EFFECTS OF SOME ELEMENTS ON THE GROWTH, AND THE DEVELOPMENT OF BANANA SHOOTS *(M. acuminata)* IN MICROPONIC SYSTEM

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SUMMARY

Tieu Hong banana variety *(Musa acuminata*) is one of the most popular bananas in Vietnam because they have not only beautiful colour, delicious taste but also high nutrition, and significantly economical value. Nowadays, this banana variety is primarily propagated in wide-scale by micropropagation which is effective with a high propagation coefficient, thus, that can meet the increasing demand of people to enjoy it. However, the method of propagation still has some limitations such as the low quality of the plantlets, the quite high cost in producing. Whereas, the micro-hydroponic (also called microponic) system is said as a promising technique that can improve the efficiency of the multiplication of many plant species *in vitro*. Therefore, this technique can improve the Tieu Hong banana micropropagation *in vitro*. With using plant growth regulators, the appropriate volume, and identifying the suitable density of shoots per a culturing box, and supplementing silver nanoparticles in the system, the microponic culturing system was successfully applied on the Tieu Hong banana shoots. These experimental results showed that Tieu Hong banana shoots cultured in the system added with specific conditions gained some positive effects with the remarkable data observed. Specifically, the average number of roots was about 4 roots/shoot, the root length was approximately 2.2 cm. Also, the addition of nano silver at a concentration of 4 ppm gave the significant results of *in vitro* Tieu Hong banana rooting with an average root number of 5.13 roots/shoot and an average root length of 4.01 after 2 weeks of culture.

Keywords: Banana *(Musa acuminata*), creating complete plants, microponic, nano silver, rooting.

1. INTRODUCTION

Bananas (*Musa acuminata*) have been claimed to be a valuable agricultural product in agriculture and traditional medicine throughout the world (Mathew & Negi, 2017). Among the banana varieties in Vietnam, Tieu Hong is one of the most popular varieties because of its beauty with the uniform color, the high nutritional value, and the high economic value. In recent years, many plant varieties like bananas have been mass-produced in everywhere by *in vitro* propagation methods (Ali et al., 2011) which has many advantages such as creating an identical population similar with the original mother plant, gaining a high multiplier coefficient, and guarantying clean seedlings with low prices.

However, *in vitro* micropropagation has one disadvantage as the low quality of the plantlets because of the low CO_2 concentration and the high humidity in the *in vitro* bottles. Therefore, it may result in deformed stomata, the vitreous glass which made the samples become weak and ineffective for propagation (Ziv, 1991), (Kozai T. et al., 1992). Also, it is a challenge for the plantlets to acclimate in nursery conditions due to seedlings were to adapt with many external adverse agents such as fungal pathogens, temperature changes, low humidity, poor nutrition. The sudden change makes the survival ability of its significantly reduced. Some studies showed that the natural conditions had affected the physiological changes inside the plant body; that led to abnormal morphological, physiological, and anatomical structures when the plantlets were transferred to the nursery. Typically, stomata did not work, the root system was weak, thin epidermal layer, and the content of sugar, starch changed (Hazarika et al., 2006).

Therefore, to overcome the disadvantages mentioned, recently, micro-hydroponic systems have been developed. Micro-hydroponic (microponic) system combined the micropropagation and hydroponic system, which was a potential method in seedling production. This method applied both advantages of hydroponics and micropropagation, and initially showed a positive effect on plant growth and development. Moreover, it needed to have without sterile conditions, supplement carbohydrates like sugar and agar to the culture medium. It was also easy to implement and practical practice (Hahn et al., 1996). However, the application only was initially applied to some crops with remarkable result in potato (Nhut et al., 2006);

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Gypsophila paniculata L (Wang et al., 2013) white daisies (Nhut et al., 2005; Hoang Thanh Tung et al., 2015). Therefore, it is necessary to research to apply on Tieu Hong banana to increase the effectiveness of asexual propagation of it.

2. RESEARCH METHODOLOGY

2.1. Materials

In vitro Tieu Hong banana shoots preserved at the Department of Biology, Faculty of Biotechnology, Vietnam National University of Agriculture.

2.2. Methods

* *Experimental location and time*: Faculty of Biotechnology, Vietnam National University of Agriculture, from June 2018 to March 2019.

* *Experiment condition*

Micro-hydroponic media: ½MS medium with pH 5.8, no need autoclaving.

Media and culture system: disposable plastic containers with a volume of 400 mL, the bottom diameter of 5.5 cm, the top diameter of 8.5 cm, and 10 cm in height. Inside of the boxes, there are 9 plastic tube plastic attached together with a diameter of 1 cm, a height of 2 cm, and were put with a layer of cotton with 1 cm thick (Nhut DT et al., 2006). The rearing boxes with lid were placed in lighting conditions of 12 hours /day, intensity of 2000 lux; humidity 55 - 60%; 25 ± 2°C.

Figure 1. Microponic system

***** *The effect of auxin on Tieu Hong banana shoots in micro-hydroponic system:* Banana shoots (3 - 4 cm) were exposed with auxin in different ways. The first sectors (CT1 (DC)), the control samples were to dip shoots with distilled water; the second one (CT2) is to dip shoots into α-NAA solution at 500 ppm for 20 minutes; and the third (CT3) is to add 1 ppm α-NAA directly to the culture media (according to the method of Hoang Thanh Tung et al. (2015) has improved). These samples were cultured in boxes with 30 ml of ½MS medium (3 samples /box) and monitored after 2 weeks of culture and counted shoots such as shoot height, root length, number of roots/shoots.

* *The effect of the medium volume on the Tieu Hong banana shoots in the micro-hydroponic system:* The banana shoots (3 - 4 cm) were treated with auxin by the most optimal method above and cultured in micro-hydroponic systems containing an MS nutrient medium with various volume (20; 30; 40 and 50 mL) in each box (3 samples/box). Samples were monitored after 2 weeks of culture and identified shoots such as shoot height, root length, number of roots/shoots.

* *The effect of the culture density on Tieu Hong banana in micro-hydroponic system:* The banana shoots (3 - 4 cm) were treated with auxin by the most optimal method above and cultured in micro-hydroponic systems containing 30 mL MS medium in each box with various density (1, 3; 5 and 7 shoots /box). The shoots were evenly spaced in the boxes. Samples were monitored after 2 weeks of culture and identified shoots such as shoot height, root length, number of roots/shoots.

* *The effect of silver nanoparticles on Tieu Hong banana in micro-hydroponic system*: The banana shoots (3 - 4 cm) were treated with auxin by the most optimal method above and cultured in micro-hydroponic systems in boxes. Each box contained 3 samples with 30 mL of ½MS medium supplemented with different concentrations (0; 2; 4; 6 ppm) of silver nanoparticles. Samples were monitored after 2 weeks of culture and identified shoots such as shoot height, root length, number of roots/shoots.

2.3. Data analysis

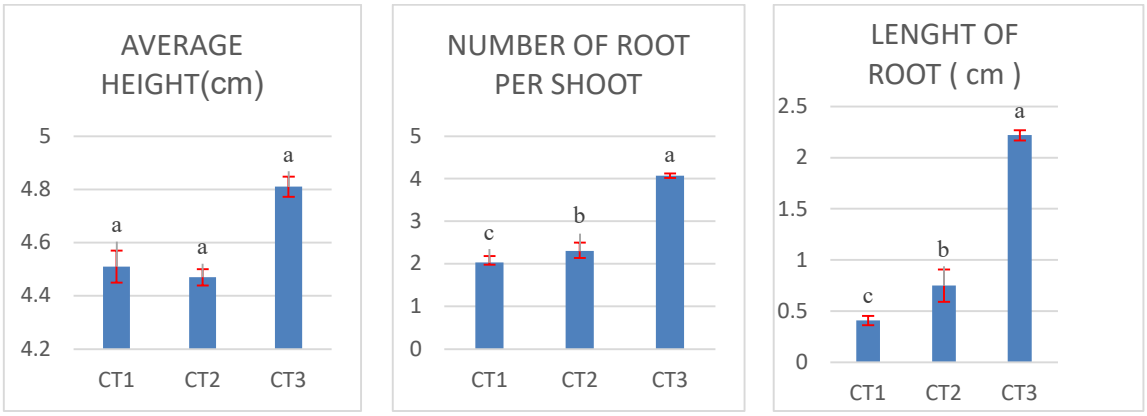
* Experimental layout: completely random CRD (Completely Randomized Design); Each experiment was repeated 3 times, each time consisted of 20 samples. Data were processed using MICROSOFT EXCEL and IRRISTAT 5.0 statistical processing software on computers.

3. RESULTS AND DISCUSSION

3.1. The effect of the method of shoot treating by auxin on Tieu Hong banana shoots in micro-hydroponic system

It was said that α - NAA could stimulate the growth, the cell division, and indeterminate the rooting in bananas (Al-Amini et al., 2009; Ali et al., 2011). In this experiment, the results in fig. 2 and fig. 3) show that, after 2 weeks of culture, different remedies of auxin treatments for shoots did not affect the average shoot height which ranged from 4.4 - 4.8 cm. However, samples cultured in the medium supplemented with 1 ppm α-NAA medium yielded the best root index with number of roots per shoot reaching 4.07 and average root length being 2.22 cm. The control samples (dipped only in water but neither treated with α-NAA 500 ppm nor added

NAA to the culture medium) also rooted after 2 weeks of culture with a small number of roots and the root length, only 2.03 roots/shoot and root length of 0.41 cm, respectively. Besides, the banana shoots were treated with α-NAA 500 ppm for 20 minutes, then cultured in the rooting medium also rooted with 2.30 roots per shoot and the average root length reached 0.75 cm. The results were different from research on *Chrysanthemum* culturing in hydroponic system as well. It demonstrated that 100% sample rooting when they were pretreated with 500 ppm IBA before culturing (Hoang Thanh Tung et al., 2015).



a *b* *c*

Figure 2. Relationship between the shoot treatment formulas by α-NAA and average shoot height (a), the average number of roots (b) and average root length (c) of Tieu Hong banana shoots in microsystem after 2 weeks of culture

(Note: CT1- samples treated with water; CT2- samples treated with 500 ppm α-NAA; CT3- samples in medium added 1 ppm α-NAA)

sample treated with water | *sample treated with 500 ppm α-NAA* | *sample in medium added 1 ppm α-NAA*

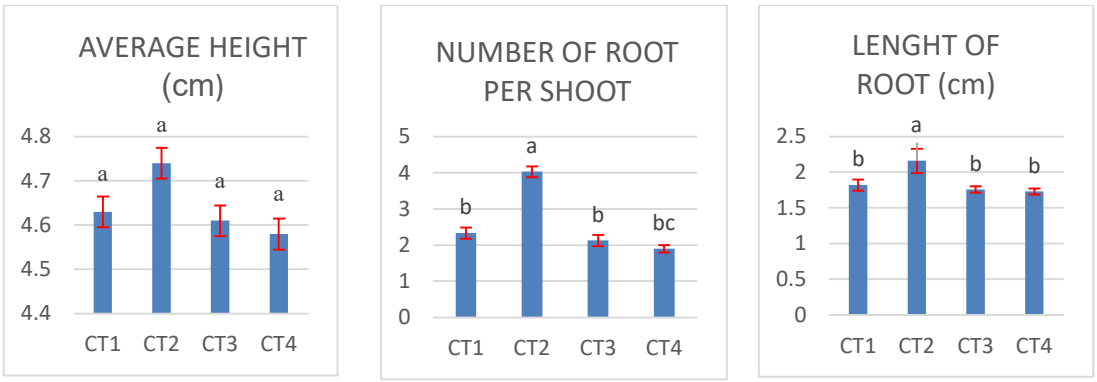
Figure 3. Tieu Hong banana shoots treated with α-NAA after 2 weeks of culture

The addition of 1 ppm α-NAA to ½MS culture medium facilitated shoot to root best. This result was similar to one of our publication (Bui Thi Thu Huong et al., 2020) which showed that Dai Hoang banana shoots in the micro-hydroponic system as well. In ½MS medium added α-NAA, 100% Tieu Hong banana shoots rooted with a high average number of roots, reaching 3.0 roots /shoot, and the average root length was 3.32 cm after 14 days of culture.

3.2. The effect of the medium volume on the Tieu Hong banana shoots in the micro-hydroponic system

The different volumes of medium culture affected the banana shoots with most characteristics. The results showed that while only the average height of shoot was not affected by the volume of culture solution (about 4.7 cm), the remaining figures of the shoots were affected. In a box with 30 ml of culture medium solution, the average number of roots per shoot and root length were the highest (4.03 root/shoot, 2.16 cm). In the formula with 20 ml medium solution, the amount of media decreased significantly that almost made the growth of plants slow down after one week of culture. Besides, the growth of the plants culturing in medium with the higher than 30 ml volume was poor. Especially, in the volume of the medium reaching 50 ml, the average number of roots of the plants decreased significantly, and these plants had bad morphological appearance such as low height, weak leaves and small stems. It can be explained that the shoots were cultured in boxes with a large volume

(over 40 ml) which led the roots of shoots were flooded. Consequently, it caused the adverse conditions for the shoots as well as the development of the root system.



a *b* *c*

Figure 4. Relationship between the culture medium volume and average shoot height (a), the average number of roots (b) and average root length (c) of Tieu Hong banana shoots in microsystem after 2 weeks of culture

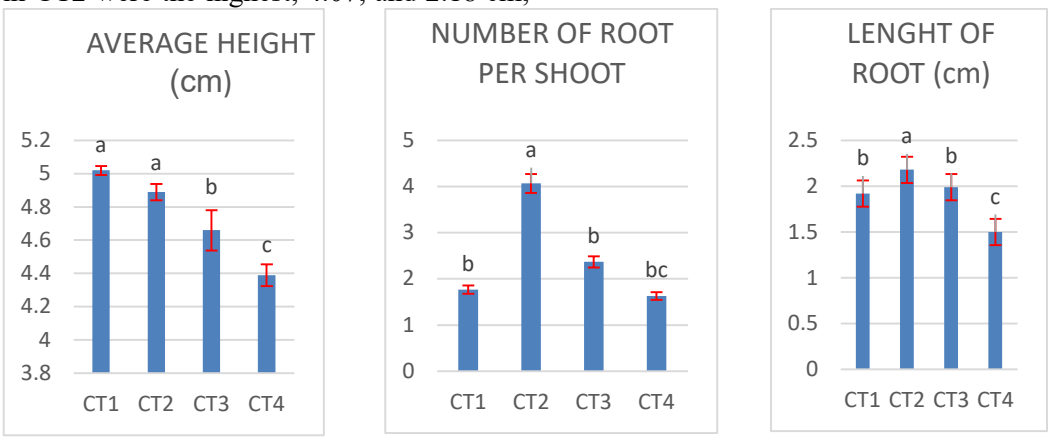
(Note: CT1: 20 ml of medium; CT2: 30 ml of medium; CT3: 40 ml of medium; CT4: 50 ml of medium)

20 ml of medium *30 ml of medium* *40 ml of medium* *50 ml of medium*

Figure 5. Tieu Hong banana shoots cultured in medium with different volume after 2 weeks of culture

3.3. The effect of the culture density on Tieu Hong banana shoots in micro-hydroponic system

The data in figure 6 shows that the sample cultured in the condition with different density got different results. In CT1 and CT2 sectors, although the average height of 4.89 cm, was higher than that of the other formulas, the number of roots and root length in CT2 were the highest, 4.07, and 2.18 cm, respectively. When the density increased to more than 5 shoots/box, all indicators decreased, including the average height of shoots, the average number of roots, and average root length. It can be explained that, when the density of shoots is high, these shoots may compete together for nutrition, light, so their growth and development were limited.



a *b* *c*

Figure 6. Relationship between density and average shoot height (a), the average number of roots (b) and average root length (c) of Tieu Hong banana shoots in microsystem after 2 weeks of culture

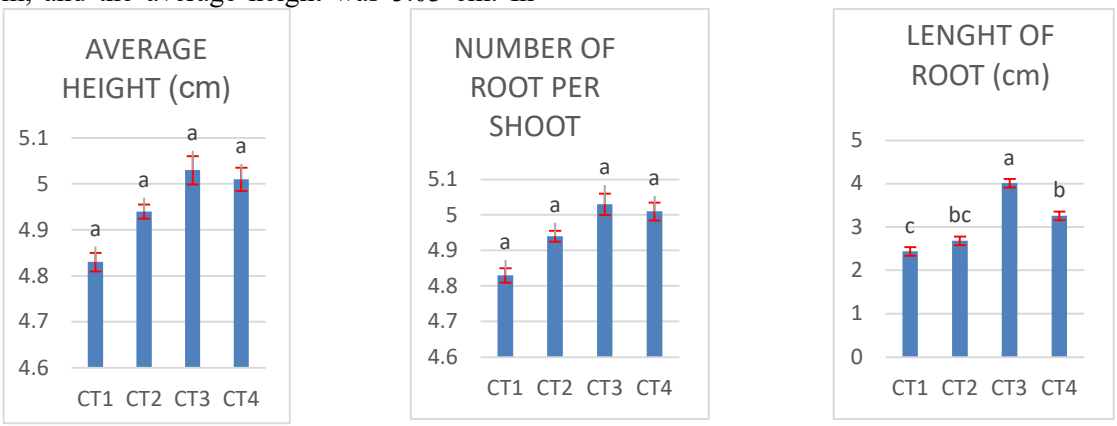
(Note: CT1: 1 shoot/box; CT2: 3 shoots/box; CT3 5 shoots/box; CT4: 7 shoots/box)

1 shoot/box *3 shoots/box* *5 shoots/box* *7 shoots/box*

Figure 7. Tieu Hong banana shoots culturing in different density after 2 weeks of culture

3.4. The effect of silver nanoparticles on Tieu Hong banana in micro-hydroponic system

Silver nanoparticles were demonstrated an effect on some plants like banana (Mai Huong Tra et al., 2017; Bui Thi Thu Huong et al., 2020). In this micro-hydroponic experiment, the Tieu Hong banana shoots cultured in the medium supplemented with silver nanoparticles of different concentrations. After 2 weeks of culture, the results showed that, excepting the shoot, all other parameters of them were higher than the control samples. Specifically, in CT3 with 4 ppm silver nano, the average number of roots was highest, 5.13 roots/shoot, and the average root length also obtained the highest with 4.01 cm, and the average height was 5.03 cm. In conclusion, silver nanoparticles at the experimented concentrations were positively affected on for Tieu Hong banana shoots. However, if being used in large doses (6 ppm), they initially inhibit the growth of the shoots. Similarly, Hoang Thanh Tung et al. (2016) also said that, in micro-hydroponic system, *Chrysanthemum* grew best in the environment supplemented with 7.5 ppm silver nanoparticles after 2 weeks of culture. A publication of Mai Huong Tra et al. (2017) also studied the effect of silver nano the growth and development of banana shoots in the *in vitro* system. They illustrated that, silver nano at small dosages, 3 ppm was had a good effect on them as well.



a *b* *c*

Figure 8. Relationship between silver nano (NS) concentration and the average shoot height (a), the average number of roots (b) and average root length (c) of Tieu Hong banana shoots in microsystem after 2 weeks of culture

(Note: CT1: 0 ppm NS; CT2: 2 ppm NS; CT3: 4 ppm NS; CT4: 6 ppm NS)

0 ppm NS *2 ppm NS* *4 ppm NS* *6 ppm NS*

Figure 9. Tieu Hong banana shoots culturing in medium added NS with different concentration after 2 weeks of culture

4. CONCLUSION

Tieu Hong banana shoots are cultured in ½ MS medium micro-hydroponic system added 1ppm α-NAA in 30 ml medium solution with the density of 3 plants/box got an average of 4.07 roots and an average root length was 2.18 cm after 2 weeks of culture. Besides, adding 4 ppm of nano silver to the culture medium of Tieu Hong micro-buds made them well-rooted, with an average of 5.13 roots after 2 weeks of culture.

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ẢNH HƯỞNG CỦA MỘT SỐ YẾU TỐ ĐẾN SINH TRƯỞNG, PHÁT TRIỂN CỦA CHỒI CHUỐI TIÊU HỒNG TRONG ĐIỀU KIỆN VI THỦY CANH

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TÓM TẮT

Chuối Tiêu Hồng (*Musa acuminata*) là một trong những loại chuối đang được ưa chuộng tại Việt Nam bởi màu sắc đẹp, ngon và có giá trị dinh dưỡng, giá trị kinh tế cao. Ngày nay, loại chuối này đang được nhân giống chủ yếu bằng vi nhân giống nhằm đáp ứng nhu cầu ngày càng tăng của con người. Tuy nhiên, phương pháp này vẫn còn một số hạn chế, như là chất lượng cây ngoài vườn ươm thấp, giá thành sản xuất cao. Trong khi đó, hệ thống nuôi cấy vi thủy canh có thể khắc phục được hiện tượng này. Bằng việc nghiên cứu ảnh hưởng một số yếu tố đến chồi nuôi cấy vi thủy canh, nghiên cứu nhằm nâng cao hiệu quả nhân giống *in vitro* cây chuối Tiêu Hồng. Kết quả thí nghiệm cho thấy, chồi chuối Tiêu Hồng nuôi cấy trong môi trường MS được bổ sung trực tiếp α- NAA 1 ppm vào môi trường nuôi cấy có thể tích 30 ml và mật độ 3 cây/hộp trong hệ thống vi thủy canh cho sự sinh trưởng của bộ rễ chuối Tiêu Hồng là tốt nhất với các chỉ tiêu về số rễ trung bình (khoảng 4 rễ/chồi), chiều dài rễ (đạt 2,2 cm). Bên cạnh đó, việc bổ sung nano bạc vào môi trường nuôi cấy ở nồng độ 4 ppm giúp tăng hiệu quả phát sinh rễ với số rễ trung bình là 5,13 rễ/chồi và chiều dài rễ trung bình là 4,01 cm sau 2 tuần nuôi cấy.

Từ khóa: Chuối Tiêu Hồng, nano bạc, ra rễ, tạo cây hoàn chỉnh, vi thủy canh.

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