**Trials for enhancing berries maturation and grapes quality of grapevine cultivar flame seedless grown under Minia region conditions**

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**Abstract:** Maturation date, yield / vine, berries colouration % berries, shattering %, cluster weight, total anthocyanins and both physical and chemical characteristics of grapevine cv., Flame seedless, grown under Minia region conditions in response to treating the clusters with protone (10% ABA) at 500ppm, ethrel at 250 ppm and amino acids at 1 % either applied alone or in different combinations were investigated during 2017 and 2018 seasons. Single and combined applications of protone at 500ppm, ethrel at 250 ppm and amino acids at 1% once at veraison stage when approximately 10% of the berries on 50% of the clusters had softened resulted in measurable advancement in maturation date and promotion on berries colouration and berries quality compared to the control. In ascending order the best materials in this respect were amino acids, protone and Ethrel. Combined application were favourable than using each material alone in hastening fruit maturation and improving berries quality. Yield as well as weights of cluster and berries were unaffected by the present treatments. For solving the problem of uneven colouration of the berries in the clusters of Flame seedless, grapevines grown under Middle Egypt region conditions, it is suggested to subject the clusters once at veraison stage when approximately 10% of the berries on 50% of the clusters on each vine had softened with a mixture of ethrel at 250 ppm, protone at 500 ppm and amino acids at 1%.

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**Keywords**: Ethrel, protone; ABA, Amino acids, Flame seedless, berries maturation and colouration berries quality, anthocyanins.

**1. Introduction**

Many efforst were done for solving the problems of irregular colouraiton of coloured grapevine cvs Flame seedless, Red Globe and Crimson seedless under hot climates Nowadays, several trials were conducted to use amino acids as replacement of Ethrel and protone for advancing maturation date and promoting berries colouration grapevine cvs (**Davies, 1980**). The occurrence of this problem had negative effects on marketing of the fruits to local and foreign markets (**Orth *et al.,* 1993; Taiz and Zeiger, 2002. Zhang *et al.,* 2009 and Dal *et al.,* 2010**).

Previous studies showed that using amino acids (**Ahmed *et al.,* 2012; Abdelaal, 2012; Abdelaal *et al.,* 2013; El- Khawaga, 2014; Mohamed, 2014; Abdel aziz et al., 2017 and Mohamed, 2017);** ethrel **( Liu *et al.,* 2002, Omar and Girgis, 2005, El- Halaby, 2006; El- Sayed, 2007; Kassem, *et al.,* 2011; Aly, 2017 and Ibrahiem, 2018)** and ABA **(Geny et al., 2005; Koyama, 2010; Giribadi *et al.,* 2010; Strydom, 2014, Ferrara *et al.,* 2015, Yamamoto *et al.,* 2015 Lichter *et al.,* 2015, Casterllarin *et al.,* 2016, Aly, 2017 and Ibrahiem, 2018**) were very effective in advancing maturation date, berries colouration and grapes quality in different grape cvs.

The merit of this study was examining the effect of single and combined applications of amino acids, ethrel and protone as a source of ABA on maturation date, berries colouration and quality of berries in grapevines cvs Flame seedless, Red Globe and Crimson seedless grown under Minia region conditions.

**2. Material and Methods**

This study was carried out during the two consecutive seasons of 2017 and 2018 on ninety uniform in vigour own rooted 11 years old of grapevine cv Flame seedless, grown in a private vineyard, namely El Karam, Talla village, Minia district, Minia Governorate where the texture of the soil is clay.

The selected vines are planted at 1.5 x 3 meters apart. The chosen vines were trained by spur pruning system leaving 72 eyes/ vine (12fruiting spurs x 5 eyes plus six replacement spurs x two eyes) using Gable supporting method. Winter pruning was conducted on the first week of Jan. during both seasons. Surface irrigation system was followed using Nile water containing 50 ppm salinity.

The selected vines (48 vines) received the same horticultural practiced that were already applied in the vineyard except application of Ethrel, proton and amino acid. Thesepractices including the application of 10 tons FYM, (0.3% N), 240 kg ammonium nitrate, 150 kg calcium superphosphate (15.5 % P2O5) and 200 kg potassium sulphate (48 % K2O) per one fedddan annually. F.Y.M was added once at the middle of Jan. Nitrogen fertilizer was added at three unequal batches as 37.5 % just after growth start, 37.5 % just after berry setting and 25% just after harvesting. Phosphate fertilizer was added once at the middle of Jan. Potassium fertilizers was applied twice at growth start (middle of Feb.) and again just after berry setting (middle of April) during both seasons. Another horticultural practices such as twice hoeings, irrigation, pinching and pest management were carried out as usual.

Soil is classified as clay in texture with water table depth not less than two meters deep. The results of orchard soil analysis according to **Wilde *et al.,* (1985)** aregiven in **Table (1).**

**Table (1): Mechanical, physical and chemical analysis of the tested orchard soil:**

|  |  |
| --- | --- |
| **Parameters** | **Values** |
| **Particle size distribution** |  |
| Sand % | 10.0 |
| Silt % | 15.0 |
| Clay % | 75.0 |
| Texture garde | Clay |
| pH (1: 2.5 extract) | 7.59 |
| E.C. (1: 2.5 extract) ( mmhos/ 1cm 25oC) | 0.81 |
| O.M. % | 2.11 |
| CaCO3% | 2.4 |
| **Macronutrients values** |  |
| Total N % | 0.09 |
| P ( Olsen method, ppm) | 4.11 |
| K ( ammonium acetate, ppm) | 419 |
| Mg (ppm) | 6.12 |
| S (ppm) | 2.20 |
| **EDTA extractable micronutrients (ppm):** |  |
| Zn | 0.79 |
| Fe | 1.11 |
| Mn | 1.9 |
| Cu | 0.72 |

This experiment included thirty treatments from two factors (A & B). The first factor (A) comprised from three grapevine cvs namely a1) Flame seedless, a2) Red Globe and a3) Crimson seedless. The second factor (B) contained the following ten treatments from single and combined applications of Ehrel at 250 ppm, proton at 500ppm and amino acids at 1%:

1- Control

2- Spraying ethrel at 250 ppm ( 0.52 ml / L)

3- Spraying protone at 500 ppm ( 5.0 ml/L)

4- Spraying amino acids at 1% ppm ( 10 ml/L)

5- Spraying ethrel at 250 ppm+ protone at 500 ppm

6- Spraying ethrel at 250 ppm + amino acids at 1%

7- Spraying protone at 500 ppm + amino acids at 1%

8- Spraying ethrel at 250 ppm + protone at 500 ppm + amino acids at 1%

Each treatment was replicated three times, one vine per each. Ethrel (48%) and Proton (10% ABA) were sprayed once at veraison stage when approximately 10% of the berries per cluster on 50% of the number of vine clusters had softened.

Triton B as a wetting agent was added at 0.05%. Spraying was done till clusters runoff.

Randomized complete block design (RCBD) was followed Each treatment was replicated three times, two vines per each.

For realizing the objectives of this study, the following parameters were recorded in response to application of Ethrel, Proton and amino acid.

**1-Maturation time**

It was calculated when T.S.S./acid reached 25:1.

**2-Yield per vine**

The yield of each vine was recorded in terms of weight (in kg.), then the average weight of cluster (g.) was recorded. Five clusters per each vine were taken at random for determinations of the following physical and chemical characteristics of the berries:

1. Percentage of berries colouration by dividing number of red coloured berries by total number of berries per cluster and multiplying the product by 100.
2. Percentage of berries shattering by counting the number of dropped berries by the total number of berries per cluster and multiple the product by 100.
3. Average berry weight (g.) and dimensions (longitudinal and equatorial, cm).
4. Percentage of total soluble solids in the juice by using handy refractometer.
5. Percentage of total acidity (as gram tartaric acid / 100 ml juice) by titration against 0.1 N NaOH using phenolphthalein as indicator (**A.O.A.C**, **2000).**
6. The ratio between T.S.S. and acid.
7. Percentage of reducing sugars in the juice by using **Lane and Eynon (1965)** volumetric method as described in **A.O.A.C. (2000).**
8. Total phenols (**A.O.A.C., 2000**) and total anthocyanins in the berries by using ethyl alcohol and HCL method (mg /100 g F.W.) **(Fulcki and Francis, 1968).**

The proper statistical analysis was done. Treatment means were compared using new L.S.D. test at 5% (according to **Mead *et al.,* 1993).**

**3. Results**

**1- Maturation time**

Data in Table (2) show the effect of single and combined application of protone, ethrel and amino acids on maturation date of grapevine cv Flame seedless, during 2017 and 2018 seasons.

Maturation time was materially hastened with using protone at 500ppm, ethrel at 250 ppm and amino acid at 1% either applied alone or in combination compared to the control treatment Using amino acids at 1%, protone at 500ppm and ethrel at 250 ppm, in ascending order effectively advanced maturation date. Combined applications of protone, ethrel and amino acids was materially advanced maturation time than using each material alone. Combined application using two materials together gave an outstanding promotion on maturation time than using each material alone. The best double application treatment was the application of protone at 500 ppm plus ethrel at 250 ppm. Using protone at 500 ppm, ethrel at 250 ppm and amino acid at 1% gave a great advancement on maturation date. These results were true during both seasons.

**2- The yield / vine**

Data in Table (2) show the effect of single and combined applications of protone, ethrel and amino acids on the yield/ vine of grapevine cv Flame seedless, during 2017 and 2018 seasons.

Single and combined applications of protone at 500ppm, ethrel at 250 ppm and amino acids at 1% caused unsignificant promotion on the yield / vine relative to the control treatment. These results were true during both seasons.

**3- The percentage of berries colouration**

Data in Table (3) show the effect of single and combined application of protone, ethrel and amino acids on berries colouraiton % of grapevine cv Flame seedless, during 2017 and 2018 seasons.

Single and combined applications of protone at 500ppm, ethrel at 250 ppm and amino acids at 1% significantly was followed by enhancing berries colouration compared to the check treatment. Treating the clusters of the grapevine cv Flame seedless with ethrel, protone and amino acids, in descending was significantly responsible for advancing berries colouration %. Combined applications of the previous three materials were significantly favourable in enhancing berries colouration than application of each material alone. Using the three materials namely ethrel at 250 ppm, protone at 500 ppm and amino acids at 1% gave the maximum values of berries colouration %. The untreated vines produced the lowest values. These results were true during both seasons.

**4- The percentage of berries shattering**

Data in Table (4) show the effect of single and combined application of protone, ethrel and amino acids on the percentage of berries shattering of grapevine cv Flame seedless, during 2017 and 2018 seasons.

Varying ethrel, protone and amino acid treatments had no significant effect on the percentage of berry shattering. These results were true during both seasons.

**5- The average cluster weight**

Data in Table (5) show the effect of single and combined application of protone, ethrel and amino acids on the average cluster weight of grapevine cv Flame seedless, during 2017 and 2018 seasons.

Average cluster weight was significantly unaffected by varying ethrel, protone and amino acid treatment during both seasons.

**6- Physical and chemical characteristics of the berries**

Data in Tables (4 & 5) show the effect of single and combined application of protone, ethrel and amino acids on Berry weight and dimensions, TSS, reducing sugars %, T.S.S/ acid, titratable acidity %, total phenols and total anthocyanins in the juice of grapevine cv Flame seedless, during 2017 and 2018 seasons.

**6-1 Berry weight and dimensions**

Average berry weight and dimensions were significantly unaffected by ethrel, protone and amino acid treatments during both seasons.

**6-2 Total soluble solids, reducing sugars % and T.S.S/ acid**

Single and combined applications of ethrel at 250 ppm, protone at 500ppm and amino acids at 1% significantly enhanced T.S.S. %, reducing sugars and T.S.S./ acid relative to control. Using ethrel, protone and amino acids, in descending order was significantly very effective in enhancing these chemical characteristics. These results were true during both seasons.

**6-3 Titratable acidity %**

It was significantly reduced with single and combined applications of ethrel at 250 ppm, protone at 500ppm and amino acids at 1% relative to the control. Using ethrel at 250 ppm was significantly favourable than the other two materials in reducing titratable acidity. Protone significantly ranked the second position and amino acids occupied the last position in this respect. Combined applications were significantly favourable than using each material alone in this connection. The lowest values were recorded on the vines treated with ethrel at 250 ppm, proton at 500 ppm and amino acids at 1%.

**6-3 Total phenols**

Total phenols was significantly declined with using ethrel at 250 ppm, protone at 500ppm and amino acids at 1% either application alone or in combination. Using ethrel was significantly favourable than applied of protone or amino acids in reducing total phenols. The lowest values were recorded on the vine that received ethrel at 250 ppm, potone at 500 ppm and amino acids at 1%. The untreated vines produced the maximum values.

**6-4 Total anthocyanins in the juice**

It is evident from the obtained data that subjecting the vines of the grapevine cv Flame seedless once with ethrel at 250 ppm, protone at 500ppm and amino acids at 1% either singly or in combinations significantly was very effective in enhancing the total anthocyanins relative to the control. The promotion was significantly associated with using amino acids, protone and ethrel, in ascending order. Combined applications were preferable than using each material alone in enhancing total anthocyanins,. The maximum values were detected on the vines that treated with ethrel at 250 ppm, protone at 500 ppm and amino acids at 1%. The untreated vines produced the lowest values.

**Table (3) Effect of single and combined applications of Ethrel, Protone and amino acids on maturation time, yield, average cluster weight and berries colouration of Flame seedless grapevines during 2017 and 2018**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Maturation time** | | **yield vine (Kg)** | | **Av. cluster weight (g)** | | **Berries colouration %** | |
| **2017** | **2018** | **2017** | **2018** | **2017** | **2018** | **2017** | **2018** |
| Control | 30/6 | 1/7 | 12.9 | 13.2 | 390.0 | 400.0 | 64.1 | 66.1 |
| Ethrel at 250 ppm | 15/6 | 15/6 | 12.8 | 13.1 | 388.0 | 398.0 | 71.9 | 65.0 |
| Protone 500 ppm | 22/6 | 22/6 | 12.8 | 13.1 | 389.0 | 398.0 | 69.0 | 71.0 |
| Amino acids at 1 % | 26/6 | 25/6 | 12.9 | 13.1 | 390.0 | 399.0 | 66.2 | 68.1 |
| Ethrel + proton | 8/6 | 7/6 | 12.8 | 13.0 | 387 | 398 | 86.0 | 89.0 |
| Ethrel + Amino acids | 10/6 | 10/6 | 12.8 | 13.0 | 388 | 398.0 | 81.0 | 84.5 |
| Protone + Amino acids | 12/6 | 11/6 | 12.9 | 13.0 | 388 | 398.0 | 77.0 | 80.0 |
| All together | 2/6 | 2/6 | 12.6 | 12.8 | 387 | 396 | 90.0 | 92.9 |
| New L.S.D at 5 % | ---- | ---- | NS | NS | NS | NS | 1.9 | 1.6 |

**Table (4) Effect of single and combined applications of Ethrel, Protone and amino acids on Av. berry weight, Av. berry longitudinal, Av. berry equatorial and berries shattering of Flame seedless grapevines during 2017 and 2018**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **Av. berry weight (g)** | | **Av. berry longitudinal (g)** | | **Av. berry equatorial (cm)** | | **berries shattering %** | |
| **2017** | **2018** | **2017** | **2018** | **2017** | **2018** | **2017** | **2018** |
| Control | 4.11 | 4.14 | 2.15 | 2.16 | 2.02 | 2.03 | 6.0 | 6.4 |
| Ethrel at 250 ppm | 14.10 | 14.11 | 2.14 | 2.15 | 2.00 | 2.02 | 6.0 | 6.5 |
| Protone 500 ppm | 14.10 | 14.12 | 2.14 | 2.15 | 2.00 | 2.02 | 6.2 | 6.5 |
| Amino acids at 1 % | 14.10 | 14.13 | 2.14 | 2.15 | 2.01 | 2.02 | 6.1 | 6.5 |
| Ethrel + proton | 14.06 | 14.10 | 2.13 | 2.14 | 2.00 | 2.01 | 6.3 | 6.8 |
| Ethrel + Amino acids | 14.07 | 14.10 | 2.13 | 2.14 | 2.00 | 2.01 | 6.3 | 6.8 |
| Protone + Amino acids | 14.08 | 14.10 | 2.13 | 2.14 | 2.0 | 2.01 | 6.2 | 6.6 |
| All together | 14.05 | 14.08 | 2.12 | 2.13 | 2.00 | 2.01 | 6.3 | 6.9 |
| New L.S.D at 5 % | NS | NS | NS | NS | NS | NS | 1.9 | NS |

**Table (5) Effect of single and combined applications of Ethrel, Protone and amino acids on T.S.S, reducing sugars, total acidity, T.S.S / acid, total Phenols and total anthocyanins of Flame seedless grapevines during 2017 and 2018**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Treatment** | **T.S.S %** | | **reducing sugars %** | | **total acidity %** | | **T.S.S / acid** | | **total Phenols (mg / g F.w)** | | **total anthocyanins (mg / g F.w)** | |
| **2017** | **2018** | **2017** | **2018** | **2017** | **2018** | **2017** | **2018** | **2017** | **2018** | **2017** | **2018** |
| Control | 17.1 | 16.9 | 15.2 | 15.0 | 0.680 | 0.682 | 25.1 | 24.7 | 9.1 | 8.7 | 5.1 | 4.7 |
| Ethrel at 250 ppm | 18.4 | 18.3 | 16.6 | 16.5 | 0.606 | 0.619 | 30.3 | 29.5 | 7.0 | 6.9 | 8.0 | 8.3 |
| Protone 500 ppm | 18.0 | 18.0 | 16.1 | 16.0 | 0.630 | 0.641 | 25.5 | 28 | 7.8 | 7.6 | 6.9 | 7.3 |
| Amino acids at 1 % | 17.5 | 17.4 | 15.6 | 15.5 | 0.659 | 0.660 | 26.5 | 26.5 | 8.4 | 8.2 | 6.0 | 6.3 |
| Ethrel + proton | 19.8 | 19.7 | 18.0 | 17.9 | 0.540 | 0.539 | 36.6 | 36.5 | 5.0 | 4.9 | 10.9 | 11.4 |
| Ethrel + Amino acids | 19.4 | 19.4 | 17.4 | 17.4 | 0.560 | 0.568 | 34.6 | 34.3 | 6.0 | 5.9 | 10.0 | 10.2 |
| Protone + Amino acids | 19.0 | 19.0 | 17.0 | 17.0 | 0.581 | 0.592 | 32.7 | 32 | 6.5 | 6.4 | 9.0 | 9.5 |
| All together | 20.4 | 20.6 | 18.9 | 19.0 | 0.520 | 0.507 | 39.2 | 40.6 | 3.7 | 3.5 | 12.1 | 13.0 |
| New L.S.D at 5 % | 0.3 | 0.2 | 0.3 | 0.3 | 0.018 | 0.015 | 1.0 | 0.9 | 0.5 | 0.4 | 0.6 | 0.7 |

**4. Discussion:**

**1- Effect of protone and Ethrel:**

The acceleration on maturation of Flame seedless, Red Globe and Crimson seedless grapes due to application of Ehrel could be attributed to the break down of Ethrel to ethylene which results in activation the hydrolytic and oxidative enzymes involved in maturation, increasing the degradation of chlorophylls and promoting the biosynthesis of plant pigments namely anthocyanins and carotenoids and hastening the compartmentation. In addition, ethrel is effective in increasing mitochondrial oxidation of malic acid (**Dal *et al.,* 2010**).

The effect of ABA in enhancing maturation of the berries might be attributed to its effect as main signal triggering the onset of the secondary metabolism in grape skine as well as enhancing the enzymes especially UPP- Glucose – Flavonic 3-O Glucose –T (**Zhang *et al.,* 2009**). The beneficial effects of ABA in reaching the plant tissues to senescence could give another explanation ( **Taiz and Zeiger, 2002**).

These results regarding the effect of ethrel are in concordance with **Liu *et al.,* (2002),** on CabernetSauvignon grapes; **Strydom (2014),** on Flame seedless grapes, **Ibrahiem (2018)** on Crimson seedless grapes. **The results of Ferrara *et al.,* (2015)** on Crimson seedless grapes**, Lichter *et al.,* (2015)** on Flame seedless grapes and **Castellarin *et al.,* (20156)** on Thompson seedless grapes supported the present results.

**2- Effect of amino acids:**

Amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins, which are formed by as process in which ribosomes catalyze the polymerization of amino acids (**Davies, 1982**). Several hypothesis have been proposed the explain for the role of amino acids in plant. Available evidence suggests several alternative routes of IAA and ethylene synthesis in plants, starting from amino acid (**Davies, 1982**). In this respect, (**Taiz and Zeiger, 2002**) suggested that the regulatory effect of certain amino acids like phenylalanine and ornithine in plant development appeared through their influence on the biosynthesis of gibberellins, tryptophane and methionine in building of IAA and ethtlene respectively.

The results regarding the promoting effect of amino acids on maturation date, berries colouration, total athocyanins and fruit quality are in agreement with those obtained by **Ahmed *et al.,* (2012); Abdelaal (2012), Abdelaal *et al.,* (2013)** on Thompson seedless grapes, **El- Khawaga (2014) and Mohamed (2014)** on Superior grapes and **Abdel- Aziz *et al.,* (2017) and Mohamed (2017)** on Flame seedless grapes.

**5. Conclusion**

For overcoming irregular colouration of clusters, advancing berries maturation and improving berries quality of the grapevine cv Flame seedless, under Minia region conditions, it is preferable for treating clusters with ethrel at 250 ppm, protone at 500 ppm and amino acids ( tryptophane + methionene + cysteine) at 1% when approximately 10% of the berries on 50% of the clusters had softened ( Veraison stage).

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