



EVALUATION OF THE EFFECT OF CHELATE CU COMPLEX AT DIFFERENT CONCENTRATIONS ON *IN VITRO* ROOT OF TWO VARIETIES OF GRAPE

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Abstract

This study was carried out to evaluate the impact of supplement $\frac{1}{2}$ MS medium (without $\text{CuSO}_4 \cdot \text{H}_2\text{O}$) with chelated Cu at the different concentrations (0.01, 0.015, 0.02 and 0.025 mg/l) on *In vitro* rooting of grape cv. 'Khasansky' and 'White Moskvsky' on the rooting and vegetative growth. The data showed that both cultivars of grape differed in response to the addition of copper on *In vitro* root medium. For Khasansk grape treated the medium with Cu at the different concentrations caused a significantly increased in the percentage of rooting. $\frac{1}{2}$ MS medium (without CuSO_4) + Cu (0.02 mg/l) was the best treatment to get the maximum percentage of rooting with increasing the number of roots per explant and their length in addition to improving the vegetative growth of seedless. As for White Moskvsky grape, treated $\frac{1}{2}$ MS medium (without CuSO_4) with the lowest concentration of Cu (0.01 mg/l) gave the highest percentage of rooting and length of roots.

Key words: grape, *In vitro* root, copper (Cu), root growth, vegetative growth.

Introduction

Grape (*Vitis vinifera*, L.) 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). For Khasansky grape.

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; Khan *et al.*, 2015).

Copper is a micronutrient essential for the normal growth and development of plants. In-plant organisms, it performs very important physiological and biochemical functions. It takes part in the processes of photosynthesis,

respiration, conversion of nitrogen compounds, transport of carbohydrates and also regulates the process of DNA formation (PodlesnaA, *et al.*, 1996). Also, it is a constituent of the protein component of several enzymes in plants, mainly those participating in electron flow, catalyzing redox reactions in mitochondria, chloroplasts, cell wall and cytoplasm of plant cells (Lolkema, 1985).

An excess of copper and copper deficiency both lead to various disorders in plants, such as growth inhibition, decrease in biomass and ultimately their death (Zenk, 1996). At high concentrations, copper is highly toxic to all living organisms. Copper deficiency in higher plants manifests itself in the activation of morphological changes in the leaves and roots. Symptoms of deficiency appear in the early stages of plant development and are characterized by leaf deformity, chlorosis and even necrosis (Marschner, 1995).

There have been reports for the effects of copper

concentrations on In vitro: (Purnhausre 1991) on wheat; Cook, *et al.*, (1997) on Phaseolus plants; Jiang, *et al.*, (2001) on Zea mays; SanskritiGautam, *et al.*, (2016) in the Safflower and Virginia.Sarropoulou and Eleni Maloupa (2017) in *Sideritisraeseri* Boiss Jiang, *et al.*, (2001) studied the effect of the different concentrations of copper sulfate (10^{-5} - 10^{-2} M) on the root growth of *Zea mays*. The data investigated that 10^{-5} M Cu stimulated root growth, but higher concentrations (10^{-4} - 10^{-2} M) inhibited it.

Sanskriti Gautam, *et al.*, (2016) studied the changes in the Safflower when exposed to different concentration of copper (25, 50 and 100 μ M) along with control (0.5 μ M) for 10 and 20 day. The data observed that 25 μ M copper treated seedling's roots were found to be increased by 25.3% (10th day) and 13.96% (20th day) while in 50 μ M and 100 μ M treated seedling's roots were found to be decreased by 61.9%, 87.3% (10th day) and 53.98%, 81.99% (20th day) respectively as compared to the control (100%). The results showed that the shoot length was found to be reduced at all the three treatments by 6.68%, 13.34% and 70% as compared to the control in 10th days harvested seedlings. In 20th day harvested seedlings, the shoot length was found to be slightly increased by 4.54% in 25 μ M copper treated seedlings, while it was reduced by 9.09% and 54.54% in the other two treatments as compared to control.

The aim of this work was to study the effect of added chelate Cu on In vitro rooting of grape cv. 'Khasansky' and White Moskvsky at the different concentrations (0.01, 0.015, 0.02 and 0.025 mg/l) on improving rooting 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 two of early ripening varieties of grape (Khasansky and White Moskvsky).

The new protective- stimulating complex "chelate Cu" was used. This complex is the production of the National Research Center "Kurchatov Institute". It's aqueous solution of copper (II) complex of hydroxyl ethylenediphosphonic acid, consists of Cu at (2.8%).

In vitro root of grape $\frac{1}{2}$ MS medium (Murashige & Skoog 1962) was used (as a control) and $\frac{1}{2}$ MS medium without ($\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$) was used when added the chelate Cu complex. The different concentrations of Cu (0.01, 0.015, 0.02 and 0.025 mg/l) were used.

Prepare the concentrations: in the first 0.85 ml of

"chelate Cu" complex was added in 1 liter of water and then taken from this solution 0.4, 0.6, 0.8 and 1 ml and added to 1 liter of $\frac{1}{2}$ MS medium without ($\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$).

In this work, the shoots of khasansky and White Moskvsky grape from multiplication stage were cut into small pieces (1-1.5 cm). These micro cuttings were planted into the jars which filled with 30 ml of the medium and 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 1 observed that for the khasansky grape added Cu at the different concentrations (0.01, 0.015, 0.02 and 0.025 mg/l) in $\frac{1}{2}$ MS medium (without CuSO_4) for in vitro rooting caused a significantly increased in the percentage of rooting either after 3 weeks or 4 weeks from cultivated and this increase ranged from (8.33 %) for the control to (41.67%) for supplement the rooting medium with Cu either at (0.01 or 0.02 mg/l) that after 3 weeks from cultivated, while after 4 weeks from cultivated this increase ranged from (9.72%) for the control to (75%) when added 0.02 mg/l Cu for In vitro rooting.

For the number of roots, the data indicated that added chelating Cu for $\frac{1}{2}$ MS medium (without CuSO_4) at the lowest concentration (0.01 mg/l) caused a significantly decreased in the number of roots compared to the control and another concentrations of copper, after that the number of roots was increased gradually with increasing the concentrations of Cu and the maximum number of roots was observed when supplementing $\frac{1}{2}$ MS medium (without CuSO_4) with 0.025 mg/l Cu (3 and 3.5) after 3 and 4 weeks, respectively.

Concerning the length of root, the data observed that after 3 weeks from cultivated the growth of these roots

were very slowly when cultivated the cutting of khasnsky grape either in ½ MS medium (control) or when added Cu at (0.01, 0.015 mg/l) in ½ MS medium (without CuSO₄), while this growth was rapid when added Cu at 0.02 mg/l and this increase reached about (159% over the control), while when added 0.025 mg/l Cu there was a very rapid increase in the length of root and this increase ranged from (0.22) cm for the control to (1.40 cm). After 4 weeks from cultivated, there were a significantly

increase when supplementing ½ MS medium (without CuSO₄) with Cu at the different concentrations compared to the control and the maximum increase in the length of the root was observed when treated the medium with Cu at 0.025 mg/l.

So that, for the total length of roots, after the three weeks from cultivated the total length of roots ranged from (0.20cm to 4.20 cm) and added Cu at (0.02 and 0.025 mg/l) in ½ MS medium (without CuSO₄)

Table 1: Effect the different concentrations of chelate Cu complex on *In vitro* root of grape cv. ‘Khasansky’ on the rooting and vegetative growth after 3 and 4 weeks from cultivated.

Treat-ment	Root-ing(%)	Number of roots per explant	Len-gth of root (Am)	Total length of root (Am)	Plants with new growth (%)	Length of new growth (cm)
After 3 weeks from cultivated						
Control	8.33 D	2.00 B	0.22 C	0.44 C	25.00 C	1.43 A
Cu 0.01 mg/l	41.67 A	1.17 C	0.17 C	0.20 C	25.00 C	1.10 A
Cu 0.015 mg/l	25.00 C	2.00 B	0.23 C	0.46 C	50.00 A	1.22 A
Cu 0.02 mg/l	41.67 A	2.60 AB	0.57 B	1.48 B	41.67 B	1.16 A
Cu 0.025 mg/l	33.33 B	3.00 A	1.40 A	4.20 A	41.67 B	1.10 A
After 4 weeks from cultivated						
Control	9.72 D	2.50 AB	0.82 B	2.05 C	41.67 AB	1.62 A
Cu 0.01 mg/l	66.67 B	1.50 B	1.63 A	2.45 C	33.33 B	1.18 A
Cu 0.015 mg/l	41.67 C	2.33 AB	1.73 A	4.03 B	62.5 A	1.55 A
Cu 0.02 mg/l	75.00 A	3.17 A	1.38 AB	4.38 B	58.33 A	1.42 A
Cu 0.025 mg/l	63.33 B	3.50 A	1.77 A	6.20 A	50.00 AB	1.35 A

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

Table 2: Effect the different concentrations of chelate Cu complex on *In vitro* root of grape cv. ‘White Moskvsky’ on the rooting and vegetative growth after 3 and 4 weeks from cultivated.

Treat-ment	Root-ing(%)	Number of roots per explant	Len-gth of root (Am)	Total length of root (Am)	Plants with new growth (%)	Length of new growth (cm)
After 3 weeks from cultivated						
Control	37.50 B	1.33 B	0.43 C	0.53 C	66.67 C	1.77 AB
Cu 0.01 mg/l	50.00 A	1.00 B	0.52 C	0.52 C	66.67 C	1.60 AB
Cu 0.015 mg/l	33.33 B	1.00 B	1.17 A	1.17 B	83.33 B	2.00 A
Cu 0.02 mg/l	33.33 B	1.50 AB	0.50 C	0.70 C	50.00 D	1.50 B
Cu 0.025 mg/l	33.33 B	1.75 A	0.88 B	1.54 A	91.67 A	1.47 B
After 4 weeks from cultivated						
Control	66.67 B	2.00 A	0.63 B	1.12 B	91.67 AB	2.67 B
Cu 0.01 mg/l	75.00 A	1.33 A	1.67 A	2.25 A	75.00 BC	2.33 C
Cu 0.015 mg/l	33.33 D	1.67 A	1.51 A	2.48 A	83.33 ABC	3.10 A
Cu 0.02 mg/l	41.67 C	1.50 A	1.42 A	2.07 A	66.67 C	1.88 D
Cu 0.025 mg/l	66.67 B	1.87 A	1.30 A	2.43 A	100.00 A	1.75 D

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

significantly increased the total length of roots when compared to the other concentrations of copper and control, while after 4 weeks from cultivated the significantly improved in the total length of roots were noticed when supplementing Cu at the different concentrations in ½ MS medium (without CuSO₄). Supplement ½ MS medium (without CuSO₄) with Cu at (0.025 mg/l) gave the highest increased in the total length of total and this increase reached about (202% over the control) after four weeks from cultivated.

Concerning the effect of using copper for improving the vegetative growth, the data showed that after 3 weeks from cultivated, added Cu at the concentrations (0.015, 0.02 and 0.025 mg/l) in ½ MS medium (without CuSO₄) caused the increase in the percentage of plants with new growth compared to the control and treated with Cu at (0.01mg/l), without a significant difference in the length of these growths. However, after 4 weeks from cultivated treated with 0.015 mg/l Cu gave the maximum percentage of plants with new growth (62.5%), while 0.01 mg/l Cu gave the minimum percentage (33.33%) and there were no significant differences between control and other treatments.

The data which noticed from khasnsky cultivars, the lowest concentration of Cu (0.01 mg/l) led to the greatest tendency of the plant to rooting growth, while when added Cu at (0.015 mg/l) led to the greatest tendency of the plant to vegetative growth, whereas added Cu higher than 0.015 mg/l caused an increase in the rate of both vegetative and root growth.

The data for khasnsky grape are in line with Purnhausre (1991) showed that Cu at concentrations 5 to 1000 times higher than in the original Murashinge and Skoog (1962) medium (0.1M CuSO₄) strikingly enhanced

shoot and root regeneration in wheat callus cultures. And also, Purnhauser and Gyulai (1993) reported that CuSO_4 (0.1-100 μM) significantly enhanced shoot regeneration from calli of wheat and triticale cultures and CuSO_4 also stimulated root formation and length of roots. And C.M. Cook, *et al.*, (1997) the root length of Bean plants was increased in the growth medium containing 0.5- 5.5 $\frac{1}{4}\text{M}$. The stem length of plants growing in up to 1.5 $\frac{1}{4}\text{M}$ Cu increased and remained optimal for those growing in up to 10.5 $\frac{1}{4}\text{M}$ Cu. In addition to, Sarropoulou and Maloupa (2017) in the *S. raeseri*, shoot length was not influenced substantially due to CuSO_4 (0.5-100 μM) application.

For the white Moskovsky grape, the data in table 2 indicated the effect of supplement $\frac{1}{2}$ MS medium (without CuSO_4) for In vitro root stage with Cu at (0.01, 0.015, 0.02 and 0.025 mg/l) for improving the root and vegetative growth of the cutting of white Moskovsky grape.

Concerning the percentage of rooting, the data showed that added Cu at the lowest concentration (0.01 mg/l) $\frac{1}{2}$ MS medium (without CuSO_4) gave the highest significantly percentage of rooting (50 and 75%) after 3 and 4 weeks from cultivated compared to control and other treatments.

Concerning the number of roots, after 3 weeks from cultivated the highest significant increase in the number of roots was observed when used the highest concentration of Cu 0.025 mg/l (1.75), followed by added Cu at 0.02 mg/l (1.50). After 4 weeks from cultivated, there were no significant differences between control and other treatments.

The length of the root was improved when using $\frac{1}{2}$ Ms medium (without CuSO_4) + Cu at (0.01, 0.015, 0.02 and 0.025 mg/l) compared to cultivated the cutting in $\frac{1}{2}$ MS medium.

For the total length of root, the data observed that generally the total length of root was improved when adding the copper in the medium. After 4 weeks from cultivated the total length of roots increased significantly when treated $\frac{1}{2}$ Ms medium (without CuSO_4) with Cu and this increase ranged from 1.12 (for the control) to 2.48 for (0.015 mg/l Cu) and there was no significant difference on the total length of roots between the different concentrations of Cu.

Concerning to the vegetative growth, data indicated that the maximum percentage of the plants with the new growth observed when using Cu at the highest concentration (0.025 mg/l) and this percentage was (91.67) after 3 weeks from cultivated, while after 4 weeks from cultivated this percentage increased and reached to 100%, however, the minimum percentage was observed

when treated with Cu at (0.02 mg/l).

The highest length of this growth was observed when treated $\frac{1}{2}$ MS medium (without CuSO_4) with 0.015 mg/l Cu, however the added Cu at the highest concentrations (0.02 and 0.025 mg/l) caused a significantly decreased in the length of the growth.

These results are in line with Jiang, *et al.*, (2001), studied the effect of the different concentrations of copper sulfate (10^{-5} - 10^{-2} M) on the root growth of *Zea mays*. The data investigated that 10^{-5} M Cu stimulated root growth, but a higher concentrations (10^{-4} - 10^{-2} M) inhibited it. And also, SanskritiGautam, *et al.*, (2016) studied the changes in the Safflower when exposed to different concentration of copper (25, 50 and 100 μM) along with control (0.5 μM) for 10 and 20 day. The data observed that 25 μM copper treated seedling's roots were found to be increased by 25.3% (10th day) and 13.96% (20th day) while in 50 μM and 100 μM treated seedling's roots were found to be decreased by 61.9%, 87.3% (10th day) and 53.98%, 81.99% (20th day) respectively as compared to the control (100%). However, in *Ailanthus altissima* Swingle (Gatti, 2008) and poplar (*P. tremula* L. \times *P. alba* L.) (Bojarczuk, 2004) vitro cultures, supplementing the culture medium with 50-200 μM and 250- 1000 μM CuSO_4 , respectively, led to a decrease in shoot length. Sanjeev *et al.*, (2003) where CuSO_4 (25-125 μM) exhibited better results in terms of shoot growth in relation to the controls on MS copper level (0.1 μM) in the medicinal plant *Tinosporacordifolia*. In accordance with our results, in *Withaniasomnifera* L., 25-200 μM CuSO_4 stimulated shoot bud formation and subsequent elongation of the in vitro nodal explants (Fatima, *et al.*, 2011).

Conclusion

For Khasansk grape, $\frac{1}{2}$ MS medium (without CuSO_4) + Cu (0.02 mg/l) was the best treatment to get the maximum percentage of rooting with increasing the number of roots per explant and their length in with improving the vegetative growth of seedless. While White Moskovsky grape, treated $\frac{1}{2}$ MS medium (without CuSO_4) + Cu (0.01 mg/l) gave the highest percentage of rooting and length of roots.

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