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EFFECT OF PHENYLALANINE, PUTRESCINE AND SPERMIDINE ON YIELD AND BERRY QUALITY THOMPSON SEEDLESS GRAPEVINES.

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ABSTRACT

This study was carried out during 2002 and 2003 seasons to investigate the effects of phenylalanine (at 100, 200 or 400 ppm), putrescine (at 200, 250 or 300 ppm) and spermidine (at 50, 100 or 150 ppm), at full bloom on yield and berry quality of Thompson Seedless grapevines. The vines were grown at vineyard of Fac. Agric., Suez Canal Univ., Ismailia, Egypt. In this respect, phenylalanine at 400ppm exhibited high yield per vine due to its effect on increasing berry weight. Contrary, pruning weight, total carbohydrates, nitrogen, potassium and total acidity were significantly decreased as concentration of the tested chemicals increased, while phosphorus content was not significantly affected. The increments of amino acids and berry volume were detected by spraying spermidine at 100 ppm. In addition, putrescine at 300ppm or phenylalanine at 400ppm recorded the highest weight of 100 berries, cluster length and width and soluble solids content.

Therefore, phenylalanine at 400 ppm or putrescine at 300 ppm may be considered as the most effective treatments in increasing vine yield and improving berry quality.

INTRODUCTION

Grape is considered one of the most important and popular fruit crops in Egypt. According to the census of 2004 in Egypt, the area occupied with vineyards is 159929 feddans.

Polyamines are small positively charged aliphatic amines that play various roles in plant physiology (Egea-Cortines and Mizrahi, 1991). Considerable research has suggested a closed connection between polyamines, such as putrescine, spermidine and spermine, and a large range of growth and developmental processes including flower induction, reproductive development, growth and fruit ripening (Applewhite *et al*.,2000; Aziz *et al*.,2001; Kakkar and Rai, 1993; Galston *et al*., 1997; Walden *et al* .,1997). Furthermore, polyamines are new class of growth substances that play an essential role in plant physiology including the regulation of DNA replication, transcription of genes, cell division, organ development, floral process, fruit ripening and leaf senescence (Bagni and Torrigiani, 1992; Baigorri *et al* ., 2001; Colin *et al*., 2002; Galston and Kaur-Sawhney., 1995).

The effectiveness of spraying grapevine with phenylalanine (amino acid) at various concentrations on different dates was investigated by Koval *et al* (1983) on Cabernet Sauvignon; by Strakhov and Sedletskii (1986) on Aligote, and by Sedletskii *et al* (1988) on Rkatisteli. They found that the best treatment was 300 ppm when applied three time (before anthesis, at full bloom and at the start of berry ripening). This treatment increased yield by 10-32 %, due to the increase in both berry and cluster weights, berry sugar content by 1.2-1.3 g/100 cm3 and decreased berry acid content.

Application of putrescine stimulated the development of seedless berries (Shiozaki *et al*., 1998), increased bud fertility (as number of clusters/bud) in the following year (Bagni and Torrigiani, 1992; Ferrara *et al*., 1998; Galston *et al*., 1997), it was found that exogenous spermidine induces an increase in soluble sugar but decreased amino acid content in both leaves and inflorescences of grapevines (Aziz *et al*., 2001; Applewhite *et al*.,2000).

The objective of the present study was to investigate the effects of phenylalanine, putrescine and spermidine on yield /vine and berry quality of Thompson Seedless grapevines.

MATERIALS AND METHODS

This investigation was carried out during the two successive seasons of 2002 and 2003 at the vineyard of faculty of Agriculture, Suez Canal University, Ismailia, Egypt, on Thompson Seedless grapevine (Vitis vinifera L). The vines are 17-year-old, planted at 2X3m apart on sandy soil, pruned according to the cane pruning under trained on overhead trellis system with 3 horizontal wires. Each vine had 56 buds resulting from 4 canes with 12 buds and 4 spurs with 2 buds. The selected vines were healthy and subjected to the recommended management of vineyards in new lands. The vines were sprayed at full bloom (when 50% of the cap fall) with phenylalanine (2-amino-3phenylpropionic acid)at 100, 200 or 400 ppm , putrescine (1,4-diamino butane) at 200, 250 or 300 ppm and Spermidine (N-[3-amino-propyl] butane – 1,4- diamine) at 50, 100or 150 ppm. Yet, the control treatment was sprayed with water containing Triton at 0.1% which added to the spray solution as a wetting agent. The experimental treatments were arranged in randomized block design, where 10 treatments were conducted, each with 4 replicates and 2 vines per replicate.

After dormant pruning, all one-year-old wood per vine was weighed. Then, samples from ripened current growth shoots were taken (At first week of January), cut into small pieces, oven dried at 70°C and ground to determine total carbohydrates (as g glucose/100g dry weight) by using the phenol sulphoric acid method as described by Smith *et al* (1956).

At mid-June, samples of 20 petioles per each replicate were taken opposite clusters to determine: total nitrogen content by using the Micro-Kjeldahl method according to Bremner (1965), phosphorus by using Spectrophotometer according to A.O.A.C. (1985) and potassium content by using the flame photometer as described by Brown and Lililand (1946). N, P and K were calculated as g/100g dry weight.

The blades of leaves were used for determining total free amino acids (as mg glutamic acid/100g dry weight) by using the method described by Selim *et al* (1978).

When SSC ranged about 17-18 % in berry juice according to Horst (1998), both number and weight of clusters per vine were recorded. Two clusters from each replicate were randomly picked to determined cluster dimensions (cm), weight of 100berries, juice SSC (%) by using hand referactometer, juice acidity (mg tartaric acid/100 ml juice) according to A.O.A.C. (1985), and SSC/acid ratio.

Statistical analysis: The obtained data was statistically analyzed as randomized complete block design according to Steel and Torrie (1980). Analysis of variance and mean comparison were done (Duncans Multiple Range test) using M-STAT program version 7 (1990).

RESULTS AND DISCUSSION

1- Number of clusters and vine yield:-

It is clear from table (1) that the number of clusters per vine did not affect by spraying phenylalanine or polyamines in the first season. These results were expected because the floral differentiation for the current year's crop takes place in the preceding year (Williams and Mathews 1990). In the second season, number of clusters per vine was significantly affected by all treatments. In each tried substance, the high number of clusters was produced by the highest concentration with no differences among them. In this respect, Ferrara *et al* (1998) demonstrated that bud fertility (measured as number of cluster/bud) in the following year was higher in vines which sprayed with putrescine (0.58 cluster/bud) followed by benzyladenine, spermidine and chlormequat, but differences among the treatments were not significant.

Spraying Thompson Seedless grapevine with phenylalanine at 400 ppm increased the vine yield significantly comparing to the other treatments and the control in both seasons (Table 1). Similar responses were obtained in some grape cultivars by Koval *et al* (1983), Strakhov and Sedletskii (1986) and Sedletskii *et al* (1988) since they were spraying phenylalanine at 300 ppm, the increments in vine yield were 10-32% due to the increase in both berry and cluster weights. The results also indicated that the differences between the three concentrations within putrescine and spermidine were insignificant during the two seasons. Although there were slightly increases in yield per vine as the concentration increased. This indicates that the best concentration may be much higher than 300 or 150 ppm.

Cluster weight was significantly influenced by the tested materials. The results revealed that phenylalanine recorded the highest cluster weight as the average of the three concentrations (414.7 and 420.1 g in the first and second seasons, respectively) followed by putrescine and spermidine (395.0, 396.4 and 385.3, 392.7 g, respectively), while the control recorded 356.5 and 384.7 g in the first and second seasons, respectively.

It should be pointed out that, number of clusters, vine yield and cluster weight as average of two seasons (Table 1), were behaved in a similar manner where they were significantly affected by phenylalanine at 400 ppm.

2- Cluster and berry physical characters:-

The application with phenylalanine or putrescine at full bloom increased significantly cluster length in the two seasons as compared to the control (Table 2). The highest increment in cluster length was recorded with putrescine at 300 ppm (20.53, 22.70 cm) followed by phenylalanine at 400 ppm (20.30, 20.53 cm) and spermidine at 150 ppm (19.28, 20.38 cm), however, the differences were insignificant among them in the first season, but putrescine at 300 ppm showed significantly in the second one.

T 1-2

Phenylalanine at 400 ppm, when applied at full bloom to Thompson Seedless grapevines, increased cluster width significantly as compared to control and other treatments (Table 2). However, in the first season, putrescine at 200 ppm or spermidine at 50 ppm showed no significant effect in this respect, while phenylalanine at 100 ppm detected similar effect in the second one.

In general, weight of 100 berries was significantly increased by spraying phenylalanine, putrescine and spermidine, which was associated with the high concentrations, inspite of the insignificancy, spermidine only at 150 ppm showed a significant effect in the first season. This clearly indicates that phenylalanine and polyamines had similar effect on berry weight. In this respect, the increment in berry weight increased cluster weight and consequently increased the vine yield. These results were supported by Koval *et al* (1983); Strakhov and Sedletskii (1986) and Sedletskii *et al* (1988) since they stated that phenylalanine at 300 ppm increased both berry and cluster weights which increased the yield.

In the first season, the highest berry volume was obtained by spermidine at 100 ppm but the inverse trend was observed in the second one, where putrescine at 300 ppm and phenylalanine at 400 ppm gave the highest berry volume (Table 2).

3- Berry chemical characters:-

Regard to soluble solids content (SSC), phenylalanine at 400 ppm and putrescine at 300 ppm had similar trend which the highest significant increase in SSC was recorded with the highest concentrations as compared to control (Table 3).

Table (3): Effect of spraying phenylalanine, putrescine and spermidine at full bloom on berry chemical characteristics of Thompson seedless grapevines during 2002 and 2003 seasons.

Treatments (ppm	Soluble solids content (SSC) (%)		Total acidity (%)		SSC/Acid ratio	
	2002	2003	2002	2003	2002	2003
Control	20.2 bc	18.3 e	0.87 a	0.79 a	23.2 e	23.2 f
Phenylalanine						
100	19.3 d	20.4 bc	0.79 b	0.75 bc	24.4 e	27.2 de
200	20.2 bc	19.5 d	0.70 de	0.73 de	28.9 bc	26.7 e
400	21.1 a	22.6 a	0.66 e	0.69 h	31.9 a	32.8 a
Putrescine						
200	18.3 e	19.9 cd	0.74 bcd	0.74 cd	24.7 e	26.9 e
250	19.0 de	20.2 bc	0.81 ab	0.70 gh	23.5 e	28.9 bc
300	21.4 a	21.9 a	0.71 cde	0.72 ef	30.1 ab	30.4 b
Spermidine						
50	19.5 cd	18.6 e	0.77 bc	0.76 b	25.3 de	24.5 f
100	20.3 b	19.9 cd	0.75 bcd	0.71 fg	27.1 cd	28.0 cde
150	20.1 bc	20.8 b	0.69 de	0.73 de	29.1 abc	28.5 cd

Means Followed by the same letter within columns are not significantly different, $P \leq 0.05$

Phenylalanine, putrescine and spermidine at all concentrations decreased significantly the total acidity compared to control in the two seasons, except putrescine at 250 ppm in the first season. These findings are accordance with that obtained by Koval *et al* (1983); Strakhov and Sedletskii (1986) and Sedletskii *et al* (1988) who reported that spraying phenylalanine at 300 ppm on some grapevine increased berry sugar content but decreased total acidity.

Also, all concentrations of the sprayed chemicals caused a marked increase in SSC/acid ratio in berry juice as compared to untreated treatment in both seasons.

In conclusion, the spray application of phenylalanine at 400 ppm was effective in increasing yield /vine and improving berry quality. However, further work is required to determine the optimal concentrations of spermidine and time of treatment in order to use it for practical improvement of grapevines.

4- N, P, K and total amino acids:-

In both seasons, nitrogen and potassium behaved in a similar manner, so, they were significantly decreased by spraying phenylalanine, putrescine and spermidine at full bloom (Table 4). The decline in N and K was more pronounced in vine treated with spermidine at 150 ppm and putrescine at 300 ppm, respectively. Phosphorus content was not significantly affected by all treatments. In this respect, various researchers have been shown that under conditions of K deficiency, putrescine was accumulated in grape leaves. They added that leaves with deficiency symptoms had 26 times more free putrescine than healthy leaves (Adams *et al*., 1990 and 1992; and Lespy-Labayette *et al*., 1994). However, Geny *et al* (1997) suggested that polyamines can be used as sensitive biochemical markers to distinguish the optimum K levels for grapevines before the appearance of K nutrient deficiency symptoms.

Table (4): Effect of spraying phenylalanine, putrescine and spermidine at full bloom on N, P, K and total amino acids of Thompson seedless grapevines during 2002 and 2003 seasons.

Treatments (ppm)	N (%)		P (%)		K (%)		Total amino acids mg/g D. W.	
	2002	2003	2002	2003	2002	2003	2002	2003
Control	2.90 a	2.98 a	0.193 a	0.198 a	1.80 a	1.88 a	0.71 d	0.93 f
Phenylalanine								
100	2.30 bc	2.89 a	0.131 a	0.146 a	1.44 b	1.50 bcd	1.23 c	1.20 e
200	2.17 cd	2.27 bc	0.136 a	0.159 a	1.46 b	1.55 b	1.35 bc	1.71 bc
400	2.14 cde	2.31 b	0.145 a	0.163 a	1.49 b	1.53 bc	1.62 bc	1.73 bc
Putrescine								
200	2.19 cd	2.22 bcd	0.164 a	0.187 a	1.23 c	1.20 e	1.42 bc	1.35 de
250	2.09 cde	2.01 de	0.174 a	0.188 a	1.25 c	1.21 e	1.45 bc	1.54 cd
300	2.03 de	2.12 bcd	0.178 a	0.199 a	1.20 c	1.22 e	1.67 bc	1.69 bc
Spermidine								
50	2.11 cde	2.10 de	0.194 a	0.202 a	1.45 b	1.43 bcd	1.65 bc	1.70 bc
100	2.00 de	2.08 cde	0.186 a	0.209 a	1.38 b	1.41 cd	1.78 ab	1.87 ab
150	1.91 e	1.88 e	0.196 a	0.215 a	1.40 b	1.38 d	2.21 a	1.95 a

Means Followed by the same letter within columns are not significantly different, $P \leq 0.05$

In recent study, Geny and Broquedis (2002) found that N deficiency led to a high content of bound spermidine and a marked decrease of conjugated putrescine.

Total amino acids were significantly increased in response to spray spermidine application at 100 or 150 ppm at full bloom, but the differences between them were insignificant during the two seasons (Table 4). It is also indicated that the differences between the three concentrations within phenylalanine and putrescine were insignificant during the first season, where the values of amino acids varied within a narrow range, i. e., 1.23-1.62 mg/g D.W. and 1.42-1.67 mg/g D.W. for phenylalanine and putrescine, respectively. Such behaviour did not confirm in the second season, where the significancy of differences among the three concentrations was rather clear particularly when low and high concentrations were compared.

5- Pruning weight and total carbohydrates:-

Pruning weight was used as a measured of vine vigour (Mukherji *et al* 1970). As shown in Table (5), pruning weight decreased as the concentrations of phenylalanine or polyamines increased, indicating that the highest concentrations tended to reduce vine vigour, which could be partly attributed to increase the fruitfullness.

In general, total carbohydrates were significantly decreased by spraying vine with phenylalanine or polyamines compared to the control (Table 5). This reduction in carbohydrates was more observed in the vines treated with phenylalanine at 200 ppm in both seasons. On the other hand, a gradually increment in total carbohydrates was detected within the three concentrations of putrescine or spermidine, which were associated with increasing concentrations, while a fluctuation trend was given by phenylalanine.

Table (5): Effect of spraying phenylalanine, putrescine and spermidine at full bloom on pruning weight and total carbohydrates of Thompson seedless grapevines during 2002 and 2003 seasons.

Treatments (ppm)	Pruning weight (g)		Total carbohydrate (g/100g D. W.)	
	2002	2003	2002	2003
Control	372.3 a	493.2 a	15.5 a	16.9 a
Phenylalanine				
100	361.1 b	378.9 d	11.2 de	10.8 d
200	344.0 cde	343.0 f	10.3 e	9.6 d
400	350.2 c	333.2 f	11.5 cde	13.0 bc
Putrescine				
200	363.2 b	401.0 c	11.3 cde	10.5 d
250	339.5 de	378.1 d	12.4 cd	12.8 bc
300	325.1 f	369.2 de	14.6 ab	13.9 b
Spermidine				
50	347.0 c	488.0 a	12.4 cd	10.7 d
100	338.0 e	432.3 b	13.8 b	12.3 c
150	345.1 cd	360.1 e	12.5 c	13.1 bc

Means Followed by the same letter within columns are not significantly different, P ≤ 0.05

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تـأثير الـرش بالفنيـل ألانـين والبيتروسـين والإسـبرمدين علـى المحصـول وصـفات الحبات فى العنب البناتى

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أجريت هذه الدراسة خلال موسمي ٢٠٠٢- ٢٠٠٣ على كرمات العنب صنف تومسون سيدليس عمر ١٧ سنة نامية في مزرعة كلية الزراعة- جامعة قناة السويس, بمحافظة الإسماعيلية بهدف معرفة تـأثير الـرش بالفنيل ألانـين (١٠٠- ٢٠٠ أو ٤٠٠ جزء في المليون) والبيتروسين (٢٠٠- ٢٥٠ أو ٣٠٠ جزء في المليون) والإسبرمدين (٥٠- ١٠٠ أو ١٥٠ جزء في المليون) على المحصول وجودة الحبات. أظهرت النتائج أن الفنيل ألانين بتركيز ٤٠٠ جزء في المليون أعطى زيادة واضحة في المحصول نتيجة زيادة وزن الحبات وقد أدت زيادة تركيز جميع المعاملات إلى انخفاض كل من وزن خشب التقليم والكربوهيدرات الكلية ومحتوى الأعناق من النيتروجين والبوتاسيوم وكذلك محتوى عصير الحبات من الحموضة. أدى الرش بالإسبرمدين بتركيز ١٠٠جزء في المليون إلى زيادة كل من حجم الحبات والأحماض الأمينية. كما سجل البيتروسين بتركيز ٣٠٠جزء في المليون أو الفينيل ألانين بتركيز ٤٠٠جزء في المليون أعلى طول وقطر للعنقود وأكبر وزن ١٠٠حبة وكذلك محتوى عصير الحبات من المواد الصلبة الذائبة.

كذا يوصى بالرش عند التزهير الكامل بالفنيل ألانين بتركيز ٤٠٠جزء في المليون أو البيتروسين بتركيز ٣٠٠جزء في المليون لزيادة المحصول وكذلك تحسين خواص وصفات الحبات.

Table (1): Effect of spraying phenylalanine, putrescine and spermidine at full bloom on number of cluster, vine yield and cluster weight of Thompson seedless grapevines during 2002 and 2003 seasons.

Treatments (ppm)	No. of clusters			Yield / vine (Kg)			Cluster weight (g)		
	2002	2003	Mean	2002	2003	Mean	2002	2003	Mean
Control	11.2 a	10.7 c	10.95 e	3.99 c	4.12 cd	4.06 d	356.5 e	384.7 de	370.6 d
Phenylalanine									
100	12.1 a	11.8 bc	11.95 cde	4.73 bc	4.51 cd	4.62 cd	391.2 bcd	381.9 e	386.6 cd
200	12.4 a	13.2 ab	12.80 bcd	4.99 bc	5.35 bc	5.17 bc	402.8 bc	405.8 bc	403.9 bc
400	14.6 a	15.3 a	14.95 a	6.57 a	7.24 a	6.91 a	450.1 a	473.2 a	461.7 a
Putrescine									
200	11.3 a	12.1 bc	11.7 de	4.35 bc	4.59 cd	4.47 cd	384.0 cd	379.5 e	381.8 cd
250	11.4 a	12.8 bc	12.10 cde	4.45 bc	5.04 bcd	4.75 bcd	390.3 bcd	394.1 cde	392.2 bcd
300	12.7 a	13.3 ab	13.00 bc	5.22 bc	5.53 bc	5.38 bc	410.8 b	415.7 b	413.3 b
Spermidine									
50	13.2 a	12.5 bc	12.85 bcd	5.03 bc	4.83 bcd	4.93 bcd	381.0 cd	386.4 cde	383.7 cd
100	11.1 a	12.9 abc	12.00 cde	4.16 bc	5.02 bcd	4.59 cd	374.7 de	389.1 cde	381.9 cd
150	13.8 a	14.2 ab	14.00 ab	5.52 ab	5.72 b	5.62 b	400.1 bc	402.6 bcd	401.4 bc

Means Followed by the same letter within columns are not significantly different, P ≤ 0.05

Table (2): Effect of spraying phenylalanine, putrescine and spermidine at full bloom on total amino acids, cluster and berry physical characteristics of Thompson seedless grapevines during 2002 and 2003 seasons.

Treatments (ppm	Cluster dimensions (cm)				100 berries			
	Length		Width		Weight (g)		Volume $(cm)^3$	
	2002	2003	2002	2003	2002	2003	2002	2003
Control	13.18 e	18.00 d	8.10 e	9.90 cd	130.7 fg	126.1 de	121.2 ef	119.3 f
Phenylalanine								
100	14.33 e	19.01 cd	9.11 cd	9.50 de	133.1 ef	121.3 e	127.4 de	117.0 f
200	17.70 cd	18.58 cd	8.58 cde	7.40 f	141.2 de	133.1 cd	132.6 c	121.3 ef
400	20.30 a	20.53 b	12.75 a	11.93 a	177.2 a	173.5 a	142.4 ab	165.4 a
Putrescine								
200	13.55 e	18.43 cd	8.00 e	7.40 f	151.2 cd	142.7 c	132.5 c	133.5 cd
250	16.70 d	19.20 c	9.27 c	10.30 bcd	145.3 d	153.6 b	123.7 de	141.2 c
300	20.53 a	22.70 a	10.48 b	11.10 ab	167.9 ab	170.0 a	143.0 ab	167.1 a
Spermidine								
50	16.78 d	18.20 cd	8.30 de	8.65 e	121.0 g	132.4 cd	117.0 f	128.3 de
100	18.83 bc	20.30 b	10.80 b	10.53 bc	151.2 cd	142.3 c	143.8 a	131.2 d
150	19.28 ab	20.38 b	10.63 b	10.38 bcd	158.4 bc	167.6 a	139.1 b	152.7 b

Means Followed by the same letter within columns are not significantly different, P ≤ 0.05