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Research Article

Foliar Application of Alanine, Glutamine and Tryptophan Improves the Growth Parameters and Bunch Quality of Early Sweet Grapevines in Clay Soils under Surface-irrigated System

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Abstract:

The grapevine (*Vitis vinifera*, L) is one of the most profitable fruit crops globally. 'Early Sweet' one of the first commercially marketed grape varieties. This study was carried out over two consecutive seasons (2021 and 2022) in Mansoura City, Dakahlia Governorate, Egypt on 6-year-old Early Sweet grapevines grown in clay soil under surface-irrigated system and trained a quadrilateral cordon trellis system with supported by Spanish baron system . Three amino acids (alanine, glutamine, and tryptophan) were applied as foliar sprays at 100, 200, and 300 ppm, at three growth stages. First, at the beginning of growth in the last week of April when the shoots were between 30 and 40 cm; second, at full bloom, 15 days after the initial application; and third, at the veraison stage, 15 days after the second application. The results indicated that spraying the vines with alanine, glutamine, and tryptophan especially 300 mg/L significantly enhanced yield per vine, bunch weight, berry weight, soluble solids content, total sugars, and carotenoid content, while reducing the incidence of shot berries and total acidity in the berries. Additionally, the treatments improved internode length and thickness, pruning wood weight, ripening wood and total carbohydrates in canes, as well as shoot length, number of leaves per shoot, and leaf area. There were also increases in the percentages of nitrogen (N), phosphorus (P), potassium (K) and total chlorophyll in the leaves as compared to the control group. Thus, these amino acids could be applied in surface-irrigated Early Sweet vineyards to enhance bunch quality and growth characteristics in clay soils.

1. Introduction

Grapevine (*Vitis vinifera*, L) is recognized as one of the most valuable fruit yields globally (Alston and Sambucci, 2019). 'Early Sweet' is among the earliest grape cultivars to be sold commercially. This white, seedless table grape variety has successfully been cultivated in Egypt, offering a mild Muscat flavor and appealing creamy color with a crisp texture, which enhances its market appeal (Uwakiem, 2015). However, this cultivar faces challenges such as low vegetative growth and formation of shot berries (Or et al., 2020). In particular, employing suitable inorganic fertilizer alternatives and amino acids to produce high-quality and nutritious grapes is vital for improving early cultivars for both domestic and foreign markets. This approach is crucial for meeting the demands of sustainable global food security (Cataldo et al., 2020). Applying amino acids as a foliar spray is considered a viable economic treatment for promoting plant development and production. Amino acids are playing a crucial role in the detoxification of heavy metals and essential for plant metabolism (Bashir et al., 2018 and Saudy et al., 2020). Besides, amino acids are considered the structural components utilized in the protein production (Opike and Rolfe, 2005). Its applications in plants are practically limitless, and new applications are always being discovered via continued research. Principal effects include promoting blooming, Improving fruit coloration, flavor, shape, and setting; enhancing the composition of nutrients; enhancing resistance to abiotic stress, improving photosynthesis, boosting stomata opening, enhancing mineral chelation, boosting pest and pathogen resistance, increasing chlorophyll production, promoting vitamin synthesis, impacting various enzymatic systems, and protein biosynthesis (Sharma and Dietz, 2006 and Souri and Hatamian, 2019). Natural amino acids serve as the primary nitrogenous compounds and form the basis for various secondary chemicals such as phenolic compounds, methoxypyrazines, esters, thiols, flavonols, anthocyanins, and higher alcohols in grapes (Guan et al., 2017).

Alanine is an important nutrient for plant growth with significant effects. It is a crucial amino acid involved in the synthesis of plant protein, enhancing enzyme activity and accelerating metabolic processes to promote overall plant health and strength. Alanine also plays a vital role in root development, improving water and nutrient absorption capacity in plants. Additionally, it increases the production of chlorophyll, which improves plants' photosynthetic efficiency and ability to use solar energy (Parthasarathy et al., 2019 and Voet et al., 2006).

In addition, glutamine also is an α- amino acid applied to promote the development of plants and protein synthesis. It exhibits beneficial effects under stress conditions by reducing physiological damage through enhancing antioxidant enzyme activity, promoting plant hormone release, carbohydrate anabolism, and increasing chlorophyll production (Al-Juthery et al., 2020 and El-Metwally et al., 2022). Glutamine also plays vital roles in various metabolic processes, particularly in pathways of nitrogen assimilation (Amin et al., 2011and Marschner, 2011). It serves as one of the most prevalent free amino acids in plants, spite of where it comes from. Glutamine is a primary amino donor for amino acids synthesis and other nitrogen-containing chemicals in plants, crucial for protein and nucleotide production (Marschner, 2011). Additionally, glutamate, derived from glutamine, serves as a signaling molecule across different organisms, contributing to metabolic processes (Cao et al., 2010 and Amin et al., 2011).

Furthermore, tryptophan is an aromatic amino acid produced when chorismate activates the shikimate pathway (Tzin and Galili, 2010). Tryptophan is essential for the synthesis of the plant hormone indole acetic acid (IAA), a type of auxin that regulates several aspects of plant growth, including fruit development, cell elongation, and tropic responses (Maeda and Dudareva, 2012). Studies by Nahed et al., (2009) and Abd-Elkader et al. (2020) showed that tryptophan acid spraying enhanced vegetative growth and production in plants. In addition, tryptophan applied topically to plants increased their overall chlorophyll and carotenoids content. It also enhances berry set by preventing early flower and berry fall as well as the generation of enzymes that catalyze the synthesis of auxin (Saburi et al., 2014). Moreover, Woodward and Bartel, (2005) noted that plants utilize an alternative pathway for IAA synthesis, which involves tryptophan. Therefore, supplying tryptophan to plants promotes the synthesis of indole acetic acid (IAA) over time.

Based on the information provided, the current study aimed to know the effect of foliar application of alanine, glutamine and tryptophan at different concentrations on the growth parameters and bunch quality of Early Sweet grapevines in clay soils under surface-irrigated system.

2. Materials and Methods

2.1 Experimental Site, Design and Treatments

This study was carried out during two consecutive years (2021 and 2022) in the vineyard of EL-Baramon experimental farm located in Mansoura, Dakahlia Governorate, Egypt (31° 26' 45.6" E, 31° 07' 19.2" N). The experiment was conducted on approximately 6-years old of Early Sweet grapevines. The vines were planted at 2 x 3 m intervals cultivated in clay soil under surface irrigation system. At the start of January each season, the grapevines were spur-pruned and loaded with 68 buds per vine, utilizing a quadrilateral cordon trellis system supported by Spanish baron system. Standard agricultural techniques, including fertilizers, irrigation, and pest and disease control, were applied to all vines following guidelines from the Ministry of Agriculture. During the experiment, climatic conditions remained consistent, with average temperatures (21–31 °C), humidity levels (58–64 %), minimal rainfall of 0–5 mm, and approximately 12 hours of sunlight daily.

Herein, the study was set up in a randomized complete block design (RCBD). Ninety vines were selected based on their uniform vigor levels. Ten treatments, three replicates of each, with three vines per replicate, were used for the experiment design. Soil physical and chemical analysis in Table 1 was assessed following the procedures outlined by Chapman and Pratt, (1987).

Table 1. Analysis of mechanical and chemical properties of the experimental soil at a depth ranging from 0 to 90 cm

Mechanical	**Clay (%)**	47.15
	Silt (%)	26.69
	Sand (%)	26.16
	Texture	Clay
	O.M. (%)	2.2
	pH	7.6
Chemical	**E.C. (1:5 extract) (Mmhos/cm)**	0.60
	$CaCo_3$ (%)	1.83
	N (ppm)	28.00
	P (ppm)	10.00
	K (ppm)	249.00

2.2 Impact of three amino acids foliar spray on grapevines

Three amino acids (alanine, glutamine, and tryptophan) (El-Gomhouria Company for Chemicals) were applied via foliar spray at concentrations of 100 ppm, 200 ppm, and 300 ppm on the grapevines using a backpack sprayer (INGCO, HSPP4202). Distilled water was used as the control. The amino acids were applied three times: first, at the beginning of growth in the last week of April when the shoots were between 30 and 40 cm; second, at full bloom, 15 days after the initial application; and third, at the veraison stage, 15 days after the second application. The following parameters were assessed:

2.3 Parameters of vegetative growth

Measurements of vegetative growth were conducted on non-bearing shoots located near the berry set to assess the following parameters;

a) Average of shoots length (cm).
b) Average of leaf surface area (cm^2). Measurements of leaf surface area were performed using a representative sample of mature leaves (6^{th} or 7^{th} leaves) collected from the tops of the same previously measured shoots on various sides of the vine, and applying the following equation cited in Montero et al., (2000);

Leaf surface area (cm^2/leaf) = 0.587 (L x W)

Where, L = Length of leaf blade, W = Width of leaf blade.

c) Number of leaves/shoot

2.4 N, P, and K contents in the leaves:

During fruit set, 20 leaf petioles per replication were collected from leaves opposite the bunch, and their N, P, and K contents were analyzed using the procedures cited by Cottenie et al. (1982).

2.5 The content of total chlorophyll in the leaves

Following berry set, the total chlorophyll content in the leaves was assessed using mature leaves (6th or 7th leaves) sampled from the tops of the same previously measured shoots. Using the techniques outlined by Mackinny (1941), the content of total chlorophyll was measured as mg/g fresh weight.

2.6 Physical properties of bunch and berries

At harvest, once the juice of the berries achieved soluble solids content (SSC) of 16% (Sabry et al., 2009), the following measurements were taken for each grapevine: the mean weight of six bunches per grapevine was calculated to determine the mean bunch weight in grams. Additionally, the mean yield per grapevine in kilograms was derived from this mean bunch weight. The average length and width of the bunch (cm), the weight of 25 berries (g), and the size of the berries (cm^3) were also recorded. Shot berries (%) were calculated as the percentage obtained by dividing the number of shot berries per bunch by the total number of berries per bunch. The number of berries per cluster was also determined.

2.7 Chemical properties of berries

The identical bunches utilized for evaluating the physical attributes of bunches and berries were also employed for analyzing the chemical traits of the berries in the following manner: The SSC (°Brix) of the berries was determined using a handheld refractometer, specifically the Master T model (ATAGO Co., Ltd., Japan). Titratable acidity, measured in grams of tartaric acid per 100 mL of juice, was assessed using the procedures of AOAC (2006). Additionally, total sugar content (%) was analyzed following the procedure of Sadasivam and Manickam (1996), and the content of total carotenoid (mg/100 g fresh weight) was determined using the method outlined in Hsia et al., (1965).

2.8 Measurements of dormant season parameters

Additionally, during the dormant season, various parameters were indicated. Internode thickness was measured using a caliper at the third basal internode and recorded in centimeters (cm). Similarly, internode length was measured using a steel ruler and expressed in centimeters (cm). To determine the wood ripening rate, ten shoots of the current season's growth were marked for each replication. The level of wood ripening was determined by dividing the ripened part length by the shoot total length, as per the procedures outlined by Rizk and Rizk, (1994). Likewise, the weight of pruning wood was measured during the winter months and recorded in kilograms per vine (kg/vine). As well, total carbohydrates in canes (g/100 g dry weight) were measured according to the method outlined by Hodge and Hofreiter (1962).

2.9 Statistical analysis

For this experiment, the RCBD was used. Data were preliminary tested for numerical normality and homogeneity of variance using the Shapiro–Wilk and Levene tests, respectively. Data calculated as percentages were first transformed to the Arcsine square root values before performing the analysis of variance (ANOVA), and results were presented as back-transformed means. All data was analyzed using analysis of variance (ANOVA) and post-hoc Tukey's test with a significance level of 0.05. A two-way hierarchical cluster analysis (HCA) was conducted using the ward minimum variance method. Additionally, a heatmap was generated to visualize multivariate similarities among treatments **(Ward, 1963).** Principal component analysis (PCA) was employed to reduce the dataset's dimensionality and reveal underlying treatment patterns. JMP Data Analysis Software Version 9 (SAS Institute Inc., 270 Cary, NC, USA) was utilized for performing ANOVA, Tukey's tests, HCA, heatmap creation, and PCA.

3. Results

3.1 Spraying Early Sweet grapevines with amino acids increased vine growth

The data in **Figure 1** illustrates the impact of spraying Early Sweet grapevines three times with amino acids (alanine, glutamine, and tryptophan) in the two seasons. Overall, all treatments significantly increased leaf area, shoot length, and leaves number compared to the control, indicating enhanced vine development and vigorous state. As the levels of amino acids increased, all growth indicators generally showed a gradual increase. The highest levels were observed in vines treated with tryptophan at 300 ppm, followed by glutamine at 300 ppm and 200 ppm, and then alanine at 300 ppm Figure 1A, B and C. However, the differences in growth indicators (shoot length and leaf area) between alanine at 100 ppm and tryptophan at 100 ppm were insignificant during both seasons. Similarly, the difference in the number of leaves per shoot between glutamine at 100 ppm and tryptophan at 100 ppm was insignificant across the two seasons. The control group recorded the lowest levels for growth parameters in 2021 and 2022 seasons (116.60 cm, 106.00 m^2, and 19.40 in 2021 and 121.60 cm, 110.00 cm^2, and 20.40 in 2022 for shoot length, leaf area, and the number of leaves, respectively) **(Figure 1).**

3.2 N, P, and K contents in leaf petiole and total chlorophyll in leaves

As shown in Figure 2, the contents of nitrogen N, P, and K in the leaf petiole and the total chlorophyll in the leaves were significantly increased by all amino acid applications (alanine, glutamine, and tryptophan) compared to the control across both seasons. Generally, these parameters increased with higher concentrations of amino acids. Tryptophan at 300 ppm was the most effective in enhancing nutrient content and total chlorophyll in leaves, followed by glutamine at 300 ppm and alanine at 300 ppm. For N, P, and K at 300 ppm of tryptophan, the values were (1.77, 0.65, and 1.82 % in 2021 and 2.17, 0.72, and 1.92 % in 2022, respectively) (Figure 2 A, B and C). Additionally, there were no significant differences between alanine at 100 ppm, 200 ppm, and tryptophan at 100 ppm regarding total nitrogen (1.53, 1.53, and1.53 % in 2021 and 1.83, 1.86, and 1.73 % in 2022, respectively) and total chlorophyll (12.00, 12.3, and 12.90 in 2021 and 12.50, 12.80, and 13.6 mg/g in 2022, respectively. The control group consistently obtained the lowest values for nutrient contents and leaf chlorophyll content in the 2021 and 2022 seasons (Figure 2).

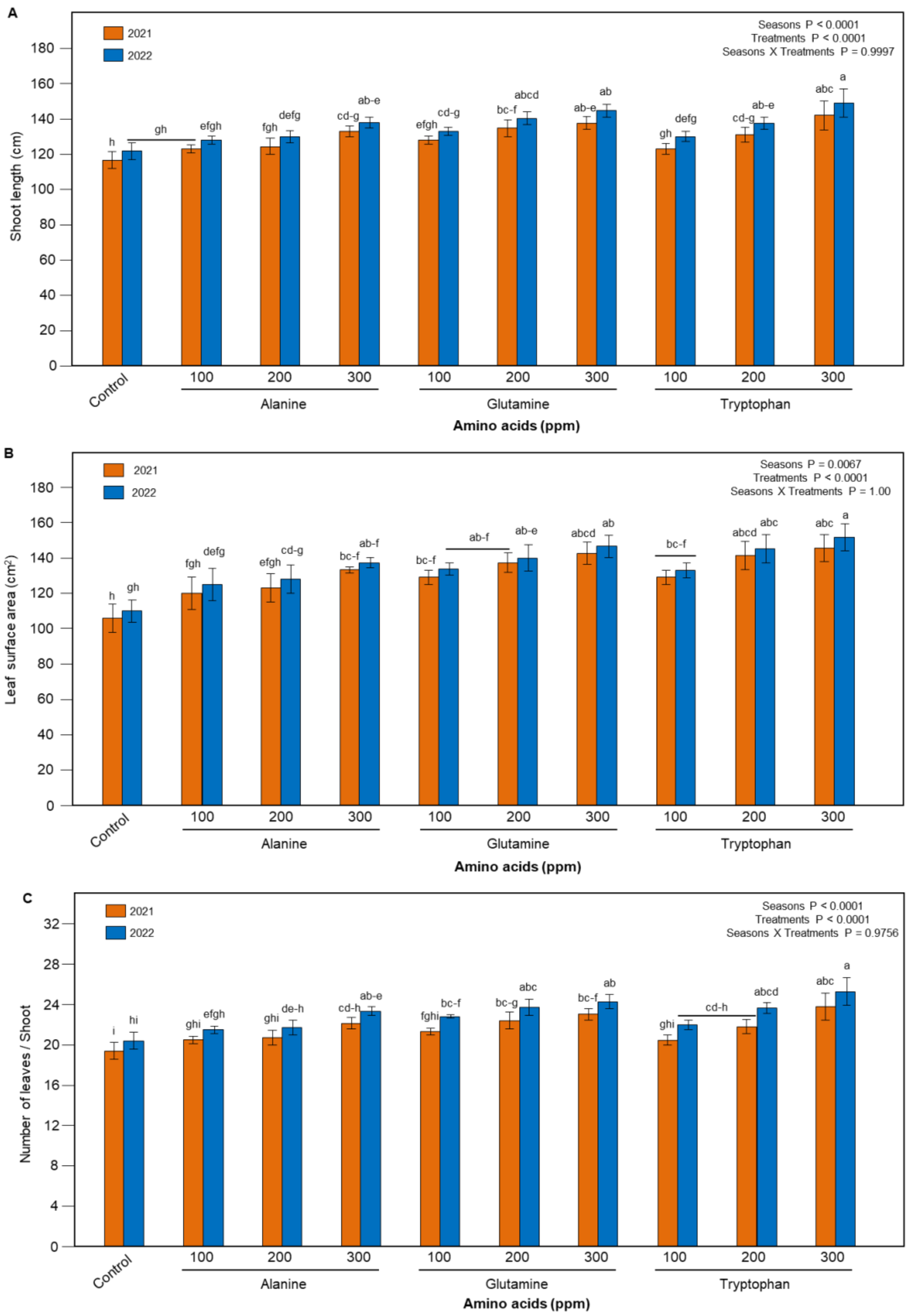

Figure 1. Impact of foliar application of alanine, glutamine and tryptophan on vine growth in the leaves of Early Sweet grapevines during the 2021 and 2022 seasons. (A) Shoot length, (B) Leaf surface area, and (C) Number of leaves/Shoot. Each bar represents the mean value of the respective parameter for each treatment, and error bars indicate the standard error of the mean. Values are the means of three replicates (n = 3). Different letters identify significant differences at $p < 0.05$, determined through analysis using two-way ANOVA followed by Tukey's test.

3.3 Yield and bunch characteristics

Figure 3 illustrates the impact of different applications on bunch characteristics and yield. The results showed that, the applying amino acids three times during the growth start, full bloom, and version stages produced the most significant improvements in bunch quality and yield compared to the control in both seasons. Tryptophan at 300 ppm resulted in the highest yield per vine and best bunch quality in both seasons. Furthermore, increasing the concentration of amino acids led to a significant rise in the parameters compared to the control (Figure 3). Additionally, there were no significant differences between alanine at 300 ppm (11.63 and 11.91 Kg, in 2021 and 2022, respectively) and glutamine at 300 ppm (12.02 and 12.52 Kg) in 2021 and 2022, respectively in yield per vine (Figure 3A). The data also showed no significant differences between alanine at 100 ppm and 200 ppm, between glutamine at 200 ppm and 300 ppm, and between tryptophan at 200 ppm and 100 ppm regarding bunch properties in both seasons. The control treatment consistently recorded the lowest values in the 2021 and 2022 seasons (Figure 3A).

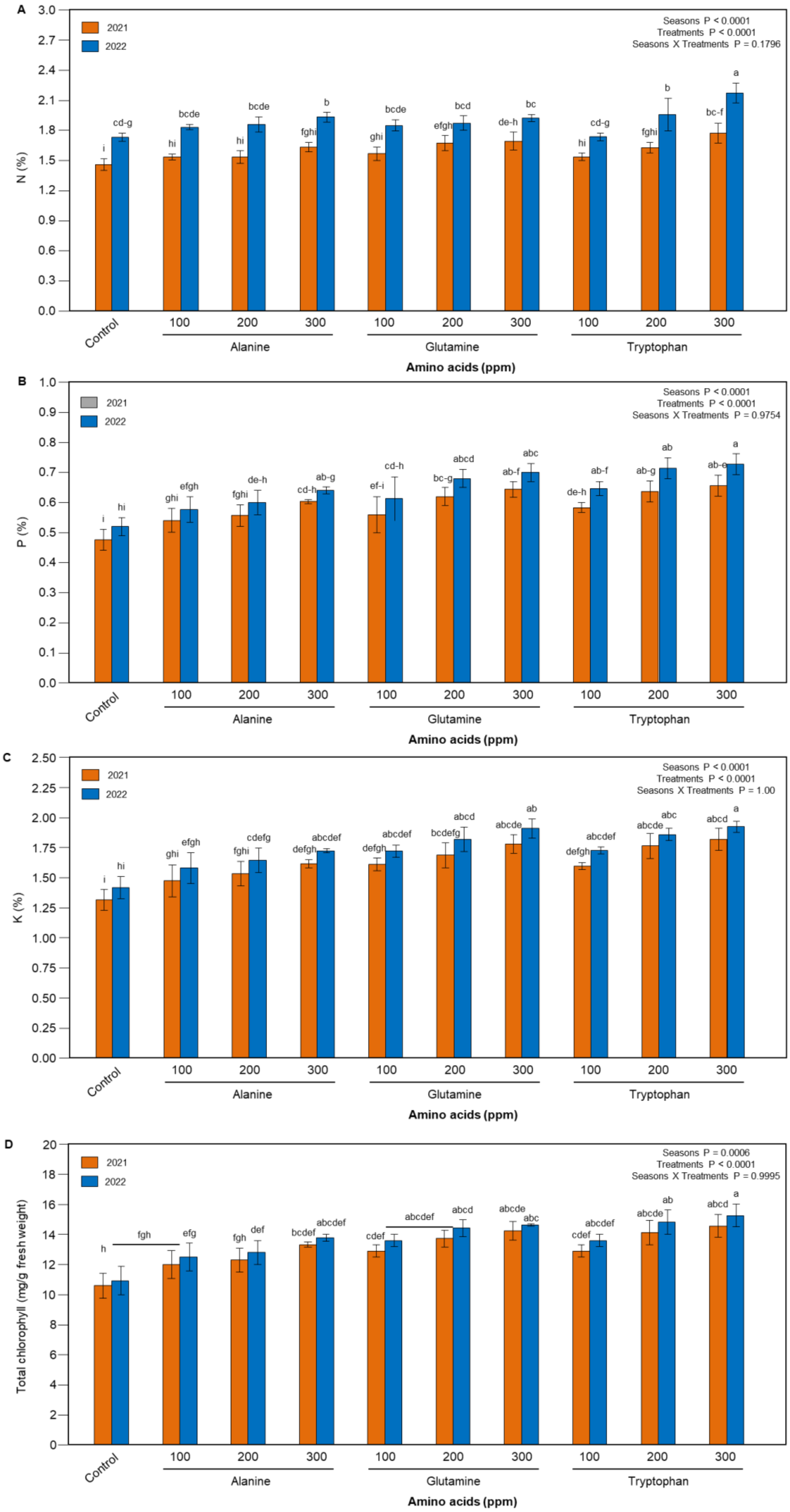

Figure 2. Impact of foliar application of alanine, glutamine and tryptophan on nutrient contents and total chlorophyll in the leaves of Early Sweet grapevines during the 2021 and 2022 seasons. (A) N (%), (B) P (%), (C) K (%), and (D) Total chlorophyll (mg/g fresh weight). Values are the means of three replicates (n = 3). Different letters in the column indicate significant differences among the treatments at $p < 0.05$ as analyzed using two-way ANOVA followed by Tukey's test.

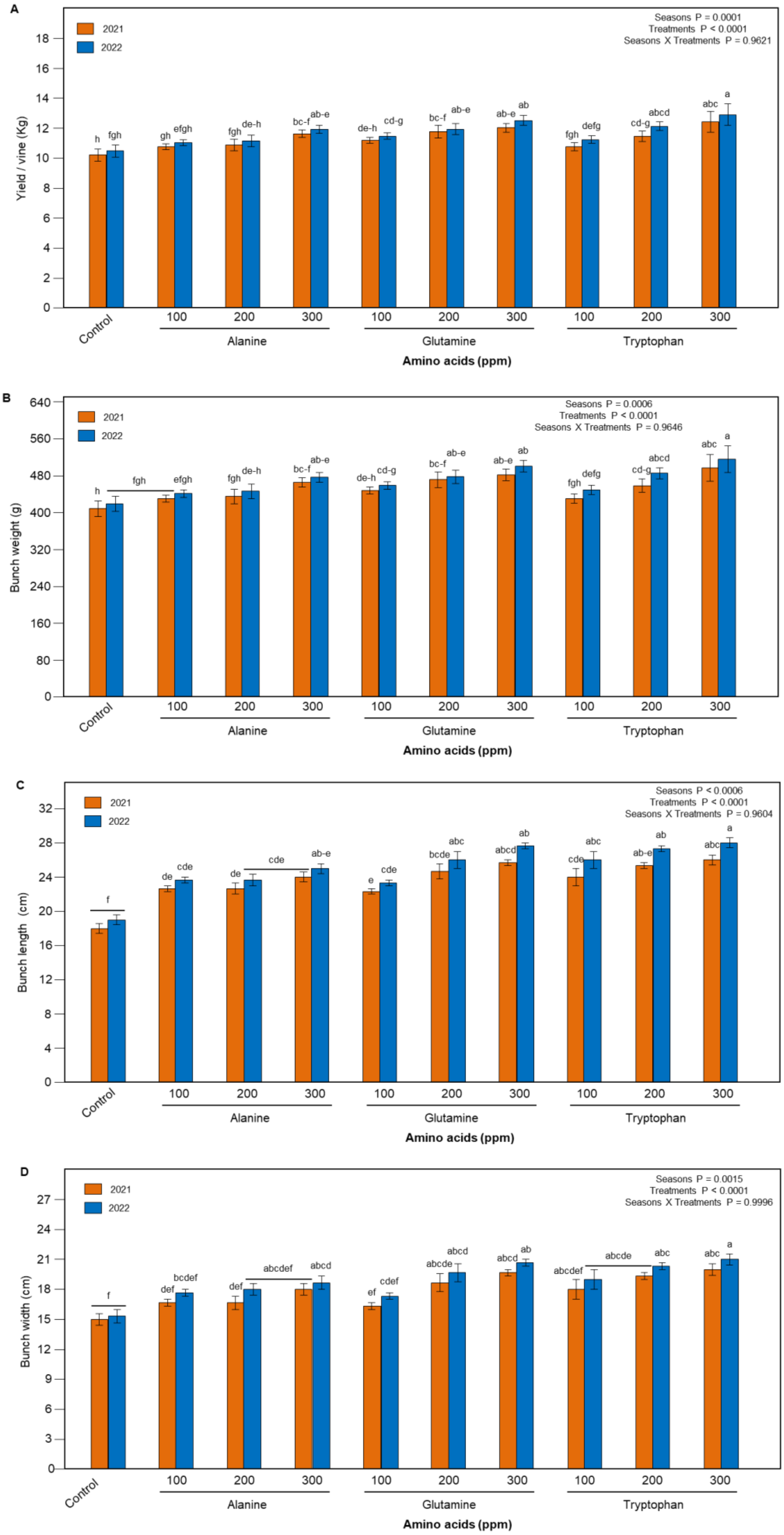

Figure 3. Impact of foliar application of alanine, glutamine **a**nd tryptophan on yield and bunch characteristics in the Early Sweet grapevines during the 2021 and 2022 seasons. (A) Yield per vine, (B) Bunch weight (g), (C) Bunch length (cm), and (D) Bunch width (cm). Values are the means of three replicates (n = 3). Different letters in the column indicate significant differences among the treatments at $p < 0.05$ as analyzed using two-way ANOVA followed by Tukey's test

3.4 Physical properties of berries

Data in Figure 4 clearly showed that applying amino acids (alanine, glutamine, and tryptophan) at three different stages, growth start, at full bloom stage, and at version stage, significantly increased 25 berry weight, berry size, and number of berries per bunch while reducing the number of shot berries compared to the untreated control in both seasons. Tryptophan at 300 ppm yielded the highest increases in 25 berry weight, berry size, and the number of berries per bunch, as well as the lowest number of shot berries in 2021 and 2022, respectively, as compared with other treatments. In contrast, the control showed the least favorable results for 25 berry weight (102.0 and 105.0 g), berry size (20.2 and 21.0 cm^3), and the number of berries per bunch (92.33 and 98) and had the highest number of shot berries (11.5 and 10.9 %) in both seasons, respectively (Figure 4). Additionally, there were no significant differences between glutamine at 300 ppm and tryptophan at 200 ppm regarding 25 berry weight and berry size in both seasons. Similarly, there were no significant differences between glutamine at 200 ppm and 100 ppm for 25 berry weight and berry size in both seasons. Furthermore, the number of shot berries did not significantly differ between alanine at 300 ppm, glutamine at 200 ppm and 300 ppm, and tryptophan at 100 ppm and 200 ppm on the two seasons (Figure 4).

3.5 Chemical properties of berries

The application of the amino acids at three different stages, as explained previously, improved the chemical properties of berries. Specifically, it increased the soluble solids content (SSC), total sugars, and carotenoids in the berry skin while reducing titratable acidity during the 2021 and 2022 seasons, as shown in Figure 5. Spraying glutamine at 300 ppm resulted in the highest values for the chemical properties of berries, including SSC (17.60 and 17.90 %), total sugars (14.08 and 14.49 %), and carotenoids in berry skin (4.90 and 5.00 mg/100g fresh weight), while also reducing titratable acidity (0.56 and 0.49 %) during the 2021 and 2022 seasons, respectively. In addition, data showed that carotenoids in berry skin significantly increased over the two years with the use of amino acids compared to the control (Figure 5D). In contrast, the control treatment recorded the lowest values for these chemical properties (SSC; 16.20 and 16.40 %, titratable acidity; 0.66 and 0.61 %, total sugars; 13.01 and 13.33 %, and carotenoids; 4.31 and 4.68 mg/100 g) in both the 2021 and 2022 seasons, respectively.

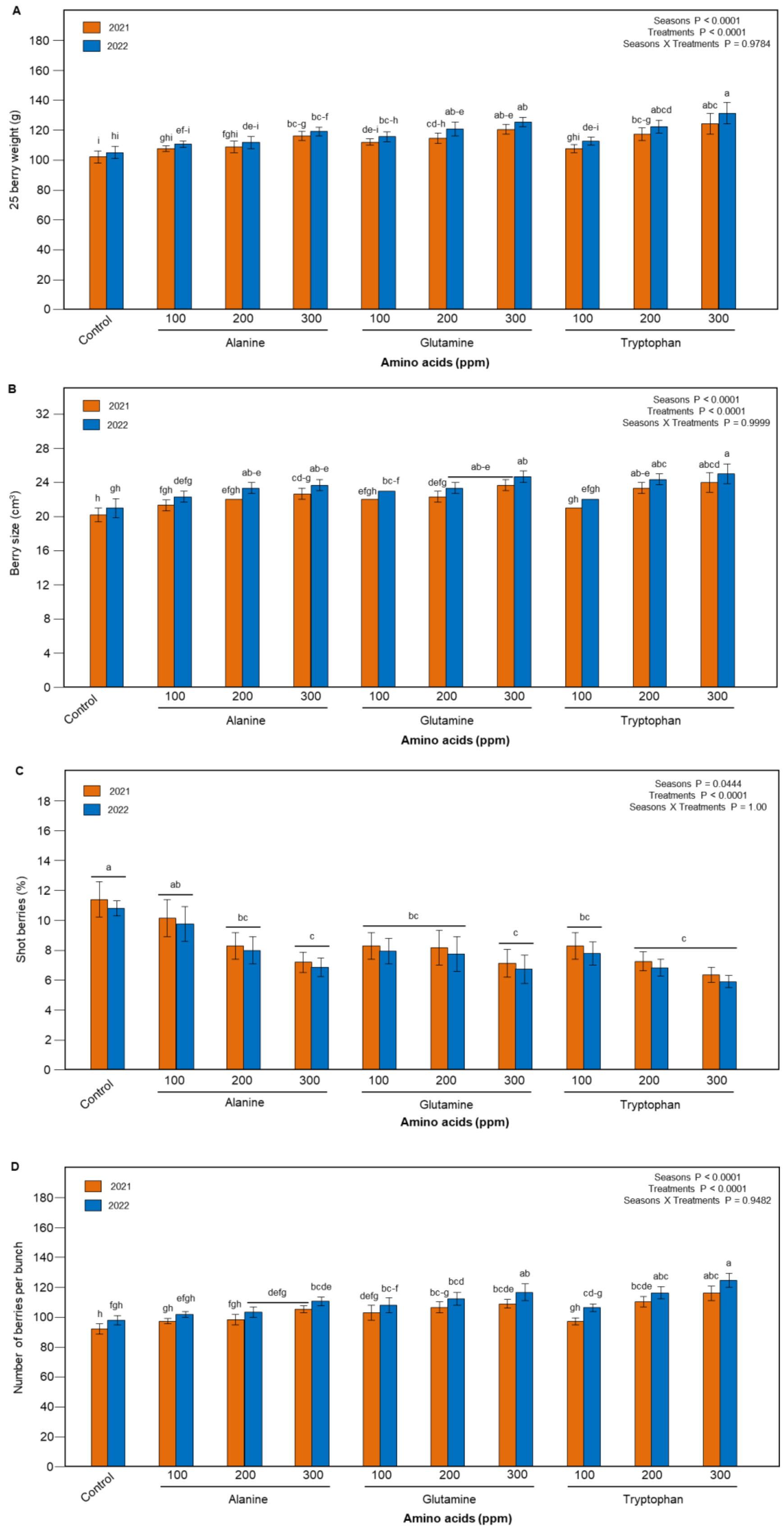

Figure 4. Impact of foliar application of alanine, glutamine and tryptophan on the physical properties of berries in the Early Sweet grapevines during the 2021 and 2022 seasons. (A) 25 berry weight (g), (B) Berry size (cm^3) (g), (C) Shot berries (%), and (D) Number of berries per bunch. Values are the means of three replicates (n = 3). Values marked with different letters specify significant differences at p < 0.05, determined through analysis by two-way ANOVA followed by Tukey's test.

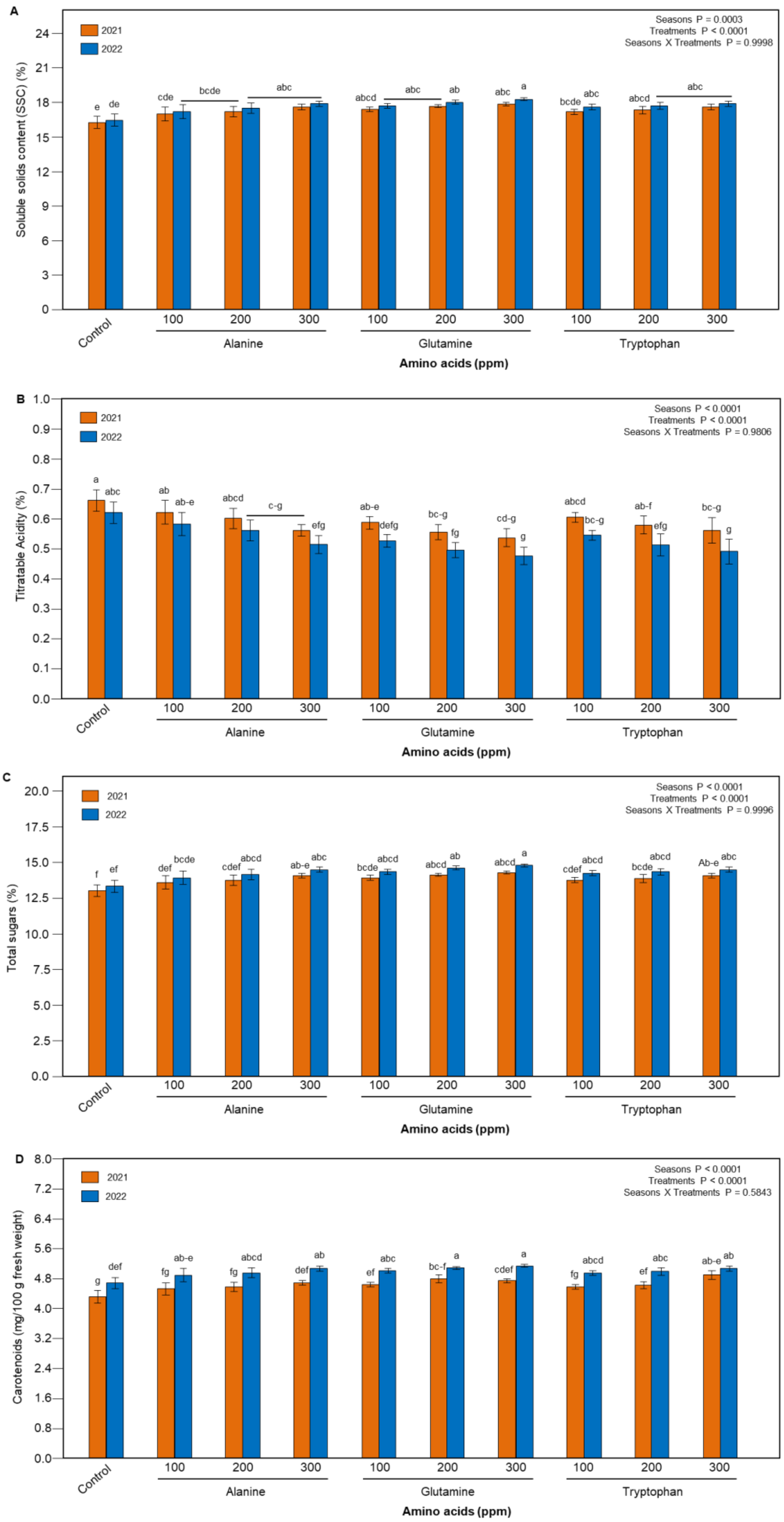

Figure 5. Impact of foliar application of alanine, glutamine, and tryptophan on the Chemical properties of berries in berry skin of the Early Sweet grapevines during the 2021 and 2022 seasons. (A) Solid soluble content (SSC %), (B) Titratable Acidity (%), (C) Total sugars (%), and (D) Carotenoids (mg/100 g fresh weight). Values are the means of three replicates (n = 3). Values marked with different letters indicate significant differences at $p < 0.05$, determined through analysis via two-way ANOVA followed by Tukey's test

3.6 Dormant season parameters

Internode length and thickness, pruning wood weight, ripening wood, and the total quantity of carbohydrates in canes are significant factors influencing vine vigor and berry quality for the following year. Data in Figures 6 and 7 indicated that all treatments applied three times, as earlier explained, and improved the previous parameters compared to the control in both seasons of the study. The most effective application was tryptophan, followed by glutamine and alanine in descending order. Vines treated with foliar application of tryptophan at 300 ppm showed the highest significant values (internode length; 7.63 and 7.23 cm, internode thickness 1.18 and 1.22 cm, pruning wood weight; 2.36 and 2.55 Kg, ripening wood; 87.40 and 89.40 %, and total carbohydrates in canes; 32.50 and 32.10 g/100g DW, respectively as compared to other treatments. As well, the control treatment recorded the lowest values (internode length; 6.20 and 6.36 cm, internode thickness 0.97 and 1.02 cm, pruning wood weight; 1.94 and 2.15 Kg, ripening wood; 63.60 and 67.90 %, and total carbohydrates in canes; 23.50 and 23.8 g/100g DW, respectively in 2021 and 2022.

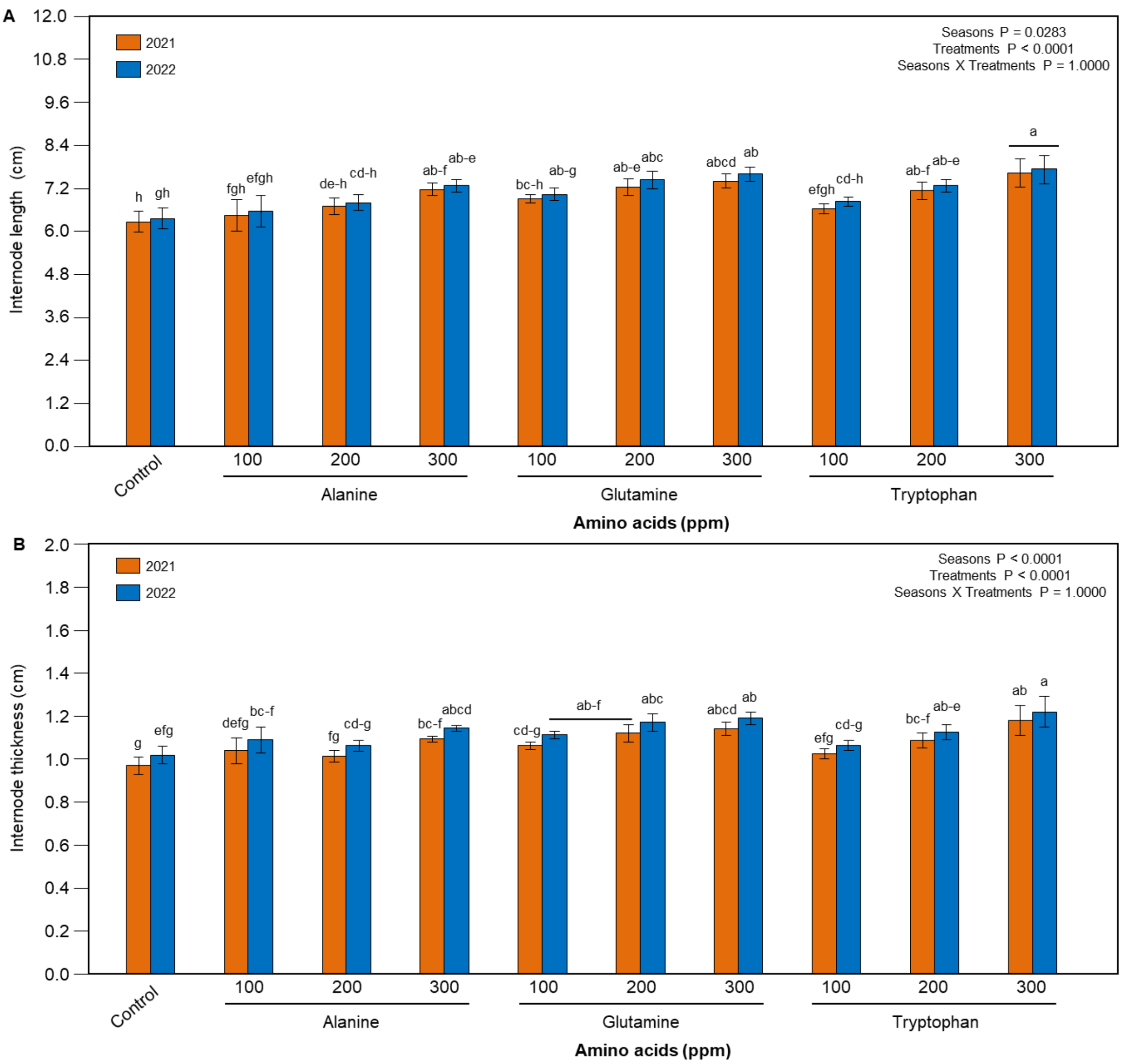

Figure 6. Impact of foliar application of alanine, glutamine and tryptophan on the dormant season parameters of the Early Sweet grapevines during the 2021 and 2022 seasons. (A) Internode length (cm) and (B) Internode thickness (cm). Values are the means of three replicates (n = 3). Values with different letters are significantly different at $p < 0.05$ as analyzed using two-way ANOVA followed by Tukey's test.

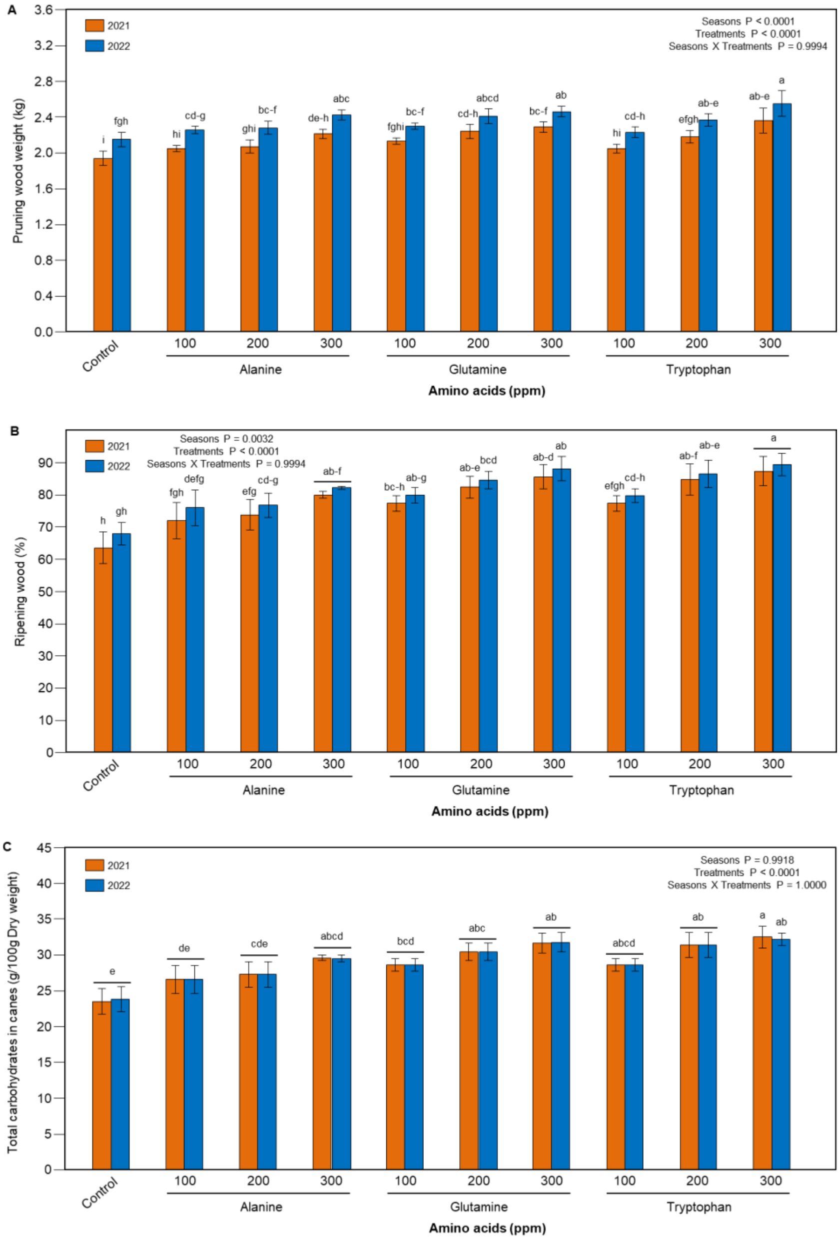

Figure 7. Impact of foliar application of alanine, glutamine and tryptophan on (A) pruning wood weight, (B) ripening wood, and (C) total carbohydrates in canes of Early sweet grapevines during 2021 and 2022 seasons. Values are the means of three replicates (n = 3). Values marked with different letters indicate significant differences at $p < 0.05$, determined through analysis using two-way ANOVA followed by Tukey's test.

3.7 Principal component analysis and heat map illustrates the impact of amino acids on Early sweet grapevines

The principal component analysis (PCA) biplot (Figure 8A) and loading plot (Figure 8B) together offer a detailed insight into the effects of foliar applications of three amino acids (alanine, glutamine, and tryptophan) at varying concentrations (100, 200, and 300 ppm) on Early Sweet grapevines over the 2021 and 2022 seasons. Combined, PC1 and PC2 explained 91.4% of the total variance in the data, with PC1 accounting for 81.2% and PC2 contributing 10.2%. The PCA biplot displayed the distribution of treatments according to their principal component scores, showing clusters of treatments that are closely situated and overlapping, indicating similar responses. Furthermore, the analysis revealed a significant impact of tryptophan on the parameters, based on a distinct cluster separate from the control was detected. Additionally, glutamine showed a noticeable impact compared to the control. Also, alanine indicated a less impact from the control compared to tryptophan and glutamine in both seasons (Figure 8A). Similarly, the loading plot's direction and length obtained each parameter's contribution to the two principal components. Total chlorophyll, total carbohydrates, P, and K showed high similarity, being positioned closely together. Conversely, TSS, total sugars, carotenoids, and N were located oppositely total chlorophyll, total carbohydrates, P, and K, indicating an inverse relationship. Post-treatment, all parameters exhibited increasing levels, whereas titratable acidity showed decreasing levels, as illustrated in Figure 8B.

Besides, Figure 8C visually illustrates the levels of various parameters under the influence of three amino acids (alanine, glutamine, and tryptophan) at different concentrations compared to the control in Early Sweet grapevines during the 2021 and 2022 seasons. Cluster 1 represents vine growth, nutrient contents (N, P, and K), total chlorophyll, yield and bunch quality, the physical properties of berries, the chemical properties of berries, the dormant season parameters, pruning wood weight, ripening wood, and total carbohydrates, while cluster 2 represents shoot berries and titratable acidity. Generally, parameters in Cluster 1 were increased compared to the control, with the highest levels observed in tryptophan, followed by glutamine and alanine, in both seasons. On the contrary, parameters in Cluster 2 decreased compared to the control, with the lowest levels recorded in tryptophan, followed by glutamine and alanine, in 2021 and 2022 seasons (Fig. 8C). The varying treatments highlighted the differential expression and activity levels, emphasizing their impact on these parameters.

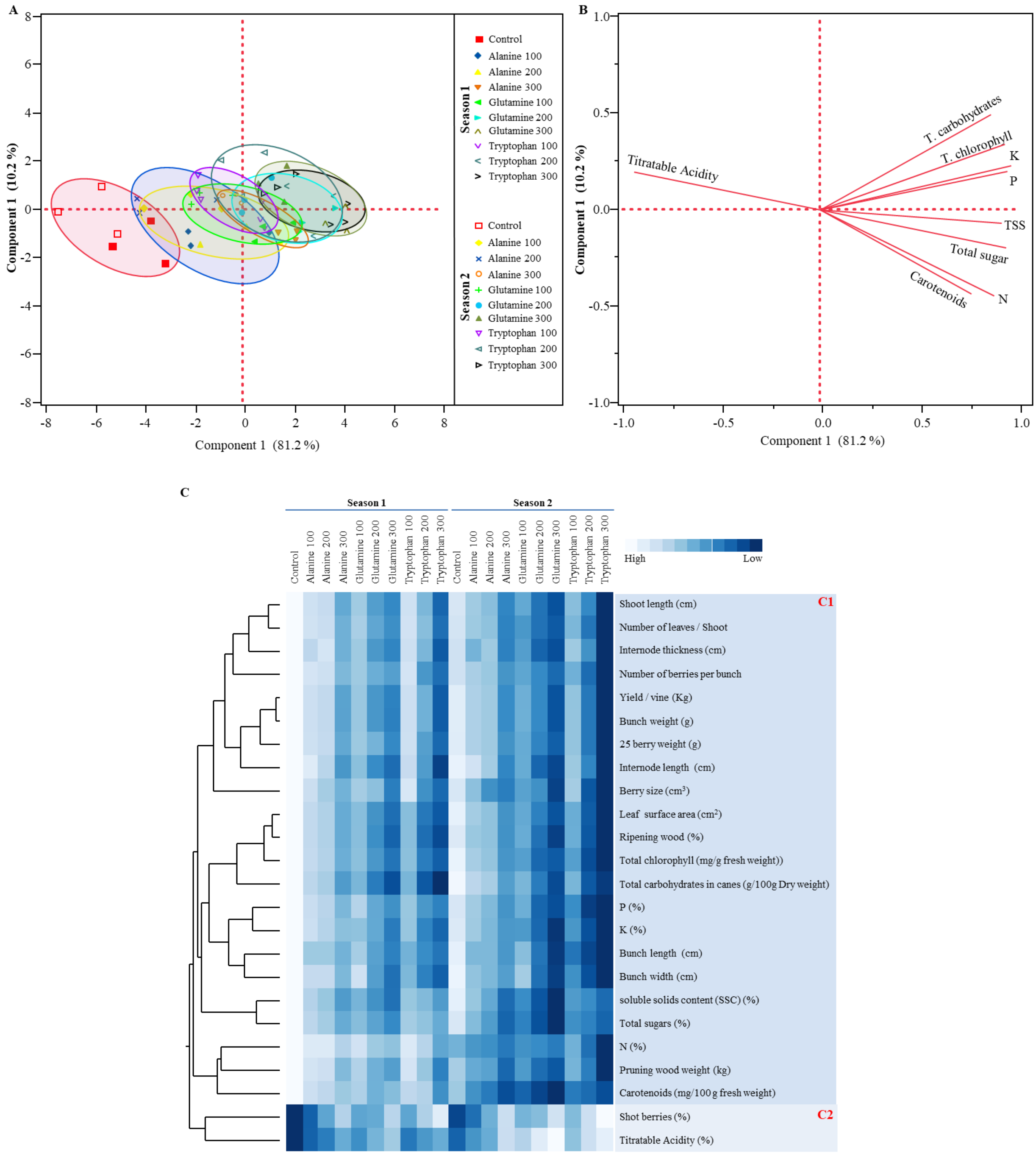

Figure 8. Principal component analysis (PCA) shows the multivariate variation among nutrient contents (N, P, and K), total chlorophyll, total carbohydrates, and chemical properties of berries after foliar application of three amino acids (alanine, glutamine and tryptophan) at different concentrations (100, 200, and 300 ppm) compared to the control on Early sweet grapevines during 2021 and 2022 seasons. (A) PCA-scatter blots. Colored symbols correspond to all previous parameters after the different treatments at different concentrations. (B) PCA-loading plot. The vectors shown in the figure indicate the direction and strength of each parameter. The two principal axes explain the variance. (C) A Two-way hierarchical cluster analysis and heat map illustrating the impact of the amino acids and control on all parameters including vine growth, nutrient contents (N, P, and K), total chlorophyll, yield and bunch quality, the physical properties of berries, the Chemical properties of berries, the dormant season parameters, pruning wood weight, ripening wood, and total carbohydrates. The cells represent the mean of the level of each parameter (n=3).

4. Discussion

The increase in shoot length, leaf area, and number of leaves per shoot resulting from the application of alanine, glutamine, and tryptophan may be attributed to the role of amino acids as organic nitrogenous compounds. These compounds participate in protein synthesis and promote enzyme activity in plants (Opike and Rolfe, 2005). Maeda and Dudareva (2012) observed that tryptophan regulates the production of auxin, thereby promoting vine growth. Additionally, tryptophan has been shown to have very positive effects on improving vegetative development characteristics in multiple crop productions. (Abbas et al., 2013). Glutamine, as an α-amino acid, is applied to promote plant development and protein manufacturing. It has beneficial effects under stressful conditions by reducing physiological damage through the promotion of antioxidant enzyme activity, the release of plant hormones, carbohydrate anabolism, and an increase in chlorophyll molecules. Glutamine is known to enhance plant growth. (Al-Juthery et al., 2020 and El-Metwally et al, 2022). Alanine, on the other hand, is a crucial amino acid that participates in protein synthesis in plants (Voet et al., 2006). The applications of alanine, glutamine, and tryptophan significantly increased the levels of N, P, and K in the leaves, as well as the leaf content of total chlorophyll, compared to the control over two years. Our findings concur with those of Abou Dahab and Abd El-Aziz (2006) and El-Kenawy (2022), who observed enhancements in N, P, and K percentages in leaves, as well as shoot length, leaf area, total chlorophyll, and total amino acid content with amino acid treatments. According to Voet et al. (2006) amino acids can enhance stress resistance, support growth and development, stimulate protein synthesis, and rejuvenate plants. The increased chlorophyll content in leaves may be attributed to a dual effect: a reduction in chlorophyll degradation and the stimulatory effect of amino acids on chlorophyll production (Souri et al., 2017 and Fahimi et al., 2016).

Subsequently, it has been demonstrated that under higher temperatures, the same system can enhance photosynthesis and increase biomass production (Fouad and Altpeter, 2009). Zewail, (2014) revealed that tryptophan facilitates the production of the plant hormone IAA. As well, amino acid foliar spraying enhances numerous physiological functions in treated plants including nutrient absorption via roots and metabolism (Zewail, 2014). Amino acids exhibit diverse applications in plants, and ongoing research continues to unveil new insights about their roles. According to Sharma and Dietz, (2006) and Souri and Hatamian, (2019), there are several significant effects, including enhanced blooming, improved fruit setting, increased nutritional content, larger, more flavorful, and more colorful fruits, and a higher brix levels (quality improvement).

Additionally, amino acids increase the efficiency of photosynthesis, thereby enhancing the rate at which tissues assimilate carbohydrates and influencing yield (Faissal et al., 2015). Studies by Shams et al., (2016) and El-Kenawy (2022) have reported that amino acid applications improve berry set, yield per vine, bunch weight, and berry weight over two seasons compared to controls. According to Abd-Elkader et al., (2020), foliar spraying of tryptophan increases production and enhances overall chlorophyll and carotenoid content in plants. Amino acid sprays increase production per fadden, bunch weight, and berry weight compared to untreated vines of Flame red grapevines as reported in Belal et al., (2016). The positive impact of amino acids on growth parameters, vine nutritional status, and the berries physical properties align with outcomes from Khan et al., (2012), El-Sayed, (2013), AL-khawaga, (2014), and Al-Wasfy, (2014). Using amino acids application (alanine, glutamine and tryptophan) at three times i.e., at growth start, at full bloom stage and at version stage significantly reduced the occurrence of shot berries compared to untreated controls. Tryptophan enhances shot berries by preventing early flower and berry drop, attributed to its role in synthesizing enzymes that catalyze responses to auxin (Saburi et al., 2014). Additionally, Zewail, (2014) highlighted that tryptophan produces the plant hormone indole 3-acetic acid (IAA). In Flame Seedless Grapevines, Belal et al., (2016) demonstrated that applying amino acids enhanced the levels of total sugars, anthocyanins content in berry skin, and total phenols, significantly. El-Kenawy, (2022) reported that spray of amino acids improved SSC, sugars, and anthocyanin content in berry skin compared to untreated vines. Also, Phenylalanine at 1000 ppm was found to be the most favorable application for improving phytochemical properties of 'Red Globe' table grape variety (Kok, 2024). These observations regarding the advantageous impacts of amino acids on the chemical characters of fruits align with previous findings by El-Sayed (2013), Al-Khawaga (2014), Faissal et al. (2014), Al-Wasfy, (2014) and Faissal et al. (2015).

Amino acids contribute positively to the increase in pruning wood weight, ripening wood thickness, and internode length by stimulating cell division and elongation, enhancing photosynthesis, facilitating nucleic acid and protein synthesis, and promoting carbohydrate assimilation (El-Sese et al., 2020). These effects reflected on enhancing vegetative parameters and physiological states, ultimately improving initial characteristics. Our findings align with those reported by El-Kenawy (2022) who observed that amino acid applications enhanced pruning wood weight, ripening wood, internode length, and thickness. Tryptophan was recently found to significantly increase shoot diameter and length in Philodendron erubescens plants (Abou Dahab and AbdEl-Aziz, 2006). According to Hassan et al. (2010) foliar amino acid spray improved the stem diameter of plum trees, while Rasmia et al. (2014), found that amino acid foliar sprays enhanced date palm stem thickness compared to controls, particularly at concentrations of 100, 200, and 300 mg/l. Additionally, Wassel et al. (2015) observed that vegetative growth parameters, such as branch diameter and length in pomegranate trees, increased with foliar sprays of amino acids combined with potassium silicate as compared to untreated trees.

5. Conclusion

In conclusion, the optimal outcomes for growth parameters, yield, biochemical parameters, berry quality, and reduction of shot berries in Early Sweet grapevines were achieved through the treatment of tryptophan, glutamine, and alanine at 300 ppm, sprayed three times at growth start, full bloom stage, and version stage. Therefore, it could be recommended for application in Early Sweet vineyards to maximize vine health and enhance berry characteristics in clay soils under flood-irrigated system.

Author Contributions

Conceptualization, S.E. and A.M.; Methodology, S.E. and A.M.; Software, A.M. and S.E.; Validation, S.E. and A.M.; Formal analysis, A.M. and S.E.; Investigation, S.E. and A.M.; Resources, S.E. and A.M; data curation, S.E. and A.M; Writing—original draft preparation, S.E. and A.M; Writing—review and editing, A.M. and S.E.; Visualization, S.E. and A.M; Supervision, S.E. and A.M; Funding acquisition, S.E. and A.M. All authors have read and agreed to the submitted version of the manuscript.

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Conflicts of Interest

No conflicts of interest are disclosed by the authors.

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