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Biochemical Regulation of Oxidative Stress to Delay Senescence in Gladiolus Cut Flowers

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ABSTRACT

The research was carried out at Arish University, Faculty of Environmental Agricultural sciences during four weeks in April 2022. It was mainly aiming at assessing the basic biochemical and molecular pathways through which multi-component preservative solutions extend the postharvest life of gladiolus cut flowers. The goal of the study was to gauge metabolic efficiency. Respiration rate and cell defense system against oxidative stress activities of Catalase (CAT), Superoxide Dismutase (SOD) and Peroxidase (POD) enzymes as well as Ethylene Sensitivity. Sixteen treatments, which included Sucrose (S), Silver Nitrate ($AgNO_3$), Gibberellic Acid (GA_3) and Benzyladenine (BA) were evaluated. It was found that high longevity is associated with the continued active production of Peroxidase (POD) enzyme specifically during the later stages (Day 28), which is essential in upholding integrity, structural rigidity of the cell wall. Treatments that provided energy (S) and ensured vascular patency ($AgNO_3$) produced the best balance T15 (S + $AgNO_3$ + GA_3 + BA) and T5 (S + $AgNO_3$) turned out to have the highest long-term POD activity. Also, T13 (S + GA_3 + BA) surprisingly had the lowest end-ethylene sensitivity (55.47%), and this indicates a strong, non-chemical physiological resistance to senescence. The study reported that a combination of controlled energy use and enforced defensive efforts with enzymes is the basic approach towards the fulfilment of the postharvest quality and marketability of Gladiolus.

Keywords: Gladiolus, Cut flowers, Antioxidant enzymes, Peroxidase (POD), Respiration rate, Ethylene sensitivity, Oxidative stress, Preservative solutions.

INTRODUCTION

1. Commercial Significance and Physiological Challenges of Gladiolus

Gladiolus (*Gladiolus* sp.), with its spectacular spikes, has a great commercial value in the flower industry especially in floriculture where it is frequently among the leading cut flowers in the world markets (Tomiozzo *et al*., 2018; 2021 and Caveião *et al*., 2022). The economic viability of the industry is reinforced by the existence of such bodies as the FAO that also acknowledge floriculture as a means of livelihood sustainability and income generation in the developing nations (Baudoin *et al*., 2007 and Canton, 2021). In addition, the activities of the FAO also incline into the identification, conservation and exploitation of plant genetic resources in food and agriculture, which supports the greater context of economic activities, based on plants (Cooper *et al*., 1996).

Although this is its natural charm, the after-harvest of Gladiolus is just too short since a complicated interplay of internal physiological processes is too restrictive (Kashyapet, 2016 and Kumarihami *et al*., 2017). The major causes of accelerated senescence are elevated rate of respiration and high rate of water loss, which drain vital carbohydrate stores necessary to open florets (Sharma & Thakur, 2020 and Jhanji & Dhatt, 2021). Moreover, the molecular pathway of senescence is oxidative stress, which is the accumulation of Reactive Oxygen Species (ROS) uncontrollably (Qian *et al*., 2021). The result of this ROS burst is irreversible cellular injury which eventually results in wilting, fading of petals and tissue decay.

2. The Physiological and Biochemical Challenges of Senescence

Although Gladiolus has a great commercial value, the postharvest life of Gladiolus is low owing to rapid senescence which is caused by a number of interconnected physiological failures (Singh *et al*., 2022). Key challenges include:

1. Metabolic Depletion, cut spikes suffer from a high respiration rate and excessive weight loss, which quickly exhaust the limited endogenous carbohydrate reserves needed to sustain vital functions and support floret opening (Kaur *et al*., 2016 and Hatibarua *et al*., 2024).
2. Ethylene and Abscission, Gladiolus also has a high ethylene sensitivity., which induces the adverse effects of wilting, fading, and floret abscision (drop) and results in very fast loss of market appeal (Iqbal *et al*., 2017 and Reid & Wu, 2018). The effects are

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commonly addressed with preservative solutions (Manzoor *et al.*, 2021).

3. Oxidative Stress, at the cellular level, senescence is ultimately driven by oxidative stress--an imbalance resulting from the overproduction of Reactive Oxygen Species (ROS) (Elansary, 2020). The subsequent degradation of the cell's structural components, such as membranes and proteins, is a direct cause of tissue breakdown (Qian *et al.*, 2021).

The plant defends against ROS using a complex enzymatic system, including Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidase (POD) (Zulfiqar *et al.*, 2022). The capacity of any preservative solution to regulate the activity of these enzymes and control the metabolic rate predetermines the maintenance of cellular integrity (Mohibe *et al.*, 2020).

3. Objective of the Study

The aim of the study was to prolong the vas life of Gladiolus with the use of improved preservative solutions as well as to determine the most effective treatment, which provides the longest preservation time (Al-Hasnawi *et al.*, 2019 and Nguyen & Lim, 2021). In particular, the study sought to examine the biochemical activities, such as: Respiration Rate, Ethylene Sensitivity, and the activity of CAT, POD and SOD enzymes, to different multi-component preservative solutions during 28 days to understand the molecular mechanisms of senescence delay.

MATERIALS AND METHODS

1. Experiment Location, Duration, and Design:

This experiment was carried out in the Faculty of Environmental Agricultural Sciences, Arish University, Egypt, in four weeks in April 2022. The experiment involved the harvesting of *Gladiolus hybrida*, Hort. spikes at the commercial stage and a Completely Randomized Design (CRD) of 16 treatments and three repetitions (Green, 1987 and Hatibarua *et al.*, 2024). Analysis of data was done statistically by use of analysis of variance (ANOVA) and the means of the treatments were compared using the least significant difference (LSD) test at $p \leq 0.05$ as explained by Gomez and Gomez (1984).

2. Preservative Solutions and Treatments:

Sixteen solutions were formulated using various combinations of four key components: Sucrose (S: 6%), Silver Nitrate ($AgNO_3$: 150 ppm), Gibberellic Acid (GA_3 : 100 ppm), and Benzyladenine (BA: 50 ppm). The control treatment (T_0) consisted of distilled water (Table 1).

3. Physiological and Biochemical Measurements:

The following traits were assessed at regular intervals (Day 7, 14, 21, and 28):

-Respiration Rate, measured as CO_2 production using the closed-system method (mg CO_2 kg^{-1} h^{-1}).

-Ethylene Sensitivity, assessed by the percentage of floret drop after exposure to C_2H_4.

Table 1. Composition of Preservative Solutions Used in the Experiment

Code	Composition	Sucrose (6%)	$AgNO_3$ (150 ppm)	GA_3 (100 ppm)	BA (50 ppm)
T0	Control (Distilled water)	-	-	-	-
T1	S	✔	-	-	-
T2	Ag	-	✔	-	-
T3	G	-	-	✔	-
T4	B	-	-	-	✔
T5	S+Ag	✔	✔	-	-
T6	S+G	✔	-	✔	-
T7	S+B	✔	-	-	✔
T8	Ag+G	-	✔	✔	-
T9	Ag+B	-	✔	-	✔
T10	G+B	-	-	✔	✔
T11	S+Ag+G	✔	✔	✔	-
T12	S+Ag+B	✔	✔	-	✔
T13	S+G+B	✔	-	✔	✔
T14	Ag+G+B	-	✔	✔	✔
T15	S+Ag+G+B	✔	✔	✔	✔

-Enzyme Activities (CAT, POD, SOD), measured using standard spectrophotometric assays at 240 nm (CAT), 470 nm (POD), and 560 nm (SOD) methods as described in Elansary (2020) and Qian *et al.* (2021).

-Individual Floret Longevity, recorded in days until the floret wilted.

RESULTS AND DISCUSSION

1. The Effect of Treatments on Respiration Rate and Energy Management:

Vital parameters of the spike metabolic health and subsequent collapse of energy are respiration rate (Thakur, 2020). The general tendency was the decrease of the respiration rate between Day 7 and Day 28 in all treatments which signified the course of senescence (Jhanji and Dhatt, 2021).

T_{13} (S+G+B) and T_1 (S) were the most metabolically efficient treatments with respiration rates of 25.60 and 25.83mg CO_2 kg^{-1} h^{-1}, respectively, which are significantly lower than that of the control (35.03) (Table 2). This emphasizes the main role of Sucrose in reducing the disastrous metabolic down turn that typifies senescence, effectively as an external source of respiration and energy efficiency (Kaur *et al.*, 2016 and Mohibe *et al.*, 2020), which is evident in the high rate of respiration found in T13 (81.33 mg CO_2 kg^{-1} h^{-1}) at the beginning of the experiment (Day 7) indicating an initial metabolic push up (Jhanji and Dhatt, 2021). The PGRs (GA_3 and BA) presumably direct this energy to aid in cell expansion and floret opening, which is an energy intensive process (Hoque *et al.*, 2021), and, when powered by sucrose, converts into initial quality and display life (Al-Hasnawi *et al.*, 2019).

The effectiveness of such integrated solutions to accommodate high early metabolic investment and at the same time attain low end respiration rate is instrumental in the prolongation of longevity.

2. The Effect of Treatments on Antioxidant Enzyme Activities:

The capacity to maintain high levels of antioxidant enzyme activity is a proxy for cellular health and resistance to oxidative damage (Elansary, 2020 and Qian *et al.*, 2021).

POD Activity: The Senescence Defense Signature:

The most significant observation is the retention of Peroxidase (POD) activity during the late-phase. T5 (S+Ag) and T15 (S+Ag+G+B) were the most successful treatments in terms of POD activities on Day 28 and were much higher than the control (Table 3).

Table 2. The Effect of Treatments on Respiration Rate (mg CO_2 kg^{-1} h^{-1})

Treatment	Respiration Rate (Day 7)	Respiration Rate (Day 14)	Respiration Rate (Day 21)	Respiration Rate (Day 28)
T0 (Control)	68.60 bns	45.67 dns	39.80 cns	35.03 ans
T1 (S)	58.53 d*	52.87 c*	45.03 b*	25.83c**
T2 (Ag)	75.90 a*	49.37 cns	41.73 cns	35.50 ans
T3 (G)	66.10 cns	57.47 b**	47.03 b*	29.73 b*
T4 (B)	64.80 cns	45.40 dns	44.83 b*	31.40 b*
T5 (S+Ag)	74.73 ans	46.70 dns	44.47 b*	32.10 bns
T6 (S+G)	52.70 d**	51.60 c*	46.43 b*	30.27 b*
T7 (S+B)	66.63 bns	65.47 a**	32.33 d*	29.83 b*
T8 (Ag+G)	72.90 bns	51.60 c*	52.50a**	27.13 c**
T9 (Ag+B)	61.90 c*	60.73a**	34.60 d*	27.93 c**
T10 (G+B)	60.03 c*	63.43 a**	45.43 b*	30.43 b*
T11 (S+Ag+G)	54.60 d**	60.00b**	43.23 bns	27.73 c**
T12 (S+Ag+B)	67.07 bns	47.17 dns	39.03 cns	31.17 b*
T13 (S+G+B)	81.33 a*	54.47 c*	34.40 d*	25.60c**
T14 (Ag+G+B)	78.20 a*	56.57 b**	35.70 dns	30.97 b*
T15(S+Ag+G+B)	66.63 bns	46.67 dns	46.90 b*	30.73 b*
LSD (5%)	6.69	5.34	4.21	3.01

Note: Values represent means of three replicates. Different letters within the same column indicate significant differences among treatments according to the LSD test at $p \leq 0.05$.

ns = not significant; * = significant at $p \leq 0.05$; ** = highly significant at $p \leq 0.01$.

Table 3. The Effect of Treatments on Antioxidant Enzyme Activities (U g^{-1} FW)

Treatment	CAT (Day 7)	CAT (Day 28)	POD (Day 7)	POD (Day 28)	SOD (Day 7)	SOD (Day 28)
T0 (Control)	14.73 cns	11.77 ans	25.60 bns	12.27 ens	24.30 ans	16.30 cns
T1 (S)	17.33 b*	11.83 ans	25.17 bns	18.27 b**	22.40 bns	15.23 cns
T5 (S+Ag)	16.80 b*	11.53 bns	21.43 c*	22.43 a**	20.23 c*	20.67 a**
T13 (S+G+B)	16.90 b*	11.80 ans	28.03 ans	17.93 b**	25.57 ans	14.00 d*
T15(S+Ag+G+B)	19.70 a**	9.17 c**	23.13 bns	21.07 a**	25.13 ans	15.43 cns
LSD (5%)	1.76	1.13	2.48	1.83	2.27	1.63

The effect of treatments on Catalase (CAT), Peroxidase (POD), and Superoxide Dismutase (SOD) activities in U g^{-1} FW over time. Data derived from.

Note: Data represent means of three replicates. Enzyme activities are expressed on a fresh weight (FW) basis. Different letters within the same column indicate significant differences among treatments according to LSD test at $p \leq 0.05$.
ns = not significant; * = significant at $p \leq 0.05$; ** = highly significant at $p \leq 0.01$.

This is crucial since, in addition to H_2O_2 scavenging, POD is important in lignification, which offers the cell walls strength and prevents physical and mechanical deterioration related to senescence (Zulfiqar *et al.*, 2022). This implies that enduring structural protection is one of the characteristics of the most effective solutions.

SOD Activity and Initial Defense:

Treatment T5 (S+Ag) showed better long-term defense as it recorded the highest SOD activity at Day 28 (Table 3). Because SOD is the primary defense against the superoxide radical ($O^-{}_2$) (Elansary, 2020), then it happens that the concerted effect of Sucrose (energy) and Silver Nitrate (anti-microbial/anti-stress) effectively reduced early oxidative load (Manzoor *et al.*, 2021 and Singh *et al.*, 2022), maintaining the enzymatic capacity during the aging process.

CAT Dynamics and T15 Efficiency:

While T15 exhibited the highest initial CAT activity (Day 7), its CAT activity dropped to the lowest value by Day 28 (Table 3). This non-linear pattern is interpreted not as a failure, but as a sign of high efficiency; the robust, integrated protection provided by T15 likely reduced overall cellular H_2O_2 production so effectively that the high-capacity CAT enzyme became less critical in the final stages, which means it is even more effective in the management of stress at the upstream level.

3. The Effect of Treatments on Ethylene Sensitivity and Floret Longevity:

Biochemical stabilization of the flower must be directly transferred to the longevity of the floret and less sensitivity to the senescence hormone, ethylene (Reid and Wu, 2018).

Floret Longevity: The longest individual floret lifespan was observed on Day 21 in T13 (S+G+B) and T15 (S+Ag+G+B) (Table 4), which suggested that energy delivery and hormonal control were achieved to sustain further floret development (Al-Hasnawi *et al.*, 2019 and Sharma & Thakur, 2020). The long-term longevity (Day 28) achieved by T13 demonstrates that a carbohydrate source with the use of PGRs offers the necessary support to the structural and metabolic needs of the floret (Hoque *et al.*, 2021), effectively, using the saved energy by reducing respiration to increase life longevity.

Table 4. The Effect of Treatments on Individual Floret Longevity (days) and Ethylene Sensitivity (%)

Treatment	Individual Floret Longevity (Day 21)	Individual Floret Longevity (Day 28)	Ethylene Sensitivity (Day 28)
T0 (Control)	1.63 dns	1.45 dns	67.10 ans
T1 (S)	1.99 c*	1.70 c*	60.70 b*
T13 (S+G+B)	2.74 a**	2.07 a**	55.47 c*
T15(S+Ag+G+B)	2.66 a**	1.64 c*	64.93 ans
LSD (5%)	0.21	0.18	6.33

The effect of treatments on Individual Floret Longevity and Ethylene Sensitivity (Drop %) at Day 28.

Note: Values represent means of three replicates. Different letters within each column indicate significant differences among treatments according to LSD test at $p \leq 0.05$.
ns = not significant; * = significant at $p \leq 0.05$; ** = highly significant at $p \leq 0.01$.

Recommendation	Best Treatment	Rationale
Optimal Performance Solution	T15 (S + $AgNO_3$ + GA3 + BA)	The most extensive biochemical support, which results in a high POD activity and a controlled metabolic breakdown (Mohibe *et al.*, 2020; Singh *et al.*, 2022 and Zulfiqar *et al.*, 2022).
Non-Silver Ethylene Strategy	T13 (S + GA_3 + BA)	Demonstrates one of the most effective silver-free ways to obtain low ethylene sensitivity and high floret longevity, which can be implemented in cases when silver use is limited (Reid & Wu, 2018; Mittal & Jhanji, 2021 and Nguyen & Lim, 2021).
Focus on POD Management	T5 (S + $AgNO_3$)	The combination of the two is effective in conserving the resources required to sustain the long-term SOD and POD activity, showing that the fundamental components of anti-microbial and energy are critical in the cell defense (Kaur *et al.*, 2016; Ahmad & Rab, 2020 and Manzoor *et al.*, 2021).

Non-Chemical Ethylene Resistance: The most enlightening finding T13 (S+G+B)-a the treatment that did not involve the chemical ethylene inhibitor ($AgNO_3$)-produced the lowest ethylene sensitivity (55.47) at Day 28 which was significantly lower than the control and even T15 (Table 4). This implies that optimal physiological fitness, metabolic activity, and strong enzymatic protection (as shown by the effective respiration rate and high initial SOD of T13) may develop a kind of physiological resistance (Nguyen and Lim, 2021); that suppresses the flower response to ethylene and provides a potential non-silver-based approach to controlling ethylene in late postharvest stages of muscular life (Mittal and Jhanji, 2021).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

This paper confirms the multi-pronged nature of an effective approach to postharvest management of Gladiolus and confirms that a multi-pronged strategy should focus on metabolic and cellular defense mechanisms in tandem. The effective solutions performed better by:

1. **Sustained Cellular Integrity:** The correlation between high POD activity and extended longevity suggests that maintaining cellular structure is the key molecular outcome of effective preservative solutions.
2. **Energy Management:** Sucrose is vital for modulating the respiration rate, preventing rapid energy depletion, and channeling metabolic resources toward defense mechanisms.
3. **Integrated Defense:** A synergist system of $AgNO_3$ (vascular patency, anti-ethylene) and PGRs (hormonal balance) reduces the stress levels of the flower, which in turn enables the antioxidant system of the flower (SOD, POD) to operate to its maximum and in a sustained manner.

The most effective treatment in general of sustaining the flower with vital biochemical functions (Respiration, POD, CAT, SOD) was the quaternary solution T15 (Sucrose 6% + Silver Nitrate 150 ppm + GA3 100 ppm + Benzyladenine 50 ppm).

Recommendations

Based on the biochemical and physiological outcomes, the following practical recommendations are

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الملخص العربي

التنظيم الكيميائي الحيوي للإجهاد التأكسدي لتأخير شيخوخة أزهار الجلاديولس المقطوفة

دينا عبد العاطى سليمان

أُجريت الدراسة في كلية العلوم الزراعية البيئية بجامعة العريش في أبريل ٢٠٢٢ على مدى أربعة أسابيع. هدفت بشكل رئيسي إلى تقييم المسارات البيوكيميائية والجزيئية الأساسية التي تساهم من خلالها المحاليل الحافظة متعددة المكونات في إطالة عمر ما بعد الحصاد لأزهار الجلاديولس المقطوفة. استهدف البحث قياس الكفاءة الأيضية (معدل التنفس) ونظام الدفاع الخلوي ضد الإجهاد التأكسدي من خلال أنشطة إنزيمات الكاتاليز (CAT)، فوق أكسيد ديسموتاز (SOD)، والبيروكسيديز (POD)، بالإضافة إلى حساسية الإيثيلين. تم تقييم ستة عشر معاملة شملت السكروز (S)، نترات الفضة ($AgNO_3$)، حمض الجبريليك (GA_3)، والبنزيل أدينين (BA). أظهرت النتائج أن طول العمر يرتبط باستمرار الإنتاج النشط لإنزيم البيروكسيديز (POD) على وجه التحديد خلال المراحل المتأخرة (اليوم ٢٨)، وهو أمر ضروري لدعم سلامة جدار الخلية وصلابته الهيكلية. المعاملات التي وفرت الطاقة (S) وضمنت نفاذية الأوعية ($AgNO_3$) أنتجت أفضل توازن، حيث أظهرت T15 ($S + AgNO_3 + GA_3 + BA$) و T5 ($S + AgNO_3$) أعلى نشاط طويل الأمد لإنزيمPOD. ووجد أيضاً أن المعاملة T13 ($S + GA_3 + BA$) سجلت أدنى حساسية للإيثيلين في المرحلة النهائية (٥٥.٤٧ %)، مما يشير إلى مقاومة فسيولوجية قوية وغير كيميائية للشيخوخة. تخلص الدراسة إلى أن الجمع بين الاستهلاك المُدار للطاقة والدفاع الإنزيمي المُحصَّن هو الإستراتيجية الأساسية لتعظيم جودة الجلاديولس بعد الحصاد و وصلاحيته التجارية.

الكلمات المفتاحية: الجلاديولس، القدرة الحفظية، الإنزيمات المضادة للأكسدة، البيروكسيديز (POD)، معدل التنفس، حساسية الإيثيلين، الإجهاد التأكسدي، المحاليل الحافظة.