Research Article

Physiological Response of Strawberry CV. Camarosa to 5-Aminolevulinic Acid and Putrescine in Relation to Growth and Reproduction

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Abstract | This study examined the individual and combined effects of foliar-applied 5-aminolevulinic acid (ALA) and putrescine (Put) on the vegetative growth and fruit quality of Fragaria × ananassa Duch. cv. Camarosa under field conditions during the 2024 growing season. A factorial experiment was conducted in a randomized complete block design with three replications, testing three concentrations each of ALA (0, 100, and 200.mg L^{-1}) and Put (0, 200, and 400 mg L^{-1}). Results revealed that the highest concentrations of both ALA (200.mg L^{-1}) and Put (400 mg.L^{-1}) significantly enhanced several key growth parameters, including chlorophyll content, leaf area, number of leaves per plant, and leaf dry matter percentage. Additionally, the combined application of 200 mg.L^{-1} ALA and 400 mg.L^{-1} Put produced the most favorable outcomes in yield components, such as fruit weight (21.99 g), fruit volume (29.89 cm^3), total soluble solids (7.533%), and sugar content, while simultaneously reducing total titratable acidity. Enhanced anthocyanin accumulation was also observed in the same treatment, indicating improved fruit quality. The synergistic interaction between ALA and Put appears to promote photosynthetic efficiency and metabolic activity, contributing to overall yield and fruit biochemical enhancement. These findings support the use of ALA and Put as sustainable and eco-friendly agents for improving strawberry productivity and quality, particularly in cultivars grown under suboptimal conditions.

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Introduction

Strawberry (*Fragaria × ananassa* Duch.) is globally esteemed for its rich flavor, nutritional value, and antioxidant capacity, making it one of the most economically important berry crops cultivated worldwide. Among its cultivars, Camarosa is particularly favored due to its high yield, fruit firmness, and shelf stability. However, to maintain its quality and productivity, the use of bio-stimulants and growth regulators is increasingly being explored as sustainable alternatives to conventional agrochemicals

(Kocaman, 2024) and can be grown in a wide variety of places, making them a favorite for many people due to their lovely scent and excellent flavor, Various kinds of strawberries are grown around the globe, but they are most often cultivated in places with a temperate climate. Strawberry fruits, in the Rosaceae family, are botanically fruits called berries (Afzal *et al*., 2024). Because they offer vitamin C, potassium, iron and many different antioxidants, strawberries are important to human health. In addition, Mushrooms are rich in dietary fiber, secondary metabolites and sugars. Having large amounts of these compounds is good for health because they can help keep blood clots from forming and lower the risk of heart disease (Masoud *et al*.,2024)

Putrescine (Put), a naturally occurring polyamine, plays critical roles in regulating cell division, stabilizing membrane integrity, and modulating stress responses. Exogenous application of Put has been reported to delay senescence, enhance chlorophyll retention, and improve fruit quality traits such as total soluble solids (TSS), fruit weight, and anthocyanin content in various fruit crops, including strawberries (Adak *et al*., 2021). Although individual applications of ALA and Put have been studied across several crops, little is known about their interactive effects on strawberry growth and fruit quality. Previous reports suggest that combinations of bio-stimulants may produce synergistic effects, enhancing physiological and biochemical attributes more effectively than single applications (Kazemi, 2013; Odeh *et al*., 2024).

Applying micro nutrients through foliar application prevents issues with nutrient availability for plants. Adding plant growth regulators and hormones to plants is a successful technique for optimizing photosynthesis. Inside plants, ALA acts as a necessary starting point for tetrapyrrole compounds and supports several functions such as making chlorophyll (Chl) and heme, as well as various hormone-related roles (Yamamoto *et al*.,2015; Bouabid *et al*., 2024). The results from prior studies indicate that ALA could have a big role in agriculture by increasing plant photosynthesis and growth, enhancing the rate at which plants store energy through photosynthesis and improving the quality of fruits by providing an environmentally friendly solution (An *et al*., 2016; Aleshkov *et al*., 2025).

Putrescine belongs to polyamines, a group of chemicals believed to control plant development. These nitrogenous molecules (Skowron and Trojak, 2021) are found in all biological things and it has been shown that foliar use of 150 ppm putrescine can increase the growth and chemical content of festival strawberry fruit (Kuru Berk *et al*., 2023). Both salicylates and polyamines have been found to stop chlorophyll breakdown and shield photosynthetic machinery so that assimilates can be produced. For these phytohormones, plant hormones can improve the natural chemicals present in fruit at both normal and difficult times (Asghari *et al*., 2021).

Therefore, the present study aimed to assess the individual and combined effects of foliar-applied ALA and Put on vegetative growth, yield components, and fruit quality of *Fragaria* × *ananassa* cv. Camarosa under field conditions. This investigation seeks to provide new insights into the use of bio-stimulants for optimizing strawberry production through physiological and metabolic enhancements.

Materials and Methods

This trial was conducted at the University of Diyala's horticulture research station (33°49^{-1}N, 44°30^{-1}E) during the 2023–2024 season. Sandy-textured soil was prepared through deep plowing, leveling, and incorporation of basal fertilizer. A drip irrigation system was installed to maintain consistent soil moisture. The experimental layout used a factorial randomized complete block design with three levels of 5-aminolevulinic acid (ALA: 0, 100, 200 mg.L^{-1}) and three levels of putrescine (Put: 0, 200, 400 mg L^{-1}), conducted over four blocks. Each plot measured 12.8 m^2 (8 m × 1.6 m) and contained five rows of strawberries, each row 2 m long, with plants spaced 0.15 m within rows and 0.25 m between rows. Camarosa seedlings, grown until they reached 3–4 true leaves, were transplanted on September 20, approximately 60 days after sowing.

Foliar treatment protocol

ALA and Put treatments were prepared by dissolving the designated concentration in warm distilled water and allowing the mixture to cool. Beginning six weeks after transplanting and repeated monthly for a total of three applications plants were sprayed until runoff using a hand-held sprayer. Applications were scheduled for early morning to optimize absorption and minimize evaporation.

Measurements and data collection

Morphological traits

Ten flowering plants were randomly selected from each plot; the average of ten plants was taken as one replicate. The following characteristics were recorded for each plant

Relative chlorophyll content (SPAD)

Relative chlorophyll in leaves was assessed with the help of chlorophyll meter. Healthy and fully expended leaves were randomly selected from each treatment as described Ali *et al*. (2021)

Leaf area (cm^2)

Leaf area was calculated from fully developed leaves selected randomly from each treatment as discussed by Ilyas *et al*. (2023) using following formula

LA = L × W × K

Number of leaves per plant

Number of leaves was counted manually from randomly selected plants from each treatment

Fruit weight (g)

For measurement of fruit weight 10 fruits were harvested from each treatment and weighed on digital electronic weight

Fruit volume (cm^3)

Fruit volume was measured through water displacement method as described by Ahmad *et al*. (2016)

Yield weight per plant

For fruit yield 5 plants were selected randomly from each treatment and mature fruits were harvested after regular intervals and cumulative weight was calculated as done by Misbah *et al*. (2024)

Biochemical parameters

Total soluble solids % (TSS)

Fully matured fruits were randomly harvested from each treatment and rinsed to remove dust particles and a drop of juice was placed on digital refractometer and reading was recorded in Brix.

Total sugars%

Total sugars (%) in strawberry fruit was assessed through homogenize mixture of fruit pulp and distilled water followed by filtering and then analyzing using the Lane and Eynon titration method after acid hydrolysis (Afzal *et al*., 2024).

Total titratable acidity %

Known volume of juice was titrated with 0.1 N NaOH, using phenolphthalein as an indicator till constant pink color appearance as done by (Ahmad and Anjum, 2022)

Anthocyanin content (mg 100g/ FW)

Anthocyanin content was measured on UV-visible spectrophotometer at 510 and 700 nm as procedure mentioned (Saqib *et al*., 2025).

Statistical analysis

Data were analyzed using ANOVA (SAS 9.4). Treatment means were separated using Duncan's Multiple Range Test at $P < 0.05$. Both main effects (ALA, Put) and their interaction were evaluated due to the factorial setup.

Results

Morphological attributes

The combined foliar application of 5-aminolevulinic acid (ALA) and putrescine exerted a significant and synergistic influence on a range of vegetative and reproductive parameters in *Fragaria × ananassa* Duch. cv. Camarosa. The data clearly show that increasing concentrations of ALA and putrescine, both individually and in combination, positively affected leaf physiology, fruit development, and overall plant productivity.

In terms of vegetative growth, the leaf dry matter percentage increased progressively with higher concentrations of both regulators. The control plants (0 $mg.L^{-1}$ ALA + 0 $mg.L^{-1}$ putrescine) exhibited the lowest dry matter (26.01%), whereas the combination of 200 mg L^{-1} ALA and 400 $mg.L^{-1}$ putrescine resulted in the maximum dry matter content (31.36%), suggesting enhanced biomass accumulation and improved water-use efficiency (Table 1). This increase in dry matter may be attributed to ALA's role in chlorophyll biosynthesis and energy metabolism, coupled with putrescine's involvement in cell division and membrane stabilization.

Fruit-related traits also exhibited marked improvements with the application of ALA and putrescine. Fruit weight and fruit volume increased significantly under combined treatments, with the highest values recorded in the 200 $mg.L^{-1}$ ALA + 400 $mg.L^{-1}$ putrescine group (21.99 g and 29.89 cm^3, respectively).

Table 1: *Interactive effects of foliar-applied 5-aminolevulinic acid (ALA) and putrescine on physiological (leaf dry matter and leaf area), reproductive (fruit weight, volume, and yield), and morphological traits (number of leaves) of strawberry (Fragaria × ananassa duch. cv. Camarosa).*

ALA (mg. L^{-1})	Putrescine (mg. L^{-1})	Leaf Dry matter (%)	Fruit weight (g)	Fruit volume (cm^3)	yield (g $plant^{-1}$)	Leaf area (cm^2)	Number of leaves per plant
0	0	26.01 C	16.84 d	15.22 d	351.0 d	193.1 b	14.47 e
0	200	26.76 bc	17.75 cd	17.89 cd	376.0 cd	220.1 a	15.13 de
0	400	30.37 ab	18.63 bcd	25.67 b	389.3 cd	206.9 ab	16.67 bc
100	0	26.78 bc	18.31 bcd	18.22 cd	439.7 bc	216.4 a	15.27 cde
100	200	28.19 abc	18.58 bcd	24.22 b	476.3 ab	218.8 a	15.80 b-e
100	400	29.67 ab	20.47 ab	26.67 b	536.6 A	209.4 ab	16.87 b
200	0	28.49 abc	19.69 abc	19.11 c	518.0 ab	211.5 a	15.20 de
200	200	29.63 ab	20.32 ab	25.22 b	551.0 a	225.5 a	16.40 bcd
200	400	31.36 a	21.99 a	29.89 a	540.7 a	214.0 a	18.60 a

Table 2: *Effect of foliar application of 5-aminolevulinic acid (ALA) and putrescine (Put), and their interaction, on biochemical attributes of Fragaria × ananassa cv. camarosa, including total soluble solids (TSS), sugars, titratable acidity, anthocyanin content, and chlorophyll concentration (SPAD values).*

ALA (mg. L^{-1})	Putrescine (mg. L^{-1})	TSS (%)	Total sugar (%)	Reducing sugar (%)	Titratable acidity (%)	Anthocyanin (mg $100g^{-1}$)	Chlorophyll content (SPAD)
0	0	5.467 f	4.071 g	2.530 e	0.655 a	18.91 d	33.90 b
0	200	6.133 de	5.441 ef	3.535 de	0.561 bc	19.54 cd	41.87 a
0	400	6.566 cd	6.181 de	3.969 d	0.500 de	19.63 cd	37.87 ab
100	0	5.700 ef	4.882 fg	3.199 de	0.606 ab	19.32 cd	42.46 a

Similarly, fruit yield per plant followed the same trend, with the maximum yield (551.0 g.$plant^{-1}$) observed under 200 mg.L^{-1} ALA + 200 mg.L^{-1} putrescine, closely followed by 200 + 400 mg.L^{-1} (540.7 g) and 100 + 400 mg.L^{-1} (536.6 g). These results suggest that the combined application of ALA and putrescine at higher doses substantially enhances productivity.

Leaf area and the number of leaves per plant, key indicators of vegetative vigor, also responded positively to the treatments. The largest leaf area (225.5 cm^2) was observed in plants treated with 200 mg.L^{-1} ALA + 200 mg.L^{-1} putrescine, while the highest number of leaves (18.60) was recorded in the 200 mg.L^{-1} ALA + 400 mg.L^{-1} putrescine treatment. These observations imply improved photosynthetic surface area, which could directly support the higher fruit yield recorded.

Biochemical attributes

The biochemical parameters of *Fragaria × ananassa* cv. Camarosa were significantly influenced by foliar applications of 5-aminolevulinic acid (ALA), putrescine (Put), and their combinations. Total soluble solids (TSS) increased markedly across treatments, with the maximum value (7.533%) observed under the combined application of 200 mg.L^{-1} ALA and 400 mg L^{-1} Put. In contrast, the control recorded the lowest TSS (5.467%). Similar trends were observed for total sugars, which increased progressively with the combined doses, peaking at 9.081% under the same treatment (Table 2). This indicates a positive effect of both ALA and Put on carbohydrate accumulation. Reducing sugars also followed this pattern, with the highest content (6.639%) obtained from the 200 mg.L^{-1} ALA + 400 mg.L^{-1} Put treatment, compared to the minimum (2.530%) in untreated plants.

Titratable acidity decreased notably in most treatments, with the lowest value (0.433%) under 200 mg L^{-1} ALA alone, while the control exhibited the highest acidity (0.655%). This inverse relationship between sugar and acid content suggests enhanced fruit ripening and improved palatability due to treatment. Anthocyanin content, a key indicator of fruit pigmentation and antioxidant potential, showed a significant increase in response to treatments. The maximum anthocyanin level (23.22 mg $100g^{-1}$) was recorded under the highest combined application, highlighting the role of these compounds in promoting secondary metabolite synthesis.

Chlorophyll concentration, expressed as SPAD values, also increased across all treatments compared to the control (33.90). The highest SPAD value (44.03) was observed with the application of 100 mg.L^{-1} ALA and 200 mg.L^{-1} Put, indicating enhanced chlorophyll biosynthesis and potential photosynthetic efficiency.

Discussion

Application of 5-aminolevulinic acid (ALA) and putrescine notably improved growth, physiological and yield parameters in *Fragaria* × *ananassa* cv. Camarosa. Significantly, combination of 200 mg.L^{-1}ALA and 400 mg L^{-1} putrescine showed most pronounced effect, indicating a synergistic association that improves both vegetative and reproductive attributes. Increased in leaf dry matter percentage, leaf area and number of leaves per plant under these treatments indicate improved carbon assimilation and photosynthetic activity. ALA plays a vital role as a precursor in the tetrapyrrole biosynthetic pathway, contributing to synthesis of chlorophyll and heme, thus improving light-harvesting efficiency and stress resilience (Sun *et al*., 2023; Ahmad *et al*., 2021).

Putrescine is a polyamine, known for its role in membrane stability, ROS scavenging and mitigation of gene expression under both optimal and stress environment. Exogenous application has been reported to stimulate cell division, improve osmotic balance and delay senescence, resulting in improved overall plant health and productivity (Li *et al*., 2022; Fariduddin *et al*., 2020). In strawberries, foliar application of putrescine has shown to improve fruit weight, firmness and sugar content while reducing physiological disorders (Adak *et al*., 2021). Enhancement in fruit weight (up to 21.99 g), fruit volume (29.89 cm^3) and yield (540.7–551.0 g.plant^{-1}) recorded in current study under combined treatments supports these results and highlights compound's contribution to sink strength and assimilate partitioning.

Synergistic action of ALA and putrescine appears to optimize both source (leaf) and sink (fruit) dynamics. Higher leaf area and chlorophyll rich tissues allow for higher carbon fixation, while polyamines improve cellular metabolism, transport and enzyme activities that promote fruit growth and development. These physiological improvements translate into higher fruit yield and better quality, consistent with recent studies that showed how biostimulants can improve crop productivity through biochemical and hormonal modulation (Kaya *et al*., 2023; Khan *et al*., 2024).

Furthermore, increase in leaf number and leaf area in treated plants suggests enhanced vegetative vigor, which supports reproductive success through greater carbohydrate supply. These results align with earlier reports where exogenous ALA and polyamines improved performance in horticultural crops like tomato, pepper and grapevine by modulating antioxidative capacity and photosynthetic potential (Zhang *et al*., 2023; Ghorbanpour *et al*., 2022). Collectively, evidence supports that foliar application of 200 mg.L^{-1} ALA along with 400 mg.L^{-1} putrescine is an effective approach to enhance physiological functions and fruit yield in strawberries.

Application of 5-aminolevulinic acid (ALA) and putrescine (Put) significantly improved biochemical traits of strawberry fruits, indicating a positive physiological response under foliar treatment. Highest values of total soluble solids (TSS), sugars, anthocyanins and chlorophyll content were recorded with combined application of 200 mg.L^{-1} ALA and 400 mg.L^{-1} putrescine. This suggests that both compounds act synergistically to enhance metabolic activities and fruit quality traits in *Fragaria* × *ananassa* cv. Camarosa.

The increase in TSS and sugars indicates enhanced carbon assimilation and sugar translocation, which may be associated with improved chlorophyll content and subsequent photosynthetic activity. ALA plays a major role in chlorophyll biosynthesis and has been reported to improve light harvesting and energy conversion in plants (Zhao *et al*., 2023; Bouabid *et al*., 2024). Enhanced photosynthetic activity due to ALA treatment could lead to higher accumulation of sugars in fruits, as observed in present study. Similarly, putrescine, a polyamine, regulates carbohydrate metabolism and delays senescence, thus facilitating extended sugar biosynthesis and retention in fruit tissues (Sheteiwy *et al*., 2021; El-Khlifi *et al*., 2024).

Anthocyanin accumulation was also significantly improved with combined treatments. Anthocyanins not only contribute to the red pigmentation in strawberry fruits but are also powerful antioxidants. The synthesis of anthocyanins is closely linked to the availability of sugar substrates and is promoted under increased photosynthetic activity and stress alleviation, both of which are influenced by polyamines and ALA (Zhou *et al*., 2022; Abolghasemi *et al*., 2023). Putrescine stabilizes cell membranes and protects cellular structures during rapid metabolic changes, which may favor pigment biosynthesis and storage.

Interestingly, the combined treatments also resulted in reduced titratable acidity, enhancing the overall flavor balance of the fruits. The reduction in acidity, especially under higher doses of ALA and putrescine, reflects a shift toward sugar acid balance, which is a desirable trait in fruit quality improvement. These findings are consistent with those of El-Sayed *et al*. (2022), who observed a decline in acidity and an increase in sugars with polyamine treatment in fruit crops.

Conclusions and Recommendations

The current results indicate that foliar application of putrescine and 5-aminolevulinic acid (ALA), not only individually but also in combination, notably improves biochemical attributes of strawberry (*Fragaria × ananassa*) cv. Camarosa fruit. Significant, improvements in total soluble solids (TSS), sugar content, anthocyanin content and relative chlorophyll (SPAD) were recorded, especially, plants treated with combination of putrescine and 5-aminolevulinic acid (ALA). These findings indicate that putrescine and ALA positively affect physiological and metabolic pathways associated with fruit quality. The synergistic effect recorded in combination may be linked to improved regulation of photosynthetic activity, biosynthesis of secondary metabolite and mitigation of stress. Such responses are important for enhancing fruit colour, sweetness and nutritional quality. Importantly, improvements were achieved without compromising titratable acidity, indicating a balanced impact on organoleptic traits. These findings highlight potential of integrating biostimulant based approaches into modern horticultural practices for quality improvement and stress resilience in strawberry production.

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Novelty Statement

Enhanced anthocyanin accumulation was also observed in the same treatment, indicating improved fruit quality. The synergistic interaction between ALA and Put appears to promote photosynthetic efficiency and metabolic activity, contributing to overall yield and fruit biochemical enhancement. These findings support the use of ALA and Put as sustainable and eco-friendly agents for improving strawberry productivity and quality, particularly in cultivars grown under suboptimal conditions

Author's Contribution

Z. S. Rashid: Writing first drift.
A. T. Homed: Data analysis.
M. D. Abdulhadi: Collect the data.
F. F. Idan: Supervisor of the project.

Generative AI and AI-assisted technology statement
The authors stated that they didn't use generative AI and AI-assisted technology in preparing this manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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