Research Article

# The Effect of Foliar Application with Glycine and Proline on the Hormonal Content and Vegetative Growth Traits of Grape Saplings

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**Abstract** | The study was conducted to investigate the impact of the amino acids Glycine and Proline on the content of grape seedlings in certain plant hormones and vegetative growth characteristics. The experiment was arranged in a factorial experiment (3×3) trail based on Randomized Complete Block Design (R.C.B.D). The experiment comprised of three levels of Glycine (0, 150, 300) mg·$L^{-1}$, and three levels of proline (0, 100, 200) mg $L^{-1}$ and their combination. The results showed significant superiority in leaf nitrogen, potassium, and protein content at the 150 mg/L Glycine level. Conversely, the 300 mg/L Glycine level achieved the highest rates for leaf phosphorus and chlorophyll content. A decrease in stem length and plant hormone levels was observed at both Glycine treatment levels. Regarding the effect of Proline, statistical analysis revealed that the 100 mg/L level was superior in increasing leaf plant hormone content and stem height. Meanwhile, plants treated with the 200 mg/L Proline level showed a significant increase in leaf phosphorus, potassium, and chlorophyll content.

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## Introduction

The cultivation of the vine has been a successful agricultural and industrial enterprise for centuries both in Iraq and globally. But continuous climate change is a serious challenge to viticulture since years, with temperature shifts and water shortage having influenced the lives of most living things, also the plants, decisively. Grapevine growth requires challenging environmental conditions, which during the summer depend on the temperatures one of the most critical factors affecting plant physiology and abiotic stressor. Its effects are expressed through a dynamic interplay of molecular, biochemical, and physiological processes (Trenti *et al.*,2021). Heat-induced drought leads to a reduction or inhibition of water and carbon uptake (Vandegehuchte *et al.*, 2015), resulting in diminished turgor pressure within plant cells due to stomatal closure (Charrier *et al.*, 2018). This, in turn, suppresses photosynthetic activity (Chaves *et al.*, 2010) and reduces carbohydrate biosynthesis, as chlorophyll degradation in leaves accelerates, causing

yellowing (Bahar *et al.*, 2011). Consequently, the vigor of bud growth declines (Kizildeniz *et al.*, 2021).

Marschner and Marschner (2011) indicated that amino acids play vital roles in plant cells, representing some of the most crucial primary metabolites. Some sources often consider certain amino acids, particularly Glycine and Proline, as secondary metabolites. The presence of these amino acids influences numerous physical and chemical properties of plant cells and tissues, subsequently impacting plant organs. Because they form the building block of proteins, which are the main component of living cells and play vital roles in many cellular metabolic reactions (Bashir *et al.*, 2018; Hussain *et al.*, 2018). They improve the absorption, transport, and metabolism of nutrients, synthesize vitamins, stimulate growth, and increase plant tolerance to environmental stresses such as drought and temperature (Souri and Hatamian., 2019). Numerous studies have indicated that spraying plants with amino acids promotes growth in various plant species (Khan *et al.*, 2012: Lahhob *et al.*, 2025; Tarmooz *et al.*, 2025). It has been observed in many previous studies that the foliar application of various amino acids has improved plant growth, due to their important role in secondary metabolic reactions in plants, and consequently, an increase in biomass production (Soliman *et al.*, 2023). Amino acids represent an optimal and highly effective form of nitrogen that plants can absorb and respond to from organic compounds. In recent years, numerous studies have focused on using amino acids for plant fertilization, especially under harsh environmental conditions (Ma *et al.*, 2017; Khan *et al.*, 2012) confirmed that amino acids are a good source of nitrogen for plants, and their addition to leaves or soil affects plant growth, stimulating the growth of shoots and roots, which is reflected in improved nutrient absorption. This may improve plant growth, especially when exposed to environmental stresses, by increasing chlorophyll synthesis and thus increasing the rate of photosynthesis (Rosa *et al.*, 2022; El-Sharabasy *et al.*, 2015) noted that the optimal concentrations of different amino acids depend on the plant species or even its cultivar. They also highlighted that the effect of amino acids is specific to the type of amino acid.

Limited research has been conducted on the impact of individual amino acids on plants. Therefore, this study aims to investigate the effect of both Glycine and Proline amino acids on the plant hormone content and vegetative growth characteristics of grape saplings.

## Materials and Methods

The study was conducted in the lath house of the University of Baghdad, College of Agricultural Engineering Sciences, Department of Horticulture and Landscape Architecture, Research Station A, from February 1, 2024, to December 1, 2024. One-year-old saplings, as homogeneous as possible in growth, were planted in plastic bags containing a medium of silt soil and peat moss at a ratio of 3:1, with three saplings per experimental unit. All maintenance procedures, including fertilization, irrigation, and insect and fungal disease control, were carried out (Jasim., 2012). The study factors included three levels of the amino acid glycine (0, 150, and 300) mg $L^{-1}$, symbolized as (G0, G150, and G300), respectively. The second factor included three levels of the amino acid proline (0, 100, and 200) mg $L^{-1}$ is coded (P0, P100, P200) respectively.

### *Experimental design*

The experiment was conducted as a factorial experiment (3x3) according to a Randomized Complete Block Design (R.C.B.D), with three replicates and three seedlings per experimental unit. Thus, the number of experimental units reached 27, and the number of saplings reached 81.

### *Studied traits*

#### *Stem length (cm)*

The length of the main stem was measured from the soil surface to the plant's apex using a measuring tape.

### *Leaf nutrient content*

#### *Nitrogen (N %) in leaves*

Determined using the Micro Kjeldahl method as described by Jackson (1958).

#### *Phosphorus (P %) in leaves*

Measured using ammonium molybdate and a spectrophotometer at a wavelength of 882 nm, following the method of (Olsen and Sommers., 1982).

#### *Potassium (K %) in leaves*

Quantified using a flame photometer according to the method of (Page *et al.*, 1982).

*Leaf Content of Auxin-like Substances, Gibberellins, and Cytokinins Using HPLC*

The content of plant hormone-like substances in leaves was determined and calculated based on the method of (Unyayar *et al.*, 1996).

*Leaf protein content*

The percentage of protein in leaves was estimated following the (1) method.

*Leaf Chlorophyll Content (mg·g$^{-1}$ fresh weight)*

Total chlorophyll in the leaves of the saplings was determined based on the method of (Goodwin., 1976).

*Branch carbohydrate content*

The percentage of carbohydrates in branches was measured at the end of November 2024, according to the method of (Joslyn., 1970).

## Results

*Stem length (cm)*

The results showed a decrease in stem length when grape saplings were treated with the amino acid glycine, especially at the 150 mg L$^{-1}$ level, which recorded the lowest stem length of 52.00 cm (Table 1). This did not significantly differ from the 300 mg L$^{-1}$ level, which recorded 52.78 cm. Meanwhile, the control treatment recorded the highest length of 54.22 cm.

Regarding the effect of Proline, its application led to a significant increase in stem length, particularly with the P100 treatment (100 mg L$^{-1}$), which yielded the highest average length of 56.44 cm. This was followed, with a significant difference, by the P200 treatment (200 mg L$^{-1}$) at 50.89 cm, marking the shortest stem length recorded by the Proline treatments. The control treatment for Proline measured 51.67 cm.

The interaction between Glycine and Proline also significantly influenced the main stem length of the grapevine saplings. The $G_0 \times P_{100}$ treatment combination produced the highest average stem length of 64.00 cm, whereas the $G_0 \times P_0$ combination (control × control) recorded the lowest average length of 46.00 cm.

The interaction of Glycine with Proline also affected the length of the main stem of saplings, as the G0*P100 treatment gave the highest average length of 64.00 cm, while the G0*P0 treatment showed the lowest average length of 46.00 cm.

**Table 1:** *Effect of Glycine, Proline and their interactions on stem length (cm) on vine saplings*

| Glycine levels (mg L$^{-1}$) G | Proline levels (mg L$^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 46.00 | 64.00 | 46.00 | 54.22 |
| G150 | 54.00 | 50.00 | 54.00 | 52.00 |
| G300 | 55.00 | 55.33 | 55.00 | 52.78 |
| Effect of P | 51.67 | 56.44 | 51.67 | |
| | | G = 1.184 | | |
| L.S.D (0.05) | | P = 1.184 | | |
| | | G * P = 2.050 | | |

*Nitrogen (N %) in leaves*

The results showed significant differences in leaf nitrogen content in response to glycine treatments (Table 2). The G150 treatment recorded the highest nitrogen percentage in the leaves at 2.280%, significantly surpassing the G300 treatment, which recorded 2.197%. The lowest nitrogen content was observed in the G0 treatment, with a value of 1.939%.

Regarding the effect of Proline foliar application, the results indicated a decrease in leaf nitrogen content with increasing Proline concentration. The $P_0$ treatment (control) showed the highest nitrogen percentage at 2.243%, while the $P_{200}$ treatment (200 mg L$^{-1}$) recorded the lowest value at 2.043%. The $P_{100}$ treatment (100 mg L$^{-1}$) showed an intermediate value of 2.120%.

**Table 2:** *Effect of Glycine, Proline and their interactions on nitrogen (N %) in leaves on vine saplings*

| Glycine levels (mg L$^{-1}$)G | Proline levels (mg L$^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 1.820 | 1.897 | 2.100 | 1.939 |
| G150 | 2.630 | 2.280 | 1.930 | 2.280 |
| G300 | 2.280 | 2.210 | 2.100 | 2.197 |
| Effect of P | 2.243 | 2.129 | 2.043 | |
| | | G = 0.0716 | | |
| L.S.D (0.05) | | P = 0.0716 | | |
| | | G * P = 0.1241 | | |

The interaction between Glycine and Proline showed a clear response. The G150*P0 treatment resulted in

the highest nitrogen percentage, reaching 2.630%, whereas the lowest leaf nitrogen content was 1.820% with the G0*P0 treatment.

*Phosphorus (P %) in leaves*

The result indicates a significant effect of glycine foliar application on the phosphorus content in grapevine leaves (Table 3). The G300 treatment recorded the highest phosphorus percentage at 0.3200%, showing a significant difference compared to both G0 (0.2800%) and G150, which recorded the lowest phosphorus level at 0.2667%.

The results also shows that leaf phosphorus content increased with increasing proline concentration. Treatment P200 yielded the highest percentage, at 0.3200%, surpassing treatment P100, which yielded a phosphorus content of 0.2800%, compared to 0.2767% in treatment P0.

The interaction between Glycine and Proline had a clear influence on phosphorus content. The $G_0 \times P_{200}$ treatment combination showed the highest phosphorus percentage at 0.330%, compared to the control combination, which recorded 0.250%. In contrast, the $G_{150} \times P_0$ treatment recorded the lowest phosphorus content at 0.220%.

**Table 3:** *Effect of Glycine, Proline and their interactions on phosphorus (p %) in leaves on vine saplings*

| Glycine levels (mg $L^{-1}$) G | Proline levels (mg $L^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 0.250 | 0.260 | 0.330 | 0.2800 |
| G150 | 0.300 | 0.220 | 0.280 | 0.2667 |
| G300 | 0.280 | 0.360 | 0.3200 | 0.3200 |
| Effect of P | 0.2767 | 0.2800 | 0.3100 | |
| | | G = 0.01522 | | |
| L.S.D (0.05) | | P = 0.01522 | | |
| | | G *P=0.02636 | | |

*Potassium (K %) in leaves*

The result indicates that increasing Glycine concentration led to a rise in leaf potassium content (Table 4). The highest content was observed with the G300 treatment, reaching 0.973%, which was not significantly different from the G150 treatment's 0.950%. The G0 (control) treatment showed the lowest leaf potassium content at 0.773%.

Regarding the effect of Proline application, the result shows no significant influence on leaf potassium content. The control treatment ($P_0$) recorded the highest potassium level at 0.973%, followed by the $P_{200}$ treatment (200 mg $L^{-1}$) with a significantly lower value of 0.883%. The $P_{100}$ treatment (100 mg $L^{-1}$) recorded the lowest potassium percentage at 0.840%.

Regarding the interaction between glycine and proline, the G150P0 combination resulted in the highest potassium content, reaching 1.200 %, compared to G0P0, which recorded 0.720 %. The lowest potassium content was observed in the G150*P100 treatment, with a value of 0.650%.

**Table 4:** *Effect of Glycine, Proline and their interactions on potassium (k %) in leaves on vine saplings*

| Glycine levels (mg $L^{-1}$) G | Proline levels (mg $L^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 0.720 | 1.000 | 0.600 | 0.773 |
| G150 | 1.200 | 0.650 | 1.000 | 0.950 |
| G300 | 1.000 | 0.870 | 1.050 | 0.973 |
| Effect of P | 0.973 | 0.840 | 0.883 | |
| | | G = 0.0680 | | |
| L.S.D (0.05) | | P = 0.0680 | | |
| | | G * P = 0.01178 | | |

*Leaf content of GA (µg)*

Table 5 reveals a decrease in plant gibberellic acid (GA) content with increasing glycine concentrations. The control treatment recorded the highest GA level at 11.157 µg, whereas the G300 treatment showed the lowest GA content at 3.177 µg.

**Table 5:** *Effect of Glycine, Proline and their interactions on leaf content of GA (µg) on vine saplings*

| Glycine levels (mg $L^{-1}$) G | Proline levels (mg $L^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 10.567 | 13.620 | 9.285 | 11.157 |
| G150 | 1.206 | 7.266 | 4.802 | 4.425 |
| G300 | 0.516 | 4.359 | 4.655 | 3.177 |
| Effect of P | 4.096 | 8.415 | 6.247 | |
| | | G = 0.1391 | | |
| L.S.D (0.05) | | P = 0.1391 | | |
| | | G * P = 0.2409 | | |

Regarding the effect of Proline application, the results revealed significant differences among Proline treatments. The P100 treatment yielded a content of 8.415 µg, surpassing the P200 treatment, which gave

6.247 µg. This is in contrast to the control treatment, which recorded the lowest content at 4.096 µg.

However, a significant increase in plant GA content was observed in the leaves due to the interaction between the two factors. The G0*P00 treatment recorded the highest percentage at 13.620 µg, while plants treated with G300*P100 showed the lowest percentage at 4.359 µg.

*Leaf content of IAA (µg kg$^{-1}$ fresh weight)*

The results in Table 6 showed that the amino acid glycine had no significant effect on this trait. The G300 treatment yielded the lowest auxin content of 0.877 µg, compared to the control treatment, which recorded the highest auxin content of 3.084 µg. It also outperformed the G150 treatment, which recorded 1.223 µg. The results showed that the P100 treatment significantly outperformed the P200 and P0 treatments, yielding 2.326 µg, while the P200 and P0 treatments recorded 1.726 and 1.132 µg, respectively. A clear interaction effect between glycine and proline was observed for this trait. The G0P100 combination yielded the highest auxin content at 3.765 µg, showing a significant increase compared to G300P0, which recorded the lowest value at 0.143 µg.

**Table 6:** *Effect of Glycine, Proline and their interactions on leaf content of IAA (µg kg$^{-1}$ fresh weight) on vine saplings*

| Glycine levels (mg L$^{-1}$) G | Proline levels (mg L$^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 2.921 | 3.765 | 3.084 | 3.084 |
| G150 | 0.333 | 2.008 | 1.223 | 1.223 |
| G300 | 0.143 | 1.205 | 0.877 | 0.877 |
| Effect of P | 1.132 | 2.326 | 3.084 | |
| | | G = 0.0899 | | |
| L.S.D (0.05) | | P = 0.0899 | | |
| | | G * P = 1557 | | |

*Leaf content of cytokinin (µg kg$^{-1}$ fresh weight)*

The results in Table 7 showed that spraying plants with glycine resulted in a significant decrease in the G150 and G300 treatments, yielding 1.439 and 1.034 µg, compared to the control treatment, which yielded the highest level of 3.634 µg.

Conversely, the result indicates that treating plants with Proline resulted in the P100 treatment significantly outperforming others with the highest cytokinin content of 2.739 µg. This was superior to the P200 treatment, which yielded an average of 2.035 µg. The control treatment for Proline gave the lowest plant cytokinin content at 1.334 µg.

The interaction between Glycine and Proline also had a significant effect on this trait. The G0*P100 treatment resulted in the highest plant cytokinin content, reaching 4.436 µg. In contrast, the lowest plant cytokinin content, 0.168 µg, was observed with the G300*P0 treatment.

**Table 7:** *Effect of Glycine, Proline and their interactions on leaf content of cytokinin (µg kg$^{-1}$ fresh weight) on vine saplings*

| Glycine levels (mg L$^{-1}$) G | Proline levels (mg L$^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 3.442 | 4.436 | 3.025 | 3.634 |
| G150 | 0.392 | 2.361 | 1.564 | 1.439 |
| G300 | 0.168 | 1.419 | 1.516 | 1.034 |
| Effect of P | 1.334 | 2.739 | 2.035 | |
| | | G = 0.0890 | | |
| L.S.D (0.05) | | P = 0.0890 | | |
| | | G * P = 1541 | | |

**Table 8:** *Effect of Glycine, Proline and their interactions on leaf protein content (%) on vine saplings*

| Glycine levels (mg L$^{-1}$) G | Proline levels (mg L$^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 11.375 | 12.063 | 13.125 | 12.188 |
| G150 | 16.438 | 14.250 | 12.063 | 14.250 |
| G300 | 14.250 | 13.813 | 13.128 | 13.730 |
| Effect of P | 14.188 | 13.375 | 12.772 | |
| | | G = 0.0794 | | |
| L.S.D (0.05) | | P = 0.0794 | | |
| | | G * P = 0.1379 | | |

*Leaf protein content (%)*

The results shown in Table 8 indicate that the $G_{150}$ treatment (150 mg·L$^{-1}$ Glycine) significantly increased protein content in grapevine saplings, reaching 14.250%. This was significantly higher than the $G_{300}$ treatment (300 mg·L$^{-1}$), which recorded 13.730%, and notably surpassed the control treatment ($G_0$), which had the lowest protein percentage at 12.188%.

Statistical analysis showed that increasing the concentration of the amino acid proline decreased plant protein content. Treatment P0 significantly

outperformed, recording the highest protein content of 14.188%, while treatment P200 recorded the lowest protein content of 12.772%, significantly behind treatment P100, which yielded 13.375%.

Regarding the interaction effect between the two factors, treatment G150*P0 significantly increased protein content to 16.438%, compared to the control treatment, which yielded the lowest protein content of 11.375%.

*Leaf chlorophyll content (mg·g$^{-1}$ fresh weight)*

Table 9 shows that foliar application of glycine exerted an evident effect on chlorophyll content in grapevine leaves. The G300 treatment contained the most chlorophyll at 184.631 mg g$^{-1}$ fresh weight, which was significantly higher than G150 at 126.853 mg g$^{-1}$ fresh weight. The minimum value was found in the G0 treatment with a value of 126.853 mg g$^{-1}$ fresh weight as well.

Regarding the effect of proline, the result revealed that P200 increased leaf chlorophyll content significantly to 206.060 mg g$^{-1}$ fresh weight. This value was markedly higher than that of P100, which was 140.975 mg g$^{-1}$ fresh weight, and the lowest was recorded under control (P0) at 127.370 mg g$^{-1}$ fresh weight.

In terms of the interaction between Glycine and Proline, the $G_{300}\times P_{200}$ treatment combination significantly enhanced chlorophyll content, reaching 240.188 mg·g$^{-1}$ fresh weight. In contrast, the $G_0\times P_0$ treatment recorded the lowest chlorophyll content at 81.758 mg·g$^{-1}$ fresh weight.

**Table 9:** *Effect of Glycine, Proline and their interactions on leaf chlorophyll content (mg·g$^{-1}$ fresh weight) on vine saplings*

| Glycine levels (mg L$^{-1}$)G | Proline levels (mg L$^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 81.758 | 145.769 | 145.769 | 126.853 |
| G150 | 126.902 | 136.902 | 136.902 | 163.921 |
| G300 | 173.452 | 140.254 | 140.254 | 184.631 |
| Effect of P | 127.370 | 140.975 | 140.975 | |
| | | G = 0.0871 | | |
| L.S.D (0.05) | | P = 0.0871 | | |
| | | G * P =0.190 | | |

*Culms carbohydrate content (mg g$^{-1}$)*

The means comparison in Table 10 showed that increasing in Glycine levels showed significantly (P< 0.05) difference in all levels of treatments. Treatment G0 produced the highest carbohydrate content of culms, up to 131,130 mg g$^{-1}$, whereas the carbohydrate content was significantly higher (119,430 mg g-1) than treatment G150, in which the lowest carbohydrate content (117,732 mg g$^{-1}$) was obtained.

Regarding the influence of Proline spraying, the results show that carbohydrate content in the culms was highest in treatment P100 (125,419 mg g$^{-1}$), whereas in treatment P0 the value was 124,973 mg g$^{-1}$. Treatment P200 produced the lowest culm carbohydrate content to 117,900 mg g$^{-1}$.

There was interaction effect observed between Glycine and Proline. The least carbohydrate content in culms (117.017 mg) was observed in the plants treated by G150*P100. g$^{-1}$. This value was greatly less than the control treatment that captured the highest culms carbohydrate amount of 137.400 mg. g$^{-1}$.

**Table 10:** *Effect of Glycine, Proline and their interactions on culms carbohydrate content (mg g$^{-1}$) on vine saplings*

| Glycine levels (mg L$^{-1}$) G | Proline levels (mg L$^{-1}$) P | | | Effect of G |
|---|---|---|---|---|
| | P0 | P100 | P200 | |
| G0 | 137.400 | 137.050 | 118.940 | 131.130 |
| G150 | 118.350 | 117.017 | 117.830 | 117.732 |
| G300 | 119.170 | 122.191 | 116.930 | 119.430 |
| Effect of P | 124.973 | 125.419 | 117.900 | |
| | | G = 0.0762 | | |
| L.S.D (0.05) | | P = 0.0762 | | |
| | | G*P=0.1320 | | |

## Discussion

The presented results clearly demonstrate variations in the vegetative growth response of 'Halwani' grape saplings to different levels of the amino acid Glycine. These differences manifested as both positive and negative effects on the studied traits. A significant impact was observed on leaf nitrogen content (Table 2). This can be attributed to amino acids serving as a nitrogen source for plant nutrition (Souri *et al.*, 2017). This could explain the increase in leaf nitrogen content (Table 2), as amino acids are considered intermediate compounds involved in nitrogen assimilation and represent the main form through which nitrogen is transported during plant growth processes (Kolota *et*

*al*., 2013). This nitrogen availability helps maintain sapling growth when Glycine is applied, either by contributing to protein synthesis or by reducing its degradation (Table 8). Moreover, nitrogen plays a role in the structure of nucleic acids (DNA and RNA) and porphyrin compounds, which are essential components in the formation of chlorophyll and cytochromes (Taiz and Zeiger., 2010). in addition to reducing the rates of chlorophyll decomposition and thus increasing the chlorophyll content of the leaves (Table 9) as amino acids play a role in protecting the components of the cell, as an anti-oxidant and anti-peroxide and decomposition of the components of the cell, especially chlorophyll, which is reflected in increasing the efficiency of the carbon metabolism process (Mohammadipour & Souri., 2019) which led to an increase in the construction and accumulation of carbohydrates in the stomata (Table 10) (Al-Hadethi., 2019). The observed effects may also be linked to potassium's role (Table 4) in stimulating cell division and elongation (Borowski & Michalek., 2009), as potassium is a key factor in plant development. It activates numerous enzymes, including those involved in carbon assimilation, redox reactions, hydrogenation, and energy metabolism. Moreover, potassium plays a crucial role in regulating cellular osmotic pressure and stomatal function, reducing respiration, conserving energy, enhancing photosynthesis through its contribution to plastid development, and boosting chlorophyll content (Table 6), protein synthesis (Table 5), and carbohydrate production (Table 7) (Prajapati & Modi., 2012). The statistical analysis also confirmed a notable increase in the concentrations of nitrogen, phosphorus, and potassium in the leaves, especially at the 300 mg $L^{-1}$ glycine level. This is likely due to glycine's role in improving nutrient uptake and enhancing their accumulation in leaf tissues (Pranckietiene *et al*., 2015).

As for the observed significant decreases in other traits, this may be attributed to the potentially toxic effect of glycine at higher concentrations. Such toxicity can produce physiological responses similar to those caused by excessive fertilization (Rose *et al*., 2022), possibly affecting the biosynthesis and transport of plant hormones (Souri and Hatamian., 2019), which in turn led to reduced main stem length in the saplings.

Regarding the effect of the amino acid proline, the study demonstrated that grapevine saplings responded differently depending on the concentration applied. The statistical analysis of the studied traits revealed a significant increase in stem length (Table 1) at the 100 mg $L^{-1}$ level of proline. This increase may be attributed to the physiological roles of proline, as it serves as a primary building block for proteins and enzymes and plays a role in energy provision (Abdel-Aziz and Balbaa., 2007). Its application promotes longer and more frequent cell divisions by elevating plant hormone levels. The increase in stem length (Table 1) may be indirectly associated with the rise in gibberellin content (Table 8), which promotes stem elongation and plant height through two distinct physiological processes: cell division and the elongation of internal tissue cells. This effect is further supported by an increase in auxin levels (Table 9), which plays a role in cell growth and is crucial for stimulating and modifying gene transcription, followed by translation, and then promoting RNA and protein synthesis. Furthermore, auxins induced by gibberellins play a vital role in promoting cell wall loosening by breaking down and rearranging cell wall bonds into new sites under turgor pressure. This contributes to increased cell volume and expansion. Auxins also influence the enzymes involved in these processes, particularly cellulase, which weakens fiber matrices and affects the construction and degradation of cell wall components. These processes may involve activation of proton ($H^+$) pumping, reduction of cell pH, and increased acidity of the cell wall, leading to altered bonding and greater flexibility of the cell wall. This process alters the plant's water relations, particularly cellular turgor and osmotic pressure, causing water influx into the cell and increased expansion (Taiz and Zeiger., 2010), ultimately leading to increased stem length. This is in addition to the synergistic interaction with cytokinin (Table 10), which is essential for cell division and elongation (Al-Mousawi *et al*., 2024).

Regarding the increase in chlorophyll content in the leaves (Table 6) following the application of 300 mg $L^{-1}$ proline, this may be attributed to proline's function as a nitrogen source essential for chlorophyll biosynthesis. Alternatively, it may serve as a respiratory substrate, thereby enhancing energy availability for anabolic processes. Proline is also known to delay leaf senescence (El-Hammady *et al*., 1999; Obaid and Hassan., 2019). or the reason may be due to the role of plant growth regulators (gibberellins, auxins, and cytokinins) in improving vegetative and root growth, which was reflected in the increase in chlorophyll

pigment (Al-Samarai *et al*., 2020), thus raising the rate of photosynthesis and the production of carbohydrates, thus increasing the materials stored in the culms. As for the increased nitrogen (Table 2) and phosphorus (Table 3) content of leaves, this may be due to the role of the amino acid proline, when added as a primary nitrogen source, as previously mentioned, in building proteins and enzymes and providing energy that encourages vegetative growth, which is reflected in root system growth and, consequently, increased nutrient absorption from the soil (Abdel-Aziz and Balbaa., 2007; Altotanje and Joody., 2019).

## Conclusions

The findings of the current study showed that glycine and proline foliar application had a noticeable effect on growth and physiological parameters of Halawni grapevine saplings. Leaf nitrogen, potassium and proteins were increased by 150 mg $L^{-1}$ glycine, whereas 300 mg $L^{-1}$ level increased the content of phosphorus and of chlorophyll. But when applied at higher levels this delayed stem elongation and hormone levels. Proline at 100 mg $L^{-1}$ enhanced stem and hormonal activity while at 200 mg $L^{-1}$ increased leaf nutrient and chlorophyll contents. The interaction between glycine and proline showed a significant synergistic effect, notably at (G0*P100); which suggests the role of balanced amino acid application in improving growth and physiological potentiality under stress conditions.

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## Novelty Statement

This experiment was able to identify the best levels of amino acids for improving plant growth. The research also aimed to determine which ones are best.

## Author's Contribution

**Hussain N. R. AL-Karaawi:** Methodology, writing, original draft preparation.
**Thaer R. Awad:** Reviewing and editing.
Author has read and agreed to the published version of the manuscript

### *Generative AI and AI-assisted technology statement*

Generative AI and AI-assisted technologies were used only to assist in searching for relevant references. The authors are fully responsible for the selection, interpretation, and use of all sources cited in this manuscript

### *Conflict of interest*

The authors have declared no conflict of interest.

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