Effect of the Different Concentrations of Protective-stimulating Complex (HFC) on the Growth and Development of Grape Seedlings in the Tissue Culture at the Different Nutrient Levels

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ABSTRACT

Background: Humic substances play a vital role in the plant tissue culture as a growth hormone for in vitro propagation of many plant seedlings. The aim of this study was to investigate the effect of added humic-fulvate complex (HFC) at the various concentrations on the growth and development of grape seedlings in in vitro at the different nutrient levels.

Methods: The cutting of khasansky grape were cultivated on ¼ Murashige and Skoog medium or ½ Murashige and Skoog medium either alone or supplemented with the humic-fulvate complex at the different concentrations at (0.1, 1 and 10 ml/l). Then, they were cultured for 4 weeks under a controlled environment.

Result: The data observed that the low concentration of Murashige and Skoog medium (¼ MS) for in vitro rooting of grape cv. 'Khasansky' either alone or combined with HFC at the various concentrations significantly increased the rooting percentages and the total length of roots and stimulating the rate of vegetative growth compared with cultivated in ½ MS medium either alone or with supplemented with HFC. ¼ MS+ 10 ml/l HFC was the best treatment for improving the growth of khasansky grape seedlings.

Key words: Grape, Humic-fulvate complex (HFC), Root initiation, Tissue culture.

INTRODUCTION

Grape (Vitis vinifera, L.) is a temperate fruit crop and also cultivated under tropical and sub-tropical regions of the world (Ramesh Kumar et al., 2015). Grape cultivation is believed to have originated in Armenia near the Caspian Sea in Russia, from where it spread westward to Europe and eastward to Iran and Afghanistan (Krithika, 2015). It is one of the most important fruit crops in the world with 79,125,982 tons produced. About 50% of grapes are used for wine, one third is consumed as fresh fruit and the rest is dried. Russia grape production was about 627739 tons produced from area 68043 ha (FAO, 2018).

Tissue culture technique particularly micropropagation, is now gaining popularity due to many reasons such as obtaining a large number of genetically identical, physiologically uniform and developmentally normal plantlets preferably with a high photosynthetic potential to survive the harsh ex vitro conditions, in a reduced time period and at a lowered cost. The performance of micropropagated plantlets should be at par in comparison to plants raised by conventional method (Singh, 2002).

Grapes propagation has been commercialized by tissue culture or micropropagation approaches around the world. Applied to selected genotypes of Vitis using the culture of intact or fragmented apical meristems of the shoot, axillary-bud micro cuttings or through the formation of adventitious bud (Kurmi et al., 2011 and Khan et al., 2015).

(HFC) it's a new protective-stimulating humic-fulvate complex, which derived from humified flax shive on its harvest humic-fulvate complex (HFC), consisting of compounds with a large number of different functional groups: carboxyl, phenolic, amino groups, amide, alcohol, aldehyde, ketone, methoxy, quinone, hydroquinone. (Перминова, 2000).

HFC is characterized by an “auxin-like” effect on plants, that is, they enhance the growth of stems by affecting on the phase of cell stretching, stimulating the growth of cambial structures and correlative growth of all plant organs.

Humic acids (HAs) and fulvic acids (FAs) have direct effects on plant cell membranes. May be humic acids have different biochemical effects either on the cell wall, membrane level or in the cytoplasm, including improved
rates of photosynthesis and respiration in the plants, improve the synthesis of protein and plant hormone-like activity (Chen and Aviad, 1990). The maximum impact of fulvic acid on the chemical reactions is due to the presence of more electronegative oxygen atoms than any other humate molecule, which enhances the membrane. HS provides the free radicals for the cell of plant. Free radicals have positive effects on the germination of the seed, initiation of the root and growth of the plant in general (Robert, 2014).

Humic substances have a positive effect on the growth of plant roots. Treated soil with humic acid and fulvic acids promoted the initiation of root and increased the growth of root (Pettit, 2004). The enhanced macronutrients absorption, like N, P, S are directly related to the stimulatory effects of humic substances (Chen and Aviad, 1990).

A small quantity of biostimulant enhances plant growth and development such that the response cannot be attributed to the application of traditional plant nutrients. It can influence numerous metabolic processes such as respiration, photosynthesis, nucleic acid synthesis and ion uptake. Sometimes they can improve nutrient availability, water-holding capacity, increase antioxidants, improve metabolism and raise chlorophyll production (Gallant, 2004).

The impact of humic substances (HS) in the tissue culture was explained by Dhanapal and Satish Sekar (2013) they showed that humic acid plays a vital role in the plant tissue culture as a growth hormone for in vitro propagation of many plant seedlings. At the concentration of 25 and 50 ppm, HA could improve the growth of eggplant seedlings in tissue cultures at low nutrient level (1/4 MS) (Obsuwan et al., 2011). Based on their differences in origin, nature and concentration, HAs can able to stimulate or inhibit plant growth. Enhanced significant growth responses can be showed in the application of humic substances to the nutrient solution, to soil or sand (Lulakis and Petsas, 1995).

The aim of this experiment is to study the effect of added humic-fульvate complex (HFC) at the different concentrations (0.1, 1 and 10 ml/l) on in vitro rooting of grape cv. ‘Khasansky’ at the different nutrient levels ½ MS and ½ MS mediums for improving root initiation and increased root growth and vegetative growth.

**MATERIALS AND METHODS**

The experiment was carried out in the Laboratory of Micro Clonal Reproduction of Russian State Agrarian University - Moscow Timiryazev Agricultural Academy at the early ripening variety of grape (Khasansky).

The new protective-stimulating humic-fulvate complex (HFC) was used and its chemical properties were observed in Table (1).

Generally, ½ MS (Murashige and Skoog 1962) medium used for in vitro rooting of grape. In this work, the shoots of khasansky grape from multiplication stage were cut into small pieces (1-1.5 cm). These micro cuttings were planted on ½ MS or ½ MS medium either alone or supplemented with the humic-fульvate complex HFC at the different concentrations at (0.1, 1 and 10 ml/l) as the following:

| Treatment                        | pH          |
|----------------------------------|-------------|
| 1- ½ MS (control)                | 6.9-7       |
| 2- ½ MS+HFC 0.1 ml/l             | 7.0±0.22    |
| 3- ½ MS+HFC 1 ml/l              | 7.0±0.22    |
| 4- ½ MS+HFC 10 ml/l             | 7.0±0.22    |
| 5- ¼ MS                         | 7.0±0.22    |
| 6- ¼ MS+HFC 0.1 ml/l            | 7.0±0.22    |
| 7- ¼ MS+HFC 1 ml/l              | 7.0±0.22    |
| 8- ¼ MS+HFC 10 ml/l             | 7.0±0.22    |

Each treatment was replicated six times and each replicate consisted of four pieces of shoots.

Then the cultures were incubated in a growth room (20±2°C), illuminated with 1000-2000 lux of light, maintained under a photoperiod of 16 h and data were recorded after 3-4 weeks.

After 3 and 4 weeks from cultivated, rooting percentage (%), number of roots per explant, root length (cm), percent of plants with new growth (%) and length of new growth (cm) were recorded.

### Statistical analyses

The experimental design consisted of a randomized complete block with four treatments and six replicates. Data were analyzed by SPSS 18 software and comparing averages was done by Duncan’s test and a probability value of 5%.

### RESULTS AND DISCUSSION

Data in the Table (2 and 3) observed that generally using the low concentration of Murashige and Skoog medium (1/4 MS) for in vitro rooting of grape cv. ‘Khasansky’ either alone or combined with HFC at the different concentrations (0.1, 1 and 10 ml/l) significantly increased the root initiation and development compared with cultivated in 1/2 MS medium either alone or with supplemented with HFC.

Good root initiation is very important for in vitro regenerated plants. Concerning the rooting percentage data showed that 1/4 MS+ HFC 10 ml/l gave the maximum percentage of roots followed by treatment with 1/2 MS+HFC 0.1 ml/l. These percentages reached about 75% and 37.50%, respectively after 3 weeks from cultivated compared.
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with 8.33% for control. While after 4 weeks from cultivated these percentages reached 83.33% and 62.50%, respectively compared with control (1/2 MS) 9.72%.

For the number of roots per explant, data noticed that the highest increase in the number of roots were observed when added higher concentrations of HFC (1 and 10 ml/l) for ¼ MS medium and these increases after three weeks from cultivated reached about (50% and 150%) over the control for two treatments, respectively while after four weeks from cultivated these increases reached about (40% and 113%) over the control, respectively.

Concerning the average length of roots data indicated that using the low nutrient level ¼ MS either alone or supplement with HFC at the different concentration and treatment with ½ MS + the lowest concentration of HFC gave a significant increase in the average length of roots compared with using the high nutrient level ½ MS medium either alone or with added HFC at the high concentrations.

For the total length of roots, the highest total length of roots was observed when used ¼ MS with added protective-stimulating complex (HFC) at the highest concentrations (10 ml/l).

Data in the Table (4) indicated that adding HFC at the different concentrations in the medium either at ½ MS or ¼ MS increased the percentage of plants with new growth and their length. Treatment ¼ MS with HFC at 10 ml/l gave the

Table 2: Effect of adding humic-fulvate complex (HFC) on *In vitro* rooting of grape cv. ‘Khasansky’ at the different nutrient levels on rooting initiation and development after three weeks from cultivated.

| Treatment             | Rooting (%) | Number of roots per explant | Length of root (cm) | Total length of roots (cm) |
|-----------------------|-------------|----------------------------|---------------------|---------------------------|
| ½ MS (control)        | 8.33 C      | 2.00 C                     | 0.22 C              | 0.44 D                    |
| ½ MS+HFC 0.1 ml/l     | 37.50 B     | 1.50 C                     | 1.22 B              | 1.82 C                    |
| ½ MS+HFC 1 ml/l       | 0.00 C      | 0.00 D                     | 0.00 C              | 0.00 D                    |
| ½ MS+HFC 10 ml/l      | 0.00 C      | 0.00 D                     | 0.00 C              | 0.00 D                    |
| ¼ MS                  | 33.33 B     | 1.33 C                     | 1.22 B              | 1.62 C                    |
| ¼ MS+HFC 0.1 ml/l     | 33.33 B     | 1.67 C                     | 1.38 B              | 2.30 C                    |
| ¼ MS+HFC 1 ml/l       | 33.33 B     | 3.00 B                     | 1.58 AB             | 4.74 B                    |
| ¼ MS+HFC 10 ml/l      | 75.00 A     | 5.00 A                     | 1.90 A              | 9.50 A                    |

Means within a column followed by different letter (s) are statistically different at 5% level.

Table 3: Effect of adding humic-fulvate complex (HFC) on *In vitro* rooting of grape cv. ‘Khasansky’ at the different nutrient levels on rooting initiation and development after four weeks from cultivated.

| Treatment             | Rooting (%) | Number of roots per explant | Length of root (cm) | Total length of roots (cm) |
|-----------------------|-------------|----------------------------|---------------------|---------------------------|
| ½ MS (control)        | 9.72 C      | 2.50 CD                    | 0.82 D              | 2.05 DE                   |
| ½ MS+HFC 0.1 ml/l     | 62.5 B      | 2.20 D                     | 1.83 BC             | 4.03 CD                   |
| ½ MS+HFC 1 ml/l       | 8.33 C      | 2.00 D                     | 1.63 C              | 3.26 CD                   |
| ½ MS+HFC 10 ml/l      | 8.33 C      | 1.00 E                     | 1.50 C              | 1.50 E                    |
| ¼ MS                  | 50.00 B     | 2.33 CD                    | 2.02 BC             | 4.71 C                    |
| ¼ MS+HFC 0.1 ml/l     | 50.00 B     | 3.00 BC                    | 2.30 B              | 6.90 B                    |
| ¼ MS+HFC 1 ml/l       | 50.00 B     | 3.50 B                     | 2.37 B              | 8.30 B                    |
| ¼ MS+HFC 10 ml/l      | 83.33 A     | 5.33 A                     | 2.95 A              | 15.72 A                   |

Means within a column followed by different letter (s) are statistically different at 5% level.

Table 4: Effect of adding humic-fulvate complex (HFC) on *In vitro* rooting of grape cv. ‘Khasansky’ at the different nutrient levels on per cent of plants with new growth (%) and length of the new growth (cm) after three and four weeks from cultivated.

| Treatment             | After 3 weeks from cultivate | After 4 weeks from cultivate |
|-----------------------|------------------------------|------------------------------|
|                       | Plants with new growth (%)   | Plants with new growth (%)   |
|                       | Length of new growth (cm)    | Length of new growth (cm)    |
| ½ MS (control)        | 25.00 B                      | 41.67 B                      |
| ½ MS+HFC 0.1 ml/l     | 33.33 B                      | 33.33 B                      |
| ½ MS+HFC 1 ml/l       | 33.33 B                      | 33.33 B                      |
| ½ MS+HFC 10 ml/l      | 33.33 B                      | 33.33 B                      |
| ¼ MS                  | 33.33 B                      | 33.33 B                      |
| ¼ MS+HFC 0.1 ml/l     | 41.67 B                      | 41.67 B                      |
| ¼ MS+HFC 1 ml/l       | 41.67 B                      | 41.67 B                      |
| ¼ MS+HFC 10 ml/l      | 83.33 A                      | 83.33 A                      |

Means within a column followed by different letter (s) are statistically different at 5% level.
maximum percentage of plants with new growth with the highest length of this new growth.

These results are in agreement with Obsuwan et al., (2011) as they found that the root length of eggplant seedlings was significantly increased when grown on ¼ MS supplemented with HAs at the concentrations of (25, 50, 75 and 100 ppm), while the highest plant was observed when treated ¼ MS with HAs at the concentrations 25, 50 ppm. And also, Aml Yousef et al., (2011) noticed that the HA increase the root length of olive seedlings. Saruhan et al., (2011) and Kumar Sootahar et al., (2019) also suggested that application of fulvic acids (FA) enhances plant growth parameters as well as uptake of mineral elements in a maize crop.

CONCLUSION

In vitro rooting of grape cv. ‘Khasansky’ the use of one-fourth of MS supplemented with HFC at 10 ml/l was the best medium for increasing the percentage of rooting with the lowest average of root length and the highest stimulation rate of vegetative growth. The results showed that appropriate amounts of HFC could be used as a growth enhancement for grape cv. ‘Khasansky’ especially when the nutrients were limited.

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