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Response of Superior Seedless Grapevines to Foliar Application with Selenium, Tryptophan and Methionine



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ABSTRACT

This study was carried out during two successive seasons (2015 & 2016) to study the effect of foliar applications of selenium (Se) at 5 mg/L, tryptophan (TRP) and methionine (MET) at (50 or 100 mg/L) for both on twelve-year old Superior Seedless grapevines. The chosen vines were irrigated under surface irrigation system, trellised by Gable supporting system, and spaced at 1.75 x 3 meters apart, whereas; the vines were pruned at the second week of Dec. during two seasons. Accordingly to the results which appear that foliar applications of Se, TRP and MET improved the growth characters, the physical characteristics of cluster and berries, also; yield/vine, leaves petioles nutrient content (N, P and K), total carbohydrates in canes and total chlorophyll in leaves as well as total free amino acids in berry juice compared with control during in two seasons. In this concern, the best results obtained by using of foliar application with TRP in combined with Se. Also, Se treatment had positive effects on T.S.S.%, but reduced total acidity% in berry juice compared with the control treatment in both seasons, while; the best results in this case obtained by foliar application with MET in combined with Se. The increasing in total free amino acids content in berry juice was increased with increasing foliar application of both TRP and MET.

Keywords: Grapevines, Superior Seedless, selenium, tryptophan, methionine



INTRODUCTION

Grape (*Vitis vinifera L.*) is one of the common grown horticulture crops in the world. In Egypt, grape ranks 2nd after citrus. In this concern, Superior Seedless grapevine is one of the most important cultivars grown in the Egyptian vineyards. Obviously, there are so many problems facing grapes growers which affect the productivity and fruit quality of grapes. But the grapes qualities depend on many factors including vineyard treatments, cultivar and harvest time (Rizzuti *et al.* 2015). Some of the vineyard treatments such as, foliar application of selenium, tryptophan and methionine influence on the grape quality.

Selenium (Se) is considered as an important trace element for humans and animals as well, involved with multiple biological functions, such as; improving immunization; antioxidation, and detoxification of heavy metals (Mao *et al.* 2016). It is chemically similar to sulfur (S), plants readily take up and metabolize Se via S transporters and pathways (Pilon-Smits and Quinn, 2010).

Nevertheless, the vital role of Se has been stated in naturally occurring, Se-accumulating plants require Se for their normal growth and in some species to act against oxidative stress (Djanaguiraman *et al.* 2005). In this case, Zhao and McGrath (2009) reported that Egypt is Se deficient. Also, Hu *et al.* (2003) reported that Se application was also beginning to apply in all kinds of fruits, vegetables and crops. The important role of Se in photosynthesis was in junction with ferredoxin, *i.e.*, NADP(+) oxidoreductase enzyme (Szczepaniak *et al.* 2013). In addition, Feng *et al.* (2015) found that spray of amino acid chelated selenium solution can improve photosynthesis in grape, pear and peach. Eventually, many researchers have been reported that

Se can enhance the activities of antioxidant enzymes as a result by providing a protection from environmental stress in crops (Feng and Wei, 2012).

Amino acids such as tryptophan and methionine are a fundamental components of the plant metabolism, which is considered as carriers of organic nitrogen between the organs of the plant and as precursors of important secondary metabolites of the plant cells (Dinkeloo *et al.* 2018). In addition to many investigations on grapevines have shown that foliar application of amino acids was essential in stimulating growth, vine nutritional status and yield and berry quality attributes (Mohamed, 2014; Hussein, 2017 and Mohamed, 2017).

Tryptophan (TRP) is one of essential amino acids, it is important for humans, animals, plants and some bacteria (Frankenberger and Arshad, 1991). It is known to be an efficient precursor of auxins (IAA) in plants. There are found that enzymes convert tryptophan to IAA such as, tryptophan aminotransferase enzyme that convert TRP to indole-3-pyruvate, and then flavin mono oxygenases enzyme convert indole-3-pyruvate to IAA (Zhao, 2012).

Also, ammonia release during TRP-metabolization, this ammonia acts as a source of nitrogen and that reflect positively effects on the plant growth (Bar and Okon 1995). Furthermore, TRP is affecting gene expression for producing the specific macromolecules required for permanent cell elongation (Vanderhoef, 1980). Eventually, Rai (2002) showed that TRP may act as an osmolyte, modulates stomatal opening and detoxify harmful effects of heavy metals, ion transport regulator and participate in cellular metabolism. However, Ayesha *et al.* (2018) reported that there is shortage in application of TRP on plants.

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Also, methionine (MET) is an essential amino acid, used at multiple levels in cellular metabolism: as a regulatory molecule in the form of S-adenosylmethionine, protein constituent, and as in the initiation of mRNA translation in plant cell (Hesse *et al.* 2004). Furthermore, MET is a precursor for the synthesis of the hormone ethylene by the formation of S-adenosyl-methionine, which is converted to 1-aminocyclopropane-1-carboxylate, and then synthesis of the ethylene, and also, cellular energy glucosinolates, cell wall biosynthesis, polyamines, chlorophyll biosynthesis, and to a large number of secondary metabolites, and provides a methyl group to DNA methylation (Rebeille *et al.* 2006; Goyer *et al.* 2007).

The objective of this study is to drawing attention to the effect of the foliar application with selenium, tryptophan and methionine, on vegetative growth, yield, cluster and berry quality of Superior Seedless grapevines.

MATERIALS AND METHODS

This study was carried out during two successive seasons 2015 and 2016 on 60 uniform vigour twelve-year old Superior seedless grapevines grown in a private vineyard located at Samaloute district, Minia Governorate. The texture of the soil is clay. Vines were irrigated under surface irrigation system, trellised by Gable supporting system, and spaced at 1.75 x 3 meters apart. For each experimental season, the vines received the usual horticultural practices. Winter pruning during both seasons was conducted on the middle of Dec. by using cane pruning system leaving 108 buds (8 fruiting canes x 12 buds + 6 renewal spurs x two buds) on each vine. Each treatment was replicated three times, two vines per each. The selected vines received four sprays from each substance as follow: the 1st date (4-to 8-inch shoots), the 2nd date (at fruit set stage), the 3rd date (after two weeks of fruit set stage) and the 4th date (at veraison stage).

The present study included ten treatments as follow:

- 1- Control.
- 2- Selenium (Se) 5 mg/L.
- 3- Tryptophan (TRP) at 50 mg/L.
- 4- TRP at 100 mg/L.
- 5- Methionine (MET) at 50 mg/L.
- 6- MET at 100 mg/L.
- 7- Se 5 mg/L+TRP 50 mg/L.
- 8- Se 5 mg/L +TRP 100 mg/L.
- 9- Se 5 mg/L + MET at 50 mg/L.
- 10- Se 5 mg/L + MET at 100 mg/L.

The following measurements were recorded during the two experimental seasons:

Some vegetative growth parameters:

- Average shoots length (cm) and Average number of leaves/shoot were measured in vegetative shoots.
- The average leaf area (cm²): sixth and seventh leaves from the tip of the growing vegetative shoot in both seasons were measured according to the method of Montero *et al.* (2000).
- Weight of pruning wood (kg): was measured at dormancy period (winter pruning) according the methods described by Selim *et al.* (1978).
- Average cane thickness (cm) : was calculated in the five basal internodes of ten vegetative canes per vine just before winter pruning by using a vernier caliper.

Chemical constitutes of grape leaves and canes

- Chemical content of N, P and K % in leaves petioles: samples of 20 leaves petioles per each replicate were taken from leaves opposite to basal cluster for the determination of N, P and K according to A.O.A.C (1995).
- Total carbohydrates of cane: total carbohydrates were measured at fruiting canes at winter pruning according the methods of Du Bois, *et al.* (1956).
- Total chlorophyll content in the leaves (mg/g fresh weight) was measured in mature 6th and 7th apical leaves using the nondestructive Minolta chlorophyll meter model SPAD 502 (Wood, 1993).

Yield and cluster characters

- Yield was recorded at the time of harvest (when total soluble solids reached about 15.5-16%, around the first week of Jun in both seasons), six clusters/ vine were weighed and the average cluster weight was multiplied by number of clusters/vine to calculation average yield/vine.
- Cluster parameters such as, average cluster number/vine, weight (g), length (cm) and width (cm) were measured.

Physical characteristics of berries were determined such as:

- Average berry weight (g).
- Average berry length and berry diameter (cm).
- Berry firmness (g/cm²) were determined in twenty berries using a push dynamometer (model FD101, Tokyo, Japan; needle diameter = 1 mm).

Chemical characteristics of berries:

- Total soluble solids content (T.S.S. %) was determined by using handrefractometer.
- Total acidity percentage and was determined by the method of (A.O.A.C, 1995).
- T.S.S/ Acid ratio.
- Total free amino acids (g/100 mg) were determined in berry juice according to Jayaraman, (1985).

Statistical analysis:

The obtained results were statistically analyzed according to analysis of variance (ANOVA) for experiment in randomized complete block design according to Snedecor and Cochran (1980) and L.S.D at 5% used to compare the variances between the treatments.

RESULTS AND DISCUSSION

Results

Vegetative growth characteristics

It is the evident from the data in Table (1), that the vegetative growth characteristics, namely, average of shoot length, number of leaves/ shoot, leaf area, cane thickness and pruning weight were significantly enhanced by all treatments compared with control in two seasons.

Meanwhile, high dose of two amino acids combined with Se has the best values in above studied characters in to both seasons. In this concern, the best results obtained from foliar application with TRP at 100 mg/L combined with Se followed by foliar application of MET at 100 mg/L when combined with Se.

Table 1. Effect of the foliar applications with selenium, tryptophan and methionine on some the vegetative growth characteristics of Superior Seedless grapevines.

Treatments	Shoot length (cm)		No. of leaves/ shoot		Leaf area (cm ²)		Cane thickness (cm)		Pruning weight (kg)	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1- Control	160.3	162.6	21.0	21.1	116.3	121.6	0.90	0.92	1.89	1.90
2- Selenium (Se) 5 mg/L	171.1	174.5	23.3	24.6	125.0	130.8	1.01	1.10	2.53	2.65
3- Tryptophan (TRP) at 50 mg/L	178.5	183.9	24.3	25.0	132.6	138.3	1.09	1.15	3.05	3.17
4- TRP at 100 mg/L	186.2	192.6	26.9	28.3	140.5	142.8	1.20	1.26	3.44	3.76
5- Methionine (MET) at 50 mg/L	177.0	183.0	23.9	24.5	131.7	136.6	1.05	1.13	2.78	2.99
6- MET at 100 mg/L	185.0	190.3	26.4	27.4	142.0	145.4	1.17	1.22	3.29	3.51
7- Se +TRP 50 mg/L	180.1	185.9	25.1	26.0	136.1	141.3	1.14	1.19	3.15	3.48
8- Se +TRP 100 mg/L	190.0	197.4	28.2	29.9	143.5	146.2	1.25	1.32	3.66	4.08
9- Se + MET at 50 mg/L	179.2	184.7	24.7	25.3	135.4	139.7	1.11	1.16	3.02	3.21
10- Se + MET at 100 mg/L	188.0	195.2	27.3	29.0	145.5	148.6	1.23	1.28	3.50	3.85
New L.S.D at 5 %	0.8	0.9	0.4	0.5	0.7	0.8	0.01	0.02	0.13	0.16

Chemical constitutes of grape leaves and canes

It is clear from the data in Table (2) that foliar application of Se, MET and TRP significantly improved N, P and K in the leaves petioles comparing with control in two seasons. Combined foliar application of Se with amino acids was favorable than using each material for both seasons. Notably, N, P and K in the leaves petioles significantly increased gradually with increasing amino acids concentration from low to high dose alone or

combined with Se. Indeed, the best results obtained by foliar spraying with TRP at 100 mg/L when combined with Se followed in a descending order by foliar spraying with MET at 100 mg/L in combined with Se during 2015 and 2016 seasons. In this case, the total chlorophyll in leaves and total carbohydrate in canes were significantly affected by above treatments for both seasons. The best results were obtained by foliar application on TRP at high dose in combined with Se, in two seasons.

Table 2. Effect of the foliar applications with selenium, tryptophan and methionine on some chemical constituents of leaves and canes in Superior Seedless grapevines.

Treatments	Petiole nutrient content (%)						Total chlorophyll (SPAD)		Total carbohydrates (g/100g)	
	N		P		K		2015	2016	2015	2016
	2015	2016	2015	2016	2015	2016				
1- Control	1.66	1.67	0.13	0.14	1.30	1.32	32.30	32.66	21.23	21.25
2- Selenium (Se) 5 mg/L	1.72	1.76	0.15	0.18	1.36	1.39	33.40	35.20	22.97	23.52
3- Tryptophan (TRP) at 50 mg/L	1.80	1.83	0.17	0.21	1.47	1.51	34.60	36.69	24.85	24.51
4- TRP at 100 mg/L	1.90	1.94	0.22	0.24	1.63	1.69	36.92	39.50	26.64	27.00
5- Methionine (MET) at 50 mg/L	1.77	1.81	0.22	0.27	1.44	1.47	33.73	36.02	23.83	24.43
6- MET at 100 mg/L	1.88	1.85	0.25	0.28	1.59	1.64	36.01	38.25	26.32	26.92
7- Se +TRP 50 mg/L	1.83	1.87	0.21	0.25	1.54	1.61	35.30	38.80	25.96	25.40
8- Se +TRP 100 mg/L	1.96	1.99	0.28	0.31	1.69	1.75	38.80	41.50	27.98	29.71
9- Se + MET at 50 mg/L	1.81	1.84	0.19	0.23	1.50	1.58	34.97	37.10	24.95	24.99
10- Se + MET at 100 mg/L	1.94	1.97	0.30	0.33	1.66	1.71	37.40	39.70	27.35	28.68
New L.S.D at 5 %	0.02	0.02	0.01	0.01	0.03	0.03	0.33	0.40	0.31	0.34

Yield and physical of clusters and berries characteristics

As shown in the Tables (3 & 4), yield cluster parameters, namely, cluster weight, cluster length and cluster width, and berries parameters, such as, berry weight, berry length, berry width were significantly improved by all treatments compared with control vines in

both seasons. The highest values of yield, cluster weight, cluster dimension, berry weight, and berry dimension were obtained by high dose of TRP when combined with Se followed by foliar application high dose of MET in combined with Se during two seasons.

Table 3. Effect of the foliar applications with selenium, tryptophan and methionine on yield/vine and physical characteristics of cluster in Superior Seedless grapevines.

Treatments	Yield / vine (kg)		cluster length (cm)		cluster width (cm)		cluster weight(g)		No. of cluster /vine	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1- Control	8.78	8.87	24.06	24.08	13.67	13.68	364.3	369.5	24.1	24.0
2- Selenium (Se) 5 mg/L	9.35	9.73	26.01	26.12	14.14	14.17	387.8	395.6	24.1	24.6
3- Tryptophan (TRP) at 50 mg/L	9.88	10.60	26.82	26.92	14.24	14.28	403.2	418.6	24.5	25.3
4- TRP at 100 mg/L	11.48	13.20	27.75	27.89	14.49	14.55	466.5	485.4	24.6	27.2
5- Methionine (MET) at 50 mg/L	9.74	10.20	26.76	26.80	14.28	14.31	394.3	407.6	24.7	25.1
6- MET at 100 mg/L	10.95	12.40	27.68	27.77	14.42	14.46	450.5	469.8	24.3	26.5
7- Se +TRP 50 mg/L	10.29	11.60	27.11	27.30	14.36	14.41	425.2	442.6	24.2	26.2
8- Se +TRP 100 mg/L	12.11	14.60	28.12	28.31	14.65	14.7	502.3	523.9	24.1	27.9
9- Se + MET at 50 mg/L	10.26	11.10	27.07	27.18	14.30	14.34	413.9	430.4	24.8	25.7
10- Se + MET at 100 mg/L	11.63	13.60	27.94	28.13	14.57	14.63	478.7	495.5	24.3	27.5
New L.S.D at 5 %	0.30	0.38	0.04	0.06	0.02	0.03	6.2	6.4	NS	0.2

On the other hand, the berry firmness (Table 4) was significantly affected by all treatments of amino acids at different doses when combined with Se compared with control in the two seasons. On the contrary, there were no

significant difference between MET at high dose alone and control during two seasons. The highest values of this parameter were obtained by foliar application of TRP at high dose in "2015" and "2016" seasons.

Table 4. Effect of the foliar applications with selenium, tryptophan and methionine on some physical characteristics of the berry in Superior Seedless grapevines

Treatments	Berry length (cm)		Berry width (cm)		Berry weight(g)		Berry firmness (g/cm ²)	
	2015	2016	2015	2016	2015	2016	2015	2016
1- Control	2.16	2.17	1.63	1.64	4.02	4.05	475.8	495.0
2- Selenium (Se) 5 mg/L	2.25	2.32	1.70	1.79	4.35	4.43	510.6	537.0
3- Tryptophan (TRP) at 50 mg/L	2.36	2.40	1.80	1.87	4.44	4.54	523.2	545.6
4- TRP at 100 mg/L	2.49	2.60	1.95	2.03	4.70	4.83	548.8	572.9
5- Methionine (MET) at 50 mg/L	2.33	2.36	1.77	1.84	4.40	4.46	480.3	501.0
6- MET at 100 mg/L	2.44	2.54	1.92	1.97	4.63	4.74	468.5	491.0
7- Se +TRP 50 mg/L	2.40	2.46	1.90	1.94	4.58	4.69	530.2	557.7
8- Se +TRP 100 mg/L	2.62	2.75	2.07	2.16	4.87	5.01	560.3	582.8
9- Se + MET at 50 mg/L	2.36	2.42	1.85	1.92	4.5	4.6	499.4	525.0
10- Se + MET at 100 mg/L	2.55	2.63	2.00	2.08	4.73	4.85	496.5	513.8
New L.S.D at 5 %	0.03	0.03	0.03	0.03	0.04	0.05	12.6	8.5

Chemical characteristics of berries

Data in Table (5) obviously show that combined foliar applications of amino acids and Se significantly enhanced T.S.S %, T.S.S / acid ratio, total free amino acids% and reducing total acidity % rather than untreated vines. Also the lowest values of T.S.S.% were obtained by the treatment with TRP at high dose alone, while the treatment with TRP at high dose in combined with Se enhanced T.S.S. compared with control. Treating the vines

with MET at high dose in combined with Se gave the highest values T.S.S.%, T.S.S./acid ratio and the lowest values of total acidity. Furthermore, total free amino acids % was increased with increasing the doses of foliar application with amino acids. In this case, the best values of total free amino acids% were obtained by foliar application of high doses of two amino acids (TRP and MET) when combined Se.

Table 5. Effect of the foliar applications with selenium, tryptophan and methionine on some chemical characteristics of the berry in Superior Seedless grapevines.

Treatments	T.S.S (%)		Acidity (%)		T.S.S/ acidity ratio		Total free amino acids (gm/100gm)	
	2015	2016	2015	2016	2015	2016	2015	2016
1- Control	15.60	15.62	0.760	0.758	20.53	20.61	0.0282	0.0283
2- Selenium (Se) 5 mg/L	15.98	16.07	0.708	0.687	22.57	23.39	0.0301	0.0304
3- Tryptophan (TRP) at 50 mg/L	16.20	16.27	0.692	0.674	23.41	24.14	0.0314	0.0319
4- TRP at 100 mg/L	15.56	15.60	0.773	0.769	20.13	20.29	0.0385	0.0390
5- Methionine (MET) at 50 mg/L	16.78	16.95	0.651	0.616	25.78	27.52	0.0320	0.0327
6- MET at 100 mg/L	16.93	17.08	0.618	0.585	27.39	29.20	0.0391	0.0396
7- Se +TRP 50 mg/L	16.52	16.63	0.668	0.632	24.73	26.31	0.0335	0.0339
8- Se +TRP 100 mg/L	15.76	15.99	0.745	0.730	21.15	21.90	0.0417	0.0422
9- Se + MET at 50 mg/L	16.85	17.01	0.637	0.593	26.45	28.68	0.0342	0.0345
10- Se + MET at 100 mg/L	16.99	17.11	0.601	0.570	28.27	30.02	0.0423	0.0427
New L.S.D at 5 %	0.06	0.05	0.014	0.013	0.66	0.74	0.0012	0.0013

Discussion

The improvement in the berry quality and yield/vine could be attribute to enhancing effect on physical and chemical characteristics of the berries as a result of enhancing vegetative growth parameters, total chlorophyll in leaves and total carbohydrates in canes and the nutritional status of the vines due to using foliar application of Se, TRP and MET.

The positive effect of Se on the vegetative growth characteristics may be due to its enhanced the activities of antioxidant enzymes as a result by providing a protection from environmental stress in crops, also; induced higher respiratory potential and higher efficiency of energy conversion of photosystem in plant leaves (Germ *et al.* 2009 and Feng and Wei, 2012). Furthermore, it may be due to the important role of Se in photosynthesis with junction ferredoxin, i.e., NADP oxidoreductase enzyme and then involved in nitrogen assimilation and lipid biosynthesis (Szczepaniak *et al.* 2013). Thus, Se may be increasing total chlorophyll in leaves, total carbohydrate in canes,leaves petioles nutrients and shoot length. Moreover, the beneficial effects of Se on berry firmness could be explained by the inhibitory effect of Se on various oxidative reactions (Zhao *et al.* 2013).

The function of TRP is well known, it has two ways for its effect; the first one on the plant growth and the second one on the IAA synthesis. TRP is an active precursor of IAA and can be used to regulate various physiological processes in plants (Ayesha *et al.* 2018). The beneficial effects of TRP on the vegetative growth characters, was by ammonia release during TRP-metabolization, and this ammonia acts as a source of nitrogen (Bar and Okon 1995). It is affecting gene expression for producing the specific macromolecules required for permanent cell elongation (Vanderhoeft, 1980). IAA has been suggested as control cell expansion in grape by its induced (VvCCEB1) gene that control cell expansion in grape and modifying the cell-wall network in grape (Nicolas *et al.* 2013), also; stimulate cell division and differentiation (Schenck *et al.* 2010). For this reasons, TRP may be enhanced berry quality of grape. However, Bottcher *et al.* (2011) mentioned that IAA delay grape berry ripening. Eventually, TRP may be reducing T.S.S.% and increasing total acidity%, but this was reflected in increasing the berry firmness.

MET was improved the vegetative growth characteristics due to its role in a regulatory molecule in the form of S-adenosylmethionine, protein constituent, and

as in the initiation of mRNA translation in plant cell (Hesse *et al.* 2004), also; synthesis of the cell wall biosynthesis, polyamines, chlorophyll biosynthesis, and to a large number of secondary metabolites (Rebeille *et al.* 2006; Goyer *et al.* 2007). Meanwhile, increasing total chlorophyll in leaves as result of the increasing of MET levels may be due its effect of phytohormones, which ultimately enhances chloroplast development and then, increases chlorophyll content in plant cells (Anne and Thomas, 2015). In addition to; there are so many reasons can clarify the effects of MET on the berry quality of grapevines such as, its role in maintaining the structure of proteins required for cell division, cell differentiation, growth, also; it provides sufficient sulfur and nitrogen according to plant needs (Khan *et al.* 2019). Moreover, MET is a precursor for the synthesis of the hormone ethylene, whereas; ethylene is as known important role in ripening of fruits, and MET may be implicated in increasing T.S.S. % and reducing total acidity % in the grape berry, but this was reflected in decreasing the berry firmness.

These results are in lines with those obtained by Mohamed, 2014; Hussein, 2017; Mohamed, 2017 and Zhua *et al.* 2017.

CONCLUSION

Treating Superior Seedless grapevines with TRP or MET at 100 mg/L combined with Se at 5 mg/L gave the best results with regard to vegetative growth, total chlorophyll in leaves and total carbohydrate in canes, physical clusters and berries. The treatment with Se had positive effects on T.S.S.% compared the control treatment in both seasons, while, the best results in this respect obtained by foliar application with MET at 100 mg/L combined with Se at 5 mg/L. Therefore, the increasing in total free amino acids in berry juice was increased with increasing foliar application of amino acids doses (TRP and MET) with combined Se.

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استجابة كرمات العنب السويبيور للرش الورقي بالسيلينيوم ، الترتوفان والميثونين

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أجريت هذه الدراسة خلال موسمي 2015 ، 2016 لدراسة تأثير الرش الورقي بالسيلينيوم بتركيز 5 ملجم / لتر ، الترتوفان والميثونين بتركيز (50 ، 100 ملجم / لتر) لكرمات العنب السويبيور عمرها 12 سنة . تم استخدام نظام الري السطحي للكرمات ، وكان التدعيم بنظام الجبيل ، ومسافة الزراعة 1.75 x 3 م ، حيث تم تقليم الكرمات في الاسبوع الثاني من ديسمبر خلال الموسم. وفقاً لذلك ، أظهرت النتائج أن الرش الورقي بكل من السيلينيوم ، الترتوفان والميثونين أدت إلى تحسين خصائص النمو الخضري ، الخصائص الفيزيائية للعنقود والحبات ، وأيضاً المحصول/الكرمة ، ومحتوى العناصر الغذائية في أعناق الأوراق (النيتروجين ، الفسفور والبوتاسيوم) ، الكربوهيدرات الكلية في القصبات والكلوروفيل الكلي في الأوراق وأيضاً الأحماض الأمينية الحرة في عصير الحبة مقارنة بالكنترول خلال الموسم. في هذا الصدد، تم الحصول على أفضل النتائج باستخدام الرش الورقي بالترتوفان مخلوطاً مع السيلينيوم. أيضاً ، تأثرت المواد الصلبة الذائبة الكلية إيجابياً بالمعاملة بالسيلينيوم ولكن انخفضت النسبة المئوية للحموضة في عصير الحبة مقارنة بالكنترول ، بينما أفضل النتائج في هذا الاتجاه تم الحصول عليها بالرش الورقي بالميثونين مخلوطاً مع السيلينيوم. وكذلك إزداد محتوى الأحماض الأمينية الحرة في عصير الحبة مع زيادة تركيز كلٍ من الترتوفان والميثونين.