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Research Article
Effects of Copper Sulphate on Shoot Multiplication and Rooting of Banana (*Musa acuminata* L.) (*In vitro* Study)

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Abstract

Background and Objectives: The efficiency of *in vitro* culture depends on the composition of the medium used. Micronutrients are essential for all stages of plant development performing a key function in the activation of several enzymes. This study focused on studying the influence of increased copper content in the Murashige and Skoog medium on shoot development, chlorophyll content and rooting response of *in vitro* cultured banana plants (*Musa acuminata* L.) cultivar (Grand Nain). **Materials and Methods:** Banana shoot explants were cultured on shoot multiplication media supplemented with various concentrations (0-8 mg L^{-1}) of copper sulphate ($CuSO_4$) and the shoot growth responses, number of shoots per explant, shoot length, leaf surface area and leaf chlorophyll content were noted 4 weeks after culture. The effect of applied $CuSO_4$ on root initiation and elongation was also examined. **Results:** A significant difference was observed between control (MS medium copper level) and higher concentrations of copper sulphate incorporated into MS medium for mean number of shoots produced per explants, mean shoot length, mean leaf surface area and chlorophyll content of developing banana shoots. Maximum number of shoots per explants, highest shoot length and chlorophyll content were observed on MS medium containing 6 mg L^{-1} copper sulphate. Rooting of micropropagated shoots was also stimulated by copper sulphate supplement. The highest number of roots per shoot and the longest roots were noticed in rooting medium supplemented with 4 mg L^{-1} copper sulphate. **Conclusion:** These findings suggest that copper may have an important role in improving the micropropagation potential of banana.

Key words: Copper sulphate, banana, *Musa acuminata* L., micropropagation, chlorophyll content

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In vitro culture technology is of great advantage for mass propagation of various plants including banana. While a number of protocols for *in vitro* propagation are available for banana[1]. The development of efficient protocols for banana tissue culture remains the foundation for producing high-quality and pathogen-free banana planting materials. Plant tissue culture medium is a major component of plant growth response which is composed of several components such as; minerals, organic compounds, plant growth regulators and a carbon source[2]. Mineral nutrients play essential roles in plant growth and development[3], but they have rarely been studied in banana culture. Generally, the Murashige and Skoog (MS)[3] formulation is used for *in vitro* culture of most plants. However, different plants have different demands and responses to nutrients present in culture media. In fact many studies have shown that the manipulation of copper in growth media can produce significant effects on the quality of plant cultures of some plant species. For instance, Joshi and Kothari[4] showed that high copper level in the medium improves shoot bud differentiation and elongation from the cultured cotyledons of *Capcicum annumn* reported by another study[5] that the number of *Stevia rebaudiana* shoot buds increased in the presence of high concentrations of copper. In addition Kowalska *et al*.[6] demonstrated that elevated concentrations of copper in culture media of *Daucus carota* improved the formation of plantlets. Copper plays a central role in photosynthesis, antioxidant reactions, metabolic respiration and hormone biosynthesis and perception. Copper sulfate is commonly used in plant tissue culture media, including the most frequently used MS medium. However, the concentration of $CuSO_4$ in culture media may need optimization for better growth performance. There have been no previous reports on the optimization of copper levels in banana tissue cultures. Therefore, in this study the effects of copper ($CuSO_4$) on the shooting and rooting response of banana were investigated in order to improve the production of banana plantlets through micropropagation.

MATERIALS AND METHODS

Study area: All experiments were conducted between March, 2019 and February, 2020, at the Department of Biological Sciences, The University of Jordan, Jordan.

Specimen collection: Shoot tip explants of banana (*Musa acuminata* L.) cultivar (Grand Nain) were excised from young suckers grown in pots. Explants were surface sterilized with 75% ethanol for 50 sec followed by 30 min with 40% commercial bleach (Clorox 5.75% NaOCl) to which few drops of Tween-20 were added. After complete washing with sterile distilled water, explants were trimmed to final size of 10-15 mm in the laminar flow cabinet.

Culturing (*in vitro*): For culture initiation, explants were cultured in screw-capped glass vessels containing 30 mL of initiation media composed of MS basal salts (Murashige and Skoog) supplemented with sucrose (40 g L^{-1}), thiamine (0.1 g L^{-1}), benzylaminopurine (BAP) (12 μM), indole-3-acetic acid (IAA) (3 μM) and cysteine HCl (40 mg L^{-1}). Medium was solidified with 2 g L^{-1} gelrite (Sigma Chemical Co., St. Louis) and its pH was adjusted to 5.8 before autoclaving at 121°C for 15 min. All cultures were incubated at 25°C under 16 h photoperiod for 4 weeks. Light intensity was 35 μmol sec^{-1} m^{-2}. To evaluate the influence of $CuSO_4$ on shoot multiplication and growth, banana shoot tip explants from *in vitro* initiated cultures were transferred to multiplication media.

Multiplication medium: Multiplication medium contained MS basal salts, sucrose (40 g L^{-1}), thiamine (0.1 g L^{-1}), BAP (20 μM) and cysteine HCl (40 mg L^{-1}) supplemented with different concentrations (0-8 mg L^{-1}) of $CuSO_4$. Cultures were arranged in a randomized block design with 10 replicates per treatments (3 explants per culture bottle) and incubated at 25°C under 16 h photoperiod for 4 weeks. Light intensity was 35 μmol sec^{-1} m^{-2}. After 4 weeks of culture, the number of shoots formed per explants, shoots length (cm) and leaf surface area (cm^2) were determined.

For evaluating the effect of $CuSO_4$ on *in vitro* rooting, uniform banana shoots formed on multiplication media were excised and transferred to rooting medium. The rooting medium consisted of MS basal slats, sucrose (40 g L^{-1}), 2-isopentenyladenine (2iP) (5 μM) and indole-3-butyric acid (IBA) (0.1 μM) supplemented with different concentrations (0-8 mg L^{-1}) $CuSO_4$. Medium was solidified with 1.8 g L^{-1} gelrite and its pH was adjusted to 5.8. Cultures, consisting of 10 replicates per treatment were incubated at 25°C under 16 h photoperiod. After 3 weeks, the number of roots formed per shoot and root lengths (cm) were estimated.

Determination of leaf chlorophyll content: For the determination of leaf chlorophyll content, 0.5 g fresh leaf material of individual treatments was extracted in 5 mL 80% acetone (v/v) and total chlorophyll content was determined according to Lichtenthaler[7].

Statistical analysis: All data were expressed as means of all replicates ± standard error. Means were separated by Duncan's Multiple Range Test (DMRT)[8] at 5% significance level.

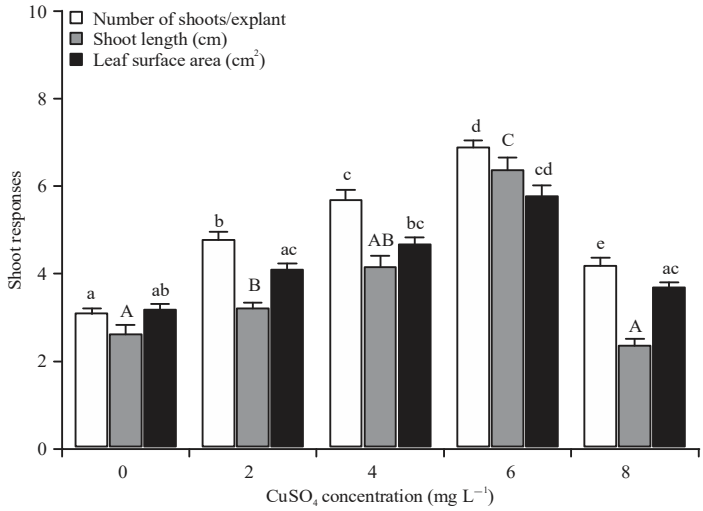

Fig. 1: Concentration effect of $CuSO_4$ on the number of shoots/explant, shoot length and leaf surface area of banana
Different letters indicate significant differences among treatments (p = 0.05)

RESULTS

Effect of $CuSO_4$ on *in vitro* shoot proliferation: The effect of different concentrations of $CuSO_4$ (2, 4, 6 and 8 mg L^{-1}) supplemented to multiplication medium was examined for *in vitro* shoot proliferation of banana shoot explants. Data presented in Fig. 1 showed that after 4 weeks culture, all concentrations of $CuSO_4$ employed significantly increased the number of shoots/explants compared to the control (without $CuSO_4$ supplementation). However, the highest number of shoots/explants was recorded in media containing $CuSO_4$ at 6 mg L^{-1}. This treatment resulted in approximately two fold increase in the rate of shoot multiplication compared to explants cultured on control media. Lower promotion of shoot multiplication response was noticed in explants cultured on media containing 4 mg L^{-1} $CuSO_4$ (approximately 80% increase over control) while the lowest response (50% increase over control) was observed in shoots cultured on media supplemented with $CuSO_4$ at the concentrations of 2 and 8 mg L^{-1}.

Strong positive effect of $CuSO_4$ was noted on shoot elongation of the *in vitro* cultured banana explants (Fig. 1 and 2). A linear increase in shoot length was observed with increasing concentrations of $CuSO_4$ from 2-6 mg L^{-1}. The best response (140% increase over control) was recorded in media supplemented with 6 mg L^{-1} $CuSO_4$. In contrast, shoot length did not change significantly in media containing 8 mg L^{-1} $CuSO_4$. Besides its positive influence on shoot length, $CuSO_4$ at the concentration of 6 mg L^{-1} was the optimal for leaf growth and resulted in an increase of 50-60% in the surface area of leaves compared to those of control cultures (Fig. 1).

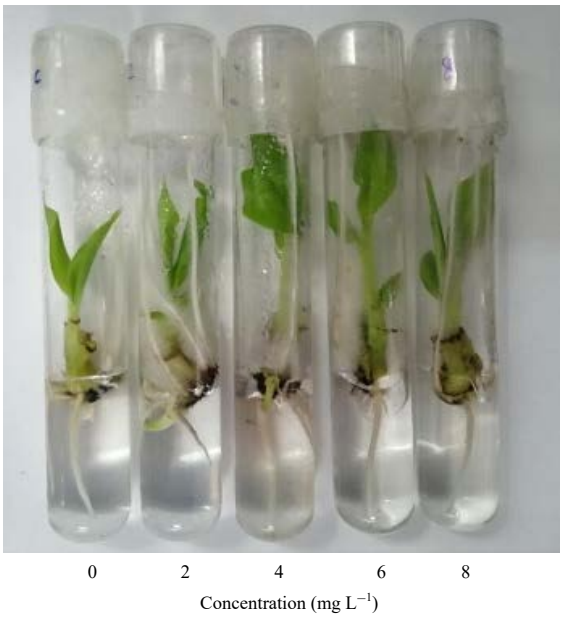

Fig. 2: Explants of banana (cultivar Grand Nain) growing in media containing 2-8 mg L^{-1} $CuSO_4$ after 3 weeks of culture compared to control (media without $CuSO_4$ supplement).

Effect of $CuSO_4$ on *in vitro* rooting: The rooting ability of *in vitro* raised banana plantlets were stimulated by the different concentrations of $CuSO_4$ supplemented into the rooting media. Data in Fig. 3 showed that among various treatments used, the highest increase in the number of roots formed per explants and root length was noticed in medium supplemented with 4 mg L^{-1} $CuSO_4$. Considering the overall

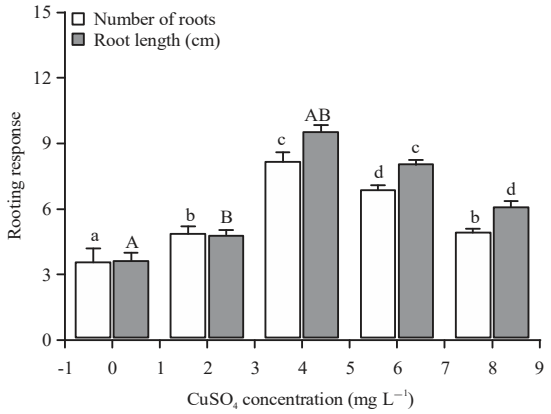

Fig. 3: Concentration effect of $CuSO_4$ on the number of roots/explant and root length of banana after 4 weeks in culture

Different letters indicate significant differences among treatments (p = 0.05)

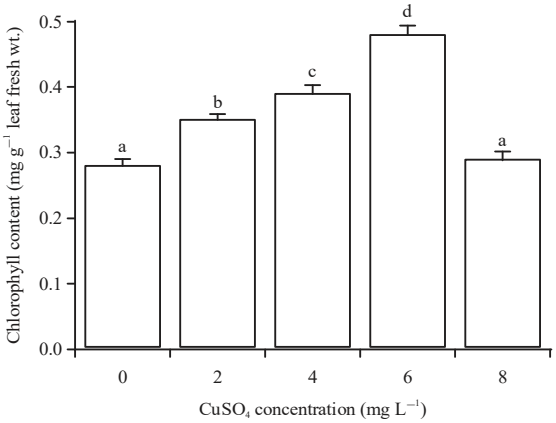

Fig. 4: Concentration effect of $CuSO_4$ on total chlorophyll content of banana leaves after 4 weeks in culture.

Different letters indicate significant differences among treatments (p = 0.05)

averages of these parameters, this treatment increased the number of roots/explants and root length by 140 and 170%, respectively, over those of control explants. The second highest result was observed with 6 mg L^{-1} $CuSO_4$ treatment and lower rooting response was recorded in shoots cultured on media supplemented with $CuSO_4$ at the concentrations of 2 and 8 mg L^{-1}.

Effect of $CuSO_4$ on leaf total chlorophyll content: The results of Fig. 4 showed that addition of $CuSO_4$ to culture media had significant positive effect on leaf chlorophyll content. The highest amount of chlorophyll was recorded with 6 mg L^{-1} $CuSO_4$ (0.47 mg g^{-1} fresh weight) which was followed by 4 and 2 mg L^{-1} $CuSO_4$ (0.39 mg and 0.34 and fresh weight), respectively. Whereas, at 8 mg L^{-1} $CuSO_4$, the total content of chlorophyll was closely similar to those recorded in untreated control.

DISCUSSION

The establishment of optimum concentrations of nutrients in plant tissue culture media is important for the production of healthy plantlets *in vitro*. The results obtained in the present study showed that increasing copper sulphate concentration in the MS-medium strongly stimulated banana shoot and root development *in vitro* as well as chlorophyll content of micropropagated plantlets. The highest number of shoots per explants and highest shoot length were observed in media supplemented with 6 mg L^{-1} $CuSO_4$. In addition, leaves of the developing shoots were healthier, more expanded and had the highest chlorophyll content compared to control cultures. The optimum rooting response of micropropagated banana shoots, in terms of root number/shoot and root length were achieved by the addition of 4 mg L^{-1} $CuSO_4$ to rooting media. These data suggested that the higher levels of copper stimulated shoot and root formation in the explants and it might be necessary to use higher levels of this medium component in order to enhance the micropropagation potential of banana. These findings are in line with previous reports emphasizing the positive influence of higher $CuSO_4$ on organogenesis of many plants. The increase of copper sulfate in the culture medium is reported to improved shoot and root development in cultures of several plants such as; hazelnut[9], *Capsicum annuum*[4], apple and pear[10], date palm[11] and orchid[12]. Besides its positive effect on shoot and root growth and development, copper sulphate was also shown to play an important roles on chlorophyll formation. In addition, result reported by AL-Mayahi[11] and Vinod *et al.*[13] demonstrated that high concentration of $CuSO_4$ in MS media increased total chlorophyll content of developing shoots.

While, it is evident that copper sulfate in the culture medium improved shoot and root development of banana, the exact basis for this is unknown, but it has been suggested that copper sulfate inhibits the formation of the ethylene precursor, 1-aminocyclopropane-1- carboxylic acid and thus, promotes regeneration[14]. However, Purnhauser and Gyulai[15] suggested that it is not through the ethylene-inhibiting action that copper ions promote organogenesis, since copper ions are activators of many enzymes involved in protein and carbohydrate biosynthesis. Thus, it is speculated that some copper enzymes might play an important role in the *in vitro*

organogenesis. Nevertheless, the results of the present study showed that copper levels in MS medium were not optimal for the micro propagation of banana since higher levels of copper enabled more efficient shoot and root formation in the explants. Based on these findings, it is recommended to supplement Murashige and Skoog (MS) medium with $CuSO_4$ to enhance the production of healthier banana plantlets through *in vitro* culture.

CONCLUSION

The results of this study indicated that $CuSO_4$ levels in MS medium is sub-optimal for *in vitro* culture of banana. Supplementing shoot multiplication media with 6 mg L^{-1} $CuSO_4$ resulted in optimum shoot multiplication and growth and improved the total chlorophyll content of developing shoots. Likewise, the addition of $CuSO_4$ at the concentration of 4 mg L^{-1} to rooting media significantly improved the rooting of micropropagated banana shoots. These findings may be useful for the development of high-frequency micropropagation culture system for banana.

SIGNIFICANCE STATEMENT

The present study demonstrated that $CuSO_4$ supplemented to Murashige and Skoog based banana multiplication and rooting media, with optimized concentrations of auxins and cytokinins, significantly improved shoot growth and rooting responses of cultured banana explants. Accordingly the results of this study may be useful for the development of efficient protocol for the micropropagation of banana.

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