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Bio-stimulant Potential of Foliar-Applied Tryptophan, Methionine, and Cystine at Graded Concentrations and Frequencies on Yield and Berry Quality of Flame Seedless Grapevines

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Abstract: A two-season field experiment was conducted during 2023 and 2024 on eight-year-old Flame Seedless grapevines grown on sandy soil in a private orchard located west of the Western Desert Road, Minia Governorate, Egypt, to investigate the effect of foliar application of a mixture of amino acids — tryptophan, methionine, and cystine — at graded concentrations (0.05, 0.1, and 0.2%) applied once, twice, or thrice per season on vine yield components, cluster physical characteristics, berry physical properties, and berry chemical quality. The experiment was arranged in a randomized complete block design (RCBD) with ten treatments and three replicates. Results demonstrated that all amino acid treatments significantly increased berry setting percentage, yield per vine, cluster weight, cluster length, and cluster shoulder in both seasons; cluster number per vine was unaffected in the first season but significantly increased in the second season, reflecting a cumulative vine conditioning effect. Berry shot percentage was significantly reduced, while berry fresh weight, longitudinal diameter, and equatorial diameter were significantly increased by all treatments. Berry TSS%, TSS/acidity ratio, total anthocyanin content, and reducing sugar percentage were all significantly elevated, while total acidity was significantly reduced. The highest values for virtually all parameters were recorded with AA at 0.2% applied thrice, which was frequently statistically comparable to AA at 0.1% applied thrice. It is concluded that three foliar applications of the amino acid mixture (tryptophan, methionine, and cystine) at 0.1% — at the onset of vegetative growth, immediately after berry set, and one month thereafter — represent the most economically favorable treatment for improving vine yield, and berry quality of Flame Seedless grapevines grown under the sandy soil conditions of Minia Governorate.

Key words: Bio-stimulants, amino acid, tryptophan, cysteine, methionine, berry quality, and Flame seedless grapevines.

1. Introduction

The grapevine, Egypt's predominant fruit crop, is cultivated globally with improved nutrition to maximize output and berry quality using various vineyard management techniques. Egypt cultivates grapes on approximately 200,000 feddans (around 84,000 hectares), ranking as the third major fruit crop after citrus and mango, its producing about 1.8 million tons annually, according to **Ministry of Agriculture (2025**). In Minia Governorate, a key viticultural region, grapevine area contributes significantly to national totals, which ranks second nationally in grapevine cultivation after Beheira, with a fruiting area of 24,085 feddans producing over 168,670 tons. The Flame seedless variety is regarded as the primary early-ripening seedless table grape with an estimated cultivated area of 50,000 to 70,000 feddans and productivity of 500-700 thousand tons in Egypt while 4-6 thousand feddans and productivity of 50-80 thousand tons in Minia Governorate (**Egyptian Ministry of Agriculture, 2025**). In the El-Minia area of Egypt, particular problems are present, including reduced crop yield and a significant prevalence of shot berries in grape clusters. These concerns adversely impact the marketing of this particular grape variety. Numerous experiments were conducted to investigate alternative approaches of mitigating these difficulties while safeguarding the environment from pollution (**Ali *et al.*, 2023**).

Elevated temperatures in Egypt adversely impact the hue and quality of fruit. Consequently, grape cultivators in this region employ many techniques to enhance the quality of their grapes. The methods employed in vineyard management significantly affect the quality of the grape yield. Organic bio-stimulants have shown efficacy in enhancing plant growth, vigor, crop output, and quality by improving the uptake of essential nutrients (**Sharma *et al.*, 2023**).

Amino acids, including tryptophan, cysteine, and methionine, are essential for plant metabolism. They act as transporters of organic nitrogen within the plant's tissues and serve as precursors for essential secondary metabolites in plant cells (**Dinkeloo *et al.*, 2018**). They act as transporters of organic nitrogen within the plant's tissues and serve as precursors for essential secondary metabolites in plant cells (**Dinkeloo *et al.*, 2018**). Numerous studies on grapevines indicate that the application of amino acids by foliar spray is crucial for promoting development, improving the nutritional state of the vines, increasing production, and enhancing berry quality (**Ali *et al.* 2025; Rashid and Al-Atrushy 2025**). **Maeda and Dudareva (2012**) shown that tryptophan acid is essential for facilitating plant growth and modulating auxin synthesis. **Abdelkader *et al.* (2021)** discovered that the use of tryptophan acid via spraying enhanced vegetative growth and augmented production. The application of tryptophan to the leaves resulted in elevated quantities of carotenoids and chlorophyll in the plants. Tryptophan is crucial in preventing the premature abscission of flowers and fruit. The development of an enzyme that facilitates auxin production is essential for enhancing berry set (**Saburi *et al.*, 2014**).

Methionine significantly influences fruit physiology as the precursor to ethylene via S-adenosylmethionine (SAM), driving climacteric ripening processes like softening, color change, and aroma development in fruits (**Zhu *et al.*, 2023 and Lin *et al.*, 2025**). Post-harvest methionine treatments enhance antioxidant enzyme activities (SOD, POD, CAT), reduce decay incidence, maintain firmness, soluble solids, and titratable acidity while boosting disease resistance against pathogens like black spot rot through lignin accumulation. In grapevines, foliar methionine promotes yield parameters, sugar accumulation, and TSS while regulating stress responses, underscoring its biostimulant potential for quality preservation amid storage and environmental challenges (**El-Sayed *et al.*, 2025**).

Cysteine, often present as cystine in its oxidized form, is critical for grapevine physiology primarily through its role as a precursor to glutathione, a key antioxidant that mitigates oxidative stress from environmental factors like UV radiation, drought, and pathogens. Cysteine is an essential amino acid characterized by the presence of an amino group, a thiol group, and a carboxylic acid group as reactive centers. The unique configuration of Cysteine allows it to function as a powerful antioxidant and effective scavenger of reactive oxygen species. A thiol side chain offers protection against oxidative damage from both biotic and abiotic stimuli by facilitating effective oxidation (**Álvarez *et al.*, 2012 and Genisel *et al.*, 2015).** S-adenosylmethionine and/or methionine in plants is vital for the production of key phytohormones, including polyamines and ethylene. Thus, these interactions significantly influence the growth and development of plants (**Sauter *et al.*, 2012 and Elkelish *et al.*, 2021**).

The primary objective of this study is to investigate the impact of foliar applications of graded methionine, cystine, and tryptophan during various times on the physiological and quality traits of Flame Seedless grapevines in Minia Governorate.

2. Material and Methods

2.1. Experimental site

This study was performed on the Flame Seedless grapevine during two successive seasons, 2023 and 2024, in a private orchard located west of Western Desert Road, Minia, Egypt. The eight-year-old grapevines were planted two meters apart in rows three meters apart on sandy soil. The vines are upheld by the Gable supporting system, accommodating a bud load of 80 buds per vine, calculated as follows: (8 fruiting spurs x 8 eyes) + (8 renewal spurs x 2 eyes).

The selected vines were healthy, devoid of any physiological abnormalities or nutrient deficits, and were treated in accordance with known vineyard protocols for this investigation. Irrigation was performed with a drip system sourced from groundwater with a concentration of 1000 ppm. Winter trimming occurred in mid-December for both seasons.

The physical and chemical properties of the soil are outlined in Table A, according to the technique established by **Wilde *et al.* (1985).**

Table (A). Analysis of tested soil physical and fertility status

Soil characters		**2023/2024**
Particle size distribution (%)	**Sand**	73.9
	Silt	14.6
	Clay	11.5
	Texture class	Sandy
EC ppm (1:2.5 extract)		2.51
pH (1:2.5 extract)		8.2
Organic matter %		0.53
$CaCO_3$ %		11.54
Soil nutrients	**Total N (%)**	0.05
	Available P (ppm)	4.9
	Available K (ppm)	132.6
	Zn (ppm)	0.3
	Fe (ppm)	2.3
	Mn (ppm)	1.1
	Cu (ppm)	0.2

Herein, the study was set up in a randomized complete block design (RCBD). Ten treatments, three replicates of each, with one vine per replicate, were used for the experiment design. At three distinct phases—specifically, the onset of development, immediately following berry set, and one month thereafter—each treatment was administered via foliar spraying utilizing the evaluated graded concentrations and frequency of various amino acids, including tryptophan, cystine, and methionine.

The vines were subjected to distinct treatments in the following manners:

1. Control (spay with tap water).

2. Amino acids (0.05%) once
3. Amino acids (0.05%) twice
4. Amino acids (0.05%) thrice
5. Amino acids (0.1%) once
6. Amino acids (0.1%) twice
7. Amino acids (0.1%) thrice
8. Amino acids (0.2%) once
9. Amino acids (0.2%) twice
10. Amino acids (0.2%) thrice

2.2. Botanical Measurements

Four clusters /vine were harvested at the ripening stage to determine the following data: Number of cluster/vine, cluster weight (g), length (cm), shoulder (cm) and yield (kg)/vine was assessed in kg for each tree/replicate by multiply the previous parameters, as well as Berry setting (%) was computed as the following: Five flower clusters were packaged per vine in perforated paper bags prior to blooming, which are released during berry set, calculated as follows:

$$\text{Fruit berry Setting\%} = \frac{\text{Number of berries /cluster}}{\text{Total number of flower /cluster}}$$

To get the shot berry proportion, the percentage of berries in each cluster was divided by the total number of berries across all clusters and then multiplied by 100. Berry weight (g) and dimensions (longitudinal and equatorial).

A hand-held refractometer utilized to measure TSS% in berries, titrating 5 ml of berry juice against 0.1 N NaOH with phenolphthalein determined the titratable acidity percentage, the TSS/acidity ratio of the berry juice was determined, the total anthocyanin content of the berry skin was quantified as mg/100g of fresh weight (**Hsia *et al.,* 1965**) and reducing sugar%.

2.3. Data Analysis

Consistent with the findings of **Mead *et al.* (1993**), the analysis of all data was conducted employing a new L.S.D. technique at significant of 5%.

3. Results and Discussion

3.1. Yield and Cluster Traits

Data presented in Table (1) reveal that foliar application of the amino acid mixture at all tested concentrations and frequencies produced consistent and significant improvements across all yield and cluster characteristics — namely berry setting percentage, number of clusters per vine, yield per vine, cluster weight, cluster length, and cluster shoulder — compared to the untreated control in both growing seasons, with the single exception of cluster number in the first season.

The control vines recorded the lowest values for all parameters in both seasons: 11.9 and 12.3% for berry setting, 25.0 clusters/vine in both seasons, 8.4 and 8.5 kg/vine for yield, 335.5 and 340.0 g for cluster weight, 16.8 and 17.0 cm for cluster length, and 10.8 and 11.1 cm for cluster shoulder. With respect to cluster number, no significant differences were detected among any of the treatments in the first season (2023), all vines recording a uniform count of 25 clusters with a non-significant, while all amino acids treatments significantly increased this parameter in the second season (2024), reflecting a cumulative vine conditioning effect from repeated seasonal amino acid applications.

The highest values across all yield and cluster parameters in both seasons were achieved with AA at 0.2% thrice, recording 19.0 and 19.2% for berry setting, 36.0 clusters/vine in 2024, 9.4 and 13.9 kg/vine for yield, 375.0 and 387.0 g for cluster weight, 21.3 and 21.5 cm for cluster length, and 13.2 and 13.6 cm for cluster shoulder. This superior treatment was not significantly different from AA at 0.1% thrice for any of these parameters in either season, confirming the statistical equivalence of the two highest-performing treatments. In the second season, both AA at 0.2% thrice and AA at 0.1% thrice were significantly superior to all remaining treatments for cluster number, while for berry setting, yield, cluster weight, cluster length, and cluster shoulder, a broader group of statistically comparable treatments was evident. AA at 0.2% twice, AA at 0.1% twice, AA at 0.05% thrice, and AA at 0.2% once formed a statistically homogeneous intermediate group for cluster weight, cluster length, and cluster shoulder in both seasons, without significant differences. AA at 0.1% once and AA at 0.05% twice were statistically comparable to each other for all cluster physical parameters in both seasons.

The significant and consistent improvements in berry setting percentage, number of clusters per vine, total yield per vine, cluster weight, cluster length, and cluster shoulder dimensions recorded in both growing seasons reflect the capacity of tryptophan, methionine, and cystine — applied foliarly at graded concentrations and frequencies — to enhance the productive performance of Flame Seedless grapevines through targeted stimulation of the hormonal, metabolic, and structural processes governing reproductive development and cluster growth.

The improvement in berry setting percentage across all treatments in both seasons is most directly attributed to the biosynthetic role of tryptophan as the universal precursor of indole-3-acetic acid (IAA), the principal auxin governing fruit set in grapevine. The exogenous supply of tryptophan through foliar applications therefore directly augments the substrate pool available for IAA biosynthesis at the critical peri-anthesis period, enhancing pollen tube growth, ovule fertilization, and the hormonal signaling cascade that commits the developing ovary to fruit growth rather than abscission (**Godoy *et al.*, 2021**). Some amino acids like tryptophan are recognized to be the precursors of phytohormones, and are also involved in the synthesis of amines, alkaloids, terpenoids, enzymes, and vitamins; they are essential to the maintenance of cellular growth and serve as sources of carbon and energy, while fertilizers containing amino acids enhance the plant's ability to absorb water and minerals and thus provide for better yield. According to **Shekari and Javanmardi (2017**), the role of methionine in fruit set is equally significant but operates through a distinct metabolic route: methionine serves as the precursor for S-adenosylmethionine (SAM), which is the obligate substrate for polyamine biosynthesis — putrescine, spermidine, and spermine — compounds that are indispensable for cell division in developing ovule and pericarp tissues. **Belal *et al.* (2016**) similarly demonstrated that amino acid foliar sprays applied to Flame Seedless grapevines markedly improved yield per vine and cluster traits, with the same cultivar as in the present study confirming the reproducibility of this response. The application of a maximum amino acid concentration of 10 ml/L topically to the leaves resulted in elevated levels in 'Thompson Seedless' yield per vine, weight and number of clusters per vine of grape cultivar (**Rashid and Al-Atrushy, 2023**).

For cluster physical characteristics — weight, length, and shoulder dimensions — the improvements reflect the synergistic stimulation by the three amino acids of cell division (driven by IAA from tryptophan and polyamines from methionine) and cell expansion (supported by turgor maintenance through osmolyte accumulation) in rachis and mesocarp tissues. Zinc increases vegetative growth by synthesizing tryptophan and regulates growth and production of grapevines, and improvements in berry diameter can be ascribed to increased chlorophyll content in the leaf, which is associated with a high production of photosynthate that supports cluster and berry physical development, (**Ali *et al.*, 2021**) further confirming the interconnected roles of tryptophan metabolism, photosynthesis, and cluster growth parameters.

Table (2). Yield and cluster traits under foliar graded amino acid applications in Flame Seedless grapevines (2023/2024)

Characteristics / Treatments	Berry setting %		Cluster number /vine		Yield/vine (kg)		Cluster weight (g)		Cluster length (cm)		Cluster shoulder (cm)	
	2023	**2024**	**2023**	**2024**	**2023**	**2024**	**2023**	**2024**	**2023**	**2024**	**2023**	**2024**
Control	11.9	12.3	25.0	25.0	8.4	8.5	335.50	340.0	16.8	17.0	10.8	11.1
AA (0.05%) once	13.6	13.9	25.0	27.0	8.6	9.5	345.0	351.0	18.0	18.2	11.4	11.7
AA (0.05%) twice	15.0	15.4	25.0	29.0	8.8	10.4	353.0	360.0	18.8	19.0	11.9	12.2
AA (0.05%) thrice	16.5	16.9	25.0	31.0	9.1	11.4	362.0	368.0	19.7	19.9	12.5	12.8
AA (0.1%) once	15.1	15.3	25.0	30.0	8.9	10.8	354.0	361.0	18.9	19.1	11.9	12.2
AA (0.1%) twice	16.5	16.7	25.0	32.0	9.1	11.9	362.0	371.0	19.9	20.0	12.4	12.8
AA (0.1%) thrice	17.9	18.1	25.0	35.0	9.3	13.3	371.0	380.0	20.8	21.0	13.0	13.3
AA (0.2%) once	16.2	16.5	25.0	31.0	9.0	11.4	360.0	368.0	19.4	19.7	12.2	12.6
AA (0.2%) twice	17.5	17.8	25.0	33.0	9.2	12.5	367.0	378.0	20.3	20.6	12.6	13.1
AA (0.2%) thrice	19.0	19.2	25.0	36.0	9.4	13.9	375.0	387.0	21.3	21.5	13.2	13.6
New LSD at 0.05	**1.2**	**1.3**	**N.S**	**1.1**	**0.2**	**0.7**	**7.0**	**8.0**	**0.6**	**0.7**	**0.4**	**0.5**

AA: Amino acids

3.2. Berry Physical Characteristics

Data presented in Table (2) reveal that foliar application of the amino acid mixture at all tested concentrations and frequencies produced significant and consistent effects on all berry physical parameters — namely shot berry percentage, berry fresh weight, berry longitudinal diameter, and berry equatorial diameter — across both growing seasons. While all amino acids treatments significantly reduced shot berry percentage relative to the control, they simultaneously and significantly increased berry weight and both dimensional measurements, confirming a broad and reproducible positive influence on berry development and cluster uniformity.

The control vines recorded the least favorable values for all parameters in both seasons: the highest shot berry percentage (8.9 and 8.5%), the lowest berry weight (3.36 and 3.48 g), the smallest longitudinal diameter (1.64 and 1.70 cm), and the smallest equatorial diameter (1.45 and 1.50 cm).

The most favorable values across all berry physical parameters were consistently achieved with AA at 0.2% thrice, recording the lowest shot berry percentage (6.2 and 5.7%), the highest berry weight (3.75 and 3.84 g), the greatest longitudinal diameter (1.86 and 1.87 cm), and equatorial diameter (1.65 and 1.72 cm). This treatment was not significantly different from AA at 0.1% thrice for any of the all parameters in either season. AA at 0.2% twice was statistically comparable to AA at 0.1% thrice for shot berry percentage, longitudinal diameter, and equatorial diameter in both seasons; however, for berry weight, AA at 0.2% thrice was significantly superior to AA at 0.2% twice in both seasons, while AA at 0.1% thrice remained non-significantly different from AA at 0.2% twice for this parameter. AA at 0.05% thrice, AA at 0.1% twice, and AA at 0.2% once formed a statistically homogeneous group for berry weight, longitudinal diameter, and equatorial diameter in both seasons. AA at 0.1% once and AA at 0.05% twice were not significantly different from each other for any of the studied parameters in either season. AA at 0.05% once was significantly superior to the control for all berry physical parameters in both seasons, yet was significantly inferior to the AA at 0.05% twice treatment for shot berries, berry weight, longitudinal diameter, and equatorial diameter. The parallel improvements in both longitudinal and equatorial diameters across all treatments and both seasons confirm that the amino acid mixture promotes isometric berry expansion, while the simultaneous reduction in shot berry incidence reflects improved hormonal and nutritional conditions for uniform berry development throughout the cluster.

Table (2). Shoot berries%, berry weight (g), berry longitudinal and berry equatorial under foliar graded amino acid applications in Flame Seedless grapevines (2023/2024)

Characteristics / Treatments	Shoot berries %		Berry weight (g)		Berry longitudinal (cm)		Berry equatorial (cm)	
	2023	2024	2023	2024	2023	2024	2023	2024
Control	8.9	8.5	3.36	3.48	1.64	1.70	1.45	1.50
AA (0.05%) once	8.0	7.4	3.47	3.58	1.69	1.74	1.50	1.55
AA (0.05%) twice	7.5	7.0	3.55	3.66	1.74	1.79	1.54	1.59
AA (0.05%) thrice	7.1	6.5	3.62	3.73	1.77	1.84	1.59	1.64
AA (0.1%) once	7.4	7.0	3.56	3.65	1.75	1.79	1.55	1.59
AA (0.1%) twice	7.0	6.5	3.63	3.72	1.79	1.82	1.59	1.64
AA (0.1%) thrice	6.5	6.0	3.70	3.79	1.84	1.86	1.63	1.69
AA (0.2%) once	7.1	6.7	3.61	3.70	1.77	1.81	1.57	1.60
AA (0.2%) twice	6.7	6.2	3.68	3.76	1.80	1.84	1.60	1.66
AA (0.2%) thrice	6.2	5.7	3.75	3.84	1.86	1.87	1.65	1.72
New LSD at 0.05	**0.4**	**0.4**	**0.06**	**0.06**	**0.03**	**0.03**	**0.03**	**0.04**

AA: Amino acids

The notable enhancements in berry weight, longitudinal and equatorial diameters, along with the substantial decrease in shot berry percentage observed across the mixture of amino acids as (tryptophan, methionine, and cystine) treatments in both seasons, affirm that these three amino acids, administered foliarly at varying concentrations and frequencies, exert a widespread and consistent stimulatory effect on berry physical development and cluster uniformity in Flame Seedless grapevines. The reduction in shot berry percentage — the most practically significant finding in Table 8 from a commercial perspective — is directly linked to the role of tryptophan as an auxin precursor. In grapevine, the application of an inhibitor of auxin action reduced mesocarp cell number and cell diameter at fruit set in a manner similar to unpollinated berries, confirming that most of the fruit sizing effect of pollination is mediated by auxin during early grapevine berry formation, with auxin content rapidly increasing after pollination through the TAR/YUCCA biosynthetic pathway from L-tryptophan. Central When tryptophan availability in floral and berry tissues is augmented through foliar supply, the rate of IAA biosynthesis in the developing ovary increases, maintaining the hormonal environment necessary for uniform ovule fertilization and synchronized berry growth throughout the cluster. The reduction in shot berry percentage observed under all tryptophan, methionine, and cystine treatments therefore reflects improved auxin homeostasis during the critical post-anthesis berry development window, resulting in more uniform cell division and consistent berry sizing across the cluster (**Godoy *et al.*, 2021**). Methionine contributes to this uniformity through a complementary pathway: as the precursor of SAM and subsequently of polyamines, methionine application at critical stages of berry development ensures adequate polyamine supply — particularly spermidine and spermine — for the stabilization of cell membranes and nucleic acids during the rapid cell division phase (S1) of berry growth. Cystine, through its reduction to cysteine and subsequent incorporation into glutathione, provides the principal antioxidant defense against the reactive oxygen species generated during the high metabolic activity of rapidly dividing berry pericarp cells, thereby protecting cellular integrity and supporting the full expression of berry growth potential. This outcome reflects the cumulative nature of the tryptophan-mediated auxin supply and the methionine-mediated polyamine pool across the full duration of berry development from fruit set to harvest, further underscoring the importance of repeated application to sustain the metabolic advantage conferred by these amino acids throughout the growing season (**Al-Saif *et al.*, 2024**).

3.3. Berry Chemical Quality Characteristics

Data presented in Table (3) reveal that foliar application of the amino acid mixture at all tested concentrations and frequencies produced significant and consistent improvements across all berry chemical quality parameters — namely total soluble solids (TSS%), total acidity, TSS/acidity ratio, total anthocyanin content, and reducing sugar percentage — in both growing seasons.

The most favorable values across all chemical quality parameters were consistently achieved with AA at 0.2% thrice, recording (21.2 - 21.8% TSS), (0.600 - 0.590% total acidity, lowest), (35.3 - 36.9 TSS/acidity ratio), (27.2 - 27.5 mg/100g FW anthocyanin), and (17.3-18.0% reducing sugars). This treatment was not significantly different from AA at 0.1% thrice for all traits in either season compared to the control vines which recorded the least favorable values for all chemical quality parameters in both seasons, (18.5-18.7% TSS), (0.718 -0.713% total acidity, highest), (25.8 - 26.2 TSS/acidity ratio), (24.9 - 25.3 mg/100g FW anthocyanin), and (15.0 - 15.5% reducing sugars). AA at 0.2% twice was statistically comparable to AA at 0.1% thrice for total acidity, TSS/acidity ratio, and reducing sugar in both seasons; notably, in the second season AA at 0.1% thrice (27.7 mg/100g FW) was significantly superior to AA at 0.2% twice (27.2 mg/100g FW) for anthocyanin content. AA at 0.05% thrice, AA at 0.1% twice, and AA at 0.2% once formed a statistically homogeneous intermediate group for TSS, total acidity, total anthocyanin, and reducing sugar in both seasons. AA at 0.05% twice and AA at 0.1% once were not significantly different from each other for any of the mentioned parameters in either season. The consistent, progressive, and dose- and frequency-dependent improvement across all mentioned chemical quality parameters in both seasons confirms the comprehensive stimulatory effect of tryptophan, methionine, and cystine on the biochemical ripening processes governing sugar accumulation, organic acid catabolism, flavonoid biosynthesis, and overall berry metabolic maturation in Flame Seedless grapevines.

Table (3). T.S.S%, total acidity, T.S.S/acidity and reducing sugar under foliar graded amino acid applications in Flame Seedless grapevines (2023/2024)

Characteristics / Treatments	TSS%		Total acidity%		TSS/acidity ratio		Total anthocyanin (mg/100g FW)		Reducing sugar%	
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
Control	18.5	18.7	0.718	0.713	25.8	26.2	24.9	25.3	15.0	15.5
AA (0.05%) once	19.1	19.4	0.695	0.686	27.5	28.3	25.5	26.0	15.6	16.2
AA (0.05%) twice	19.6	20.0	0.670	0.661	29.3	30.3	26.0	26.5	16.1	16.7
AA (0.05%) thrice	20.2	20.7	0.643	0.635	31.4	32.6	26.6	27.1	16.6	17.2
AA (0.1%) once	19.7	20.1	0.669	0.660	29.4	30.5	26.1	26.5	16.2	16.8
AA (0.1%) twice	20.3	20.7	0.644	0.635	31.5	32.6	26.5	27.0	16.6	17.3
AA (0.1%) thrice	20.8	21.3	0.621	0.611	33.5	34.9	27.0	27.7	17.1	17.8
AA (0.2%) once	20.1	20.6	0.649	0.637	31.0	32.3	26.4	26.8	16.5	17.1
AA (0.2%) twice	20.6	21.1	0.620	0.613	33.2	34.4	26.8	27.2	16.9	17.5
AA (0.2%) thrice	21.2	21.8	0.600	0.590	35.3	36.9	27.2	27.5	17.3	18.0
New LSD at 0.05	**0.5**	**0.6**	**0.022**	**0.024**	**1.9**	**1.9**	**0.4**	**0.4**	**0.4**	**0.4**

AA: Amino acids

The comprehensive improvements in berries quality represent the culmination of the cascading physiological and biochemical enhancements generated throughout the vine by tryptophan, methionine, and cystine applications. These three amino acids, each with distinct and complementary roles in plant metabolism, collectively accelerate and deepen the ripening process in Flame Seedless berries through their influence on sugar biosynthesis and transport, organic acid catabolism, phenylpropanoid secondary metabolism, and cellular antioxidant capacity. The significant and dose-dependent increase in TSS content across both seasons reflects the combined stimulation of photosynthetic carbon fixation — enhanced through the improved chlorophyll and leaf area. Tryptophan, beyond its role in auxin biosynthesis, serves as a metabolic link between the shikimate pathway and secondary metabolism, supporting the biosynthesis of a range of carbon-containing compounds that contribute to the vine's assimilate pool. Methionine, through SAM, donates methyl groups in the biosynthesis of lignin precursors, betaines, and nucleic acid methylation, indirectly regulating the expression of genes involved in sugar transport and accumulation in berry mesocarp cells. **Rashid and Al-Atrushy (2023)** confirmed that amino acid foliar applications to Thompson Seedless grapevines significantly increased TSS content of berries compared to untreated controls, with higher amino acid concentrations producing progressively greater berry sugar accumulation. Single and combined applications of amino acids in Flame Seedless grapevines improved TSS alongside total sugars, total anthocyanin, and total phenols while reducing total acidity in berries compared to the untreated control.

The significant reduction in total acidity under all treatment combinations in both seasons reflects the stimulatory effect of tryptophan, methionine, and cystine on the enzymatic catabolism of malic acid — the principal titratable acid in ripening grapevine berries — through the enhanced activity of NAD-malic enzyme and malate dehydrogenase in the berry mesocarp. Methionine, by serving as the sulfur source for coenzyme A and other sulfur-containing enzyme cofactors, supports the activity of the decarboxylative enzymes involved in malic acid breakdown. Cystine, through its reduction to cysteine and subsequent glutathione biosynthesis, maintains the reductive cellular environment that favors malate catabolism over its storage (**Masoud *et al.*, 2025**).

Tryptophan occupies a central position in anthocyanin biosynthesis as the precursor of the indole pool that feeds into the shikimate pathway, ultimately generating phenylalanine — the direct entry point of the phenylpropanoid pathway from which all flavonoids including anthocyanins are derived. By augmenting the upstream carbon flow through the shikimate and aromatic amino acid pathways, tryptophan supply indirectly enhances the substrate availability for phenylalanine ammonia-lyase (PAL), the rate-limiting enzyme of phenylpropanoid metabolism and of anthocyanin biosynthesis. Cystine further supports anthocyanin accumulation through the glutathione-mediated transport mechanism: in grape, glutathione conjugates anthocyanin precursors (anthocyanin–glutathione conjugates) for vacuolar transport, and a well-maintained glutathione pool — sustained by the cystine supply — enhances the efficiency of this transport step and the stability of accumulated anthocyanins in berry skin vacuoles. The spray of amino acids improved SSC, sugars, and anthocyanin content in berry skin compared to untreated vines, with observations confirming the advantageous impacts of amino acids on the chemical characters of grape fruits in alignment with multiple previous findings in Egyptian viticulture (**Kok, 2024**). Sugar accumulation in grape berries is a finely tuned outcome of numerous physiological processes including photosynthesis in the leaves, long-distance transport in the phloem, and unloading in sink organs, with the efficiency of each step directly influencing the final sugar content at harvest. PubMed Central The consistent, progressive, and dose-dependent enhancement of reducing sugar content across both seasons — with AA at 0.1 or 0.2% applied thrice recording the maximum values — confirms that the synergistic action of tryptophan, methionine, and cystine on multiple steps of the sugar accumulation pathway produces a cumulative benefit that scales with both the concentration and the frequency of application.

4. Conclusion

The results indicate that any of the tested concentrations and frequencies yielded superior outcomes compared to the control, with higher concentrations and more frequent applications consistently producing greater improvements across all studied parameters. The triple application of AA at 0.2% recorded the highest values for virtually all traits across both seasons, though it was frequently statistically comparable to AA at 0.1% applied thrice. Accordingly, it could be concluded that treating Flame Seedless grapevines cultivated in the Minia region three times — at the onset of vegetative growth, immediately after berry set, and one month thereafter — with a mixture of amino acids (tryptophan, methionine, and cystine) at 0.1 % yielded the most favorable economic outcomes concerning vine vigor, leaf nutritional and pigment status, yield, cluster quality, and berry physical and chemical characteristics of Flame seedless under the same condition.

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الإمكانيات التحفيزية الحيوية للرش الورقي بالتريبتوفان والميثيونين والسيستين بتركيزات ومواعيد رش مختلفه على المحصول وجودة الحبات في كروم عنب فليم سيدلس

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الملخص

أُجريت تجربة حقلية على موسمين متتاليين خلال عامَي 2023 و2024 على كروم عنب فليم سيدلس يبلغ عمرها ثماني سنوات، مزروعة في تربة رملية بمزرعة خاصة تقع غرب الطريق الصحراوى الغربي بمحافظة المنيا، مصر ، بهدف دراسة تأثير الرش الورقي بخليط من الأحماض الأمينية ــــ التريبتوفان والميثيونين والسيستين ــــ بتركيزات متدرجة (0.05 و0.1 و0.2%) تُطبَّق مرةً أو مرتين أو ثلاث مرات ، على مكونات المحصول، والصفات الفيزيائية للعنقود، والخصائص الفيزيائية والكيميائية للحبات. أجريت التجربة في تصميم القطاعات العشوائية الكاملة مع عشرة معاملات وثلاثة مكررات. أوضحت النتائج أن جميع معاملات الأحماض الأمينية أدّت إلى زيادة معنوية في نسبة عقد الثمار، والمحصول الكلي / كرمة، ووزن العنقود وطوله وعرضه في كلا الموسمين، فيما لم يتأثر عدد العناقيد لكل كرمة بصورة معنوية في الموسم الأول بينما ارتفع ارتفاعًا معنويًا في الموسم الثاني، انخفضت نسبة الحبات الصغيرة انخفاضًا معنويًا. ارتفعت كذلك نسبة المواد الصلبة الذائبة الكلية، ونسبة المواد الصلبة الذائبة / الحموضة، ومحتوى الأنثوسيانين الكلي، ونسبة السكريات المختزلة ارتفاعًا معنويًا، بينما انخفضت الحموضة الكلية انخفاضًا معنويًا. سجّلت أعلى القيم لغالبية الصفات المدروسة مع معاملة 0.2% ثلاث مرات، التي لم تسجل اى فرق معنوى إحصائيًا مع معاملة 0.1% ثلاث مرات. وخُلص إلى أن الرش الورقي الثلاثي بخليط الأحماض الأمينية (التريبتوفان والميثيونين والسيستين) بتركيز 0.1% ــــ في بداية النمو الخضري، وما يعقب عقد الثمار مباشرةً، وبعد شهر من ذلك ــــ يمثّل المعاملة الأمثل اقتصاديًا لتحسين قوة الكرمة الخضرية وحالتها الغذائية ومحصولها وجودة حباتها في ظل الظروف البيئية للتربة الرملية بمحافظة المنيا.

الكلمات المفتاحيه: المحفزات الحيويه، الاحماض الامينيه، تربتوفان، سيستين، ميثيونين، جوده الحبات، المحصول، عنب فليم سيدلس.