mT), T10= SMF; 25 mT, and T11= Sucrose (1%, w/v). Means with the same letter are not significantly different
 639 from each other (P> 0.05).

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641 **Figure 4.** Changes of mean Relative Fresh Weight (RFW) of cut rose flowers treated with T1 (Nano-Ag (5 ppm),
 642 (a), T2 (Nano-Ag (10 ppm) (b), T9 (c), T10 (d) and T11 (e; solid lines) and the controls (dotted lines). Values are
 643 means±SE.
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 664 **Figure 5.** Changes of mean Water Uptake (g g⁻¹ initial FW) of cut rose flowers treated with T1 (a), T2 (b), T9
 665 (c), T10 (d), and T11 (e; dotted lines) and the controls (solid lines). Values are means±SE, but where bars are
 666 absent, the variation about the mean was less than the diameter of the symbol.
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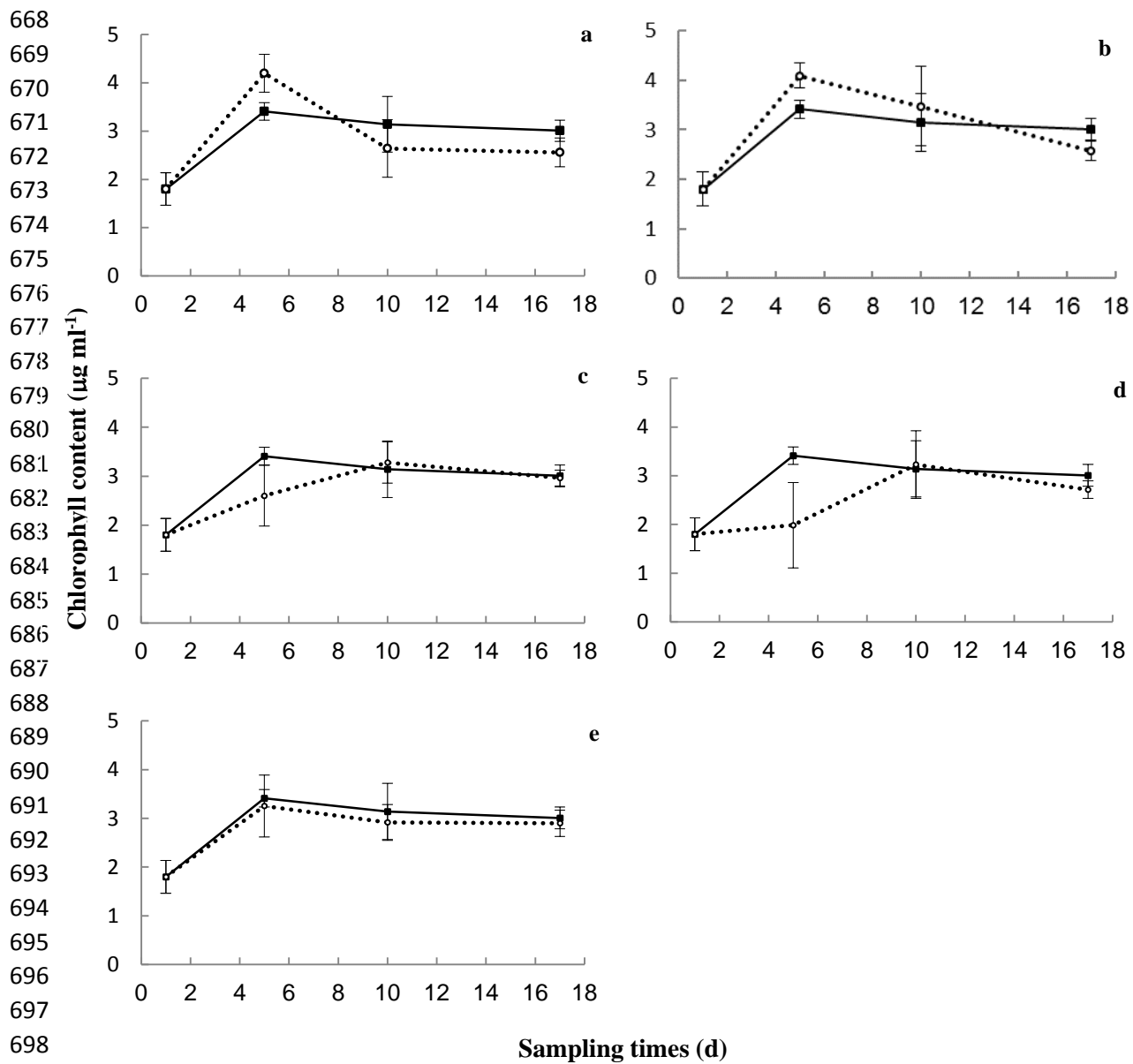


Figure 6. Changes of mean chlorophyll content ($\mu\text{g ml}^{-1}$) of cut rose flowers treated with T1 (a), T2 (b), T9 (c), T10 (d), and T11 (e; dotted lines) and the controls (solid lines). Values are means \pm S.E.

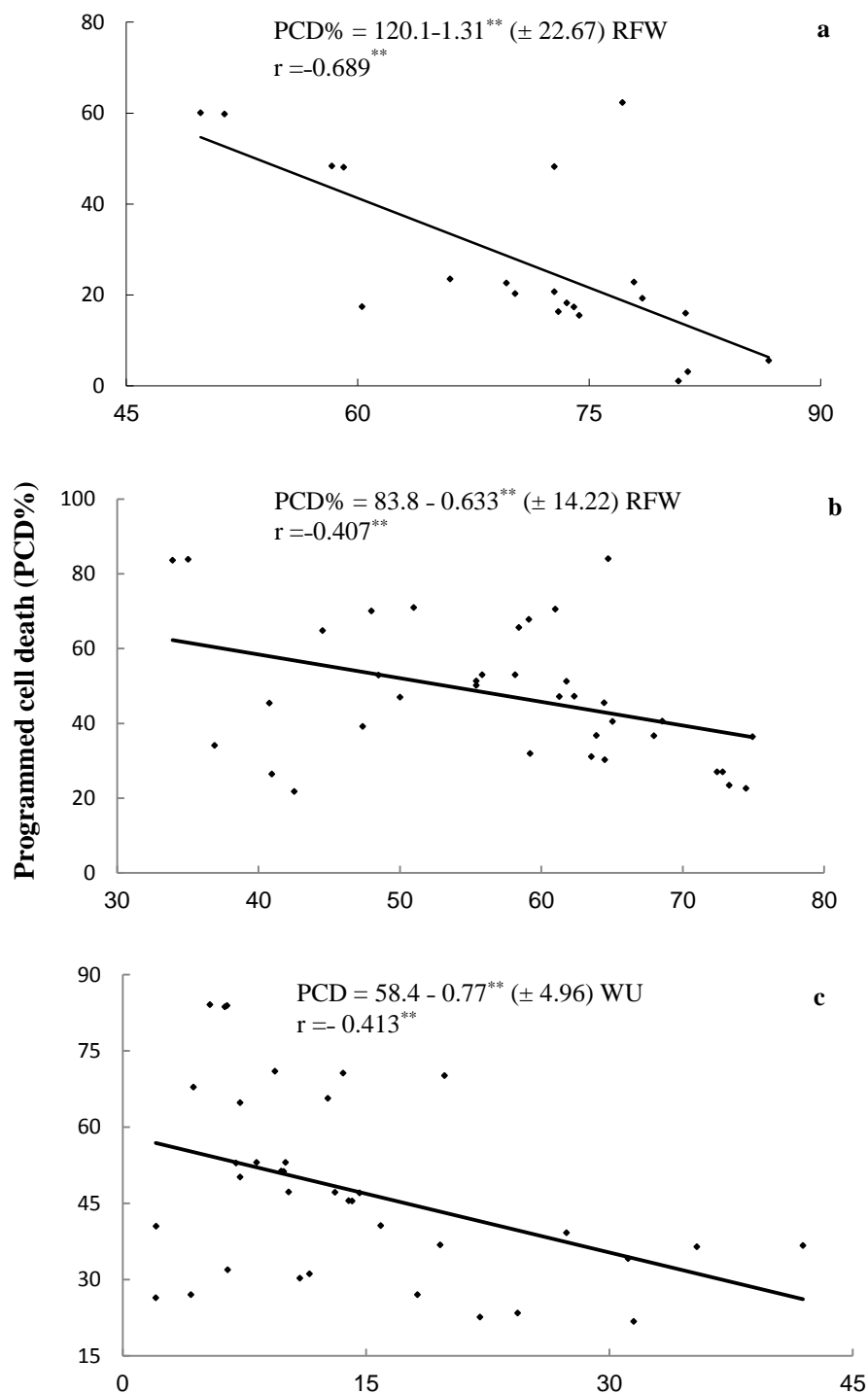


Figure 7. The relationship between the PCD% of treated cut rose flowers and either Relative Fresh Weight (RFW) on 10th day (a) and on 17th day (b), or Water Uptake (WU) on 17th day (c).

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

قاسم کریم زاده، سعید فرهادی، امین باقی زاده، و وحید صیادی

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 فیزیکیوشیمیایی نانو ذرات نقره (Nano-Ag؛ ۵ و ۱۰ پی‌پی‌ام)، 6-بنزیل آدنین (BA؛ ۲۵ و ۵۰ میلی گرم 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 قابل توجهی 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 فیزیکی SMF 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 PCD را 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.