STYDYING ON THE EFFECT OF ELECTROLYTIC NANOSILVER ON THE EFFICIENCY OF CLEAN- FORMING AND SHOOT REGENERATION OF *(Bletilla striata*) IN VITRO

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TÓM TẮT

NGHIÊN CỨU ẢNH HƯỞNG CỦA NANO BẠC ĐIỆN HÓA ĐẾN HIỆU QUẢ TẠO MẪU SẠCH VÀ KHẢ NĂNG TÁI SINH CHỒI CỦA LAN BẠCH CẬP (*Bletilla striata*) TRONG ĐIỀU KIỆN INVITRO

Bạch Cập là một trong những loài lớn nhất thuộc họ lan có nhiều ứng dụng trong y học và nghiên cứu khoa học. Các phương pháp khử trùng mô thực vật truyền thống thường hay sử dụng các loại hoá chất, có hiệu quả khử trùng nhưng phần nào đó sẽ ảnh hưởng đến sự sinh trưởng của mô nuôi cấy và gây ô nhiễm môi trường. Trong nghiên cứu này nano bạc điện hóa được điều chế bằng phương pháp điện hóa sử dụng điện thế thấp với hàm lượng bạc 100mg/L bước sóng hấp thụ cực đại UV-Vis 410nm và có trị thế zeta trung bình 40mV được sử dụng làm chất khử trùng mới mẫu đốt thân cây lan Bạch Cập so sánh với các chất khử trùng cũ là $HgCl_2$, $Ca(ClO)_2$ ở các nồng nồng độ khác nhau tương ứng với các mốc thời gian khác nhau. Kết quả thu được sau 30 ngày nuôi cấy cho thấy, mẫu được khử trùng bằng nano bạc điện hóa ở nồng độ 75 mg/L trong 30 phút cho tỷ lệ mẫu sống và tỷ lệ mẫu sinh chồi đạt hiệu quả tốt nhất là 76,1 % và 69,34 %. Đây cũng là nghiên cứu bước đầu về việc sử dụng nano bạc điện hóa không dư lượng hóa chất trong việc khử trùng nuôi cấy có thể thay thế các chất khử trùng độc hại.

Từ khóa: *Lan Bạch Cập**,** nano bạc điện hóa, khả năng sinh trưởng, sự sinh chồi, hiệu quả khử trùng.*

1. INTRODUCTION

Orchid **(***Bletilla striata***)** has not only been known as a precious medicinal plant but also been capable of curing many diseases, especially in homostasis, antibacterial, plasma exchange effect, treatment of pulmonary tuberculosis, bronchiectasis, heal burns, injuries, fight cancer and many other diseases. Therefore, the demand for high-quality (uniform size, disease-free) and a large amount of *Bletilla striata* seedlings is becoming more and more urgent and the advanced process of planting tissue culture meets that demand.

Since its inception, techniques of planting tissue culture have played an important role in propagation. This method helps rapidly increase the quantity and quality of crops, provide a source of disease-free plants with a huge number of seedlings in a short time and can be produced all year round. Bringing samples from ex vitro environment into in vitro is a very difficult stage because at this stage, common samples will susceptibly be caused by fungal, bacterial, dead or slow growth, which are costly and time consuming. There are many causes for this situation, one of which is the manipulations in the

sample disinfection process [1]. Inappropriate type of disinfectant, concentration and time of explant disinfection are the main causes of failure in the initial sample entry stage. Most of the sample disinfectants being used today [$HgCl_2$, $Ca(ClO)_2$…] are agents with high detergent properties, as well as antimicrobial resistance by the mechanism of corrosion of bacterial cell walls and fungi so often affect the explants but are still not effective in sample disinfection [10]. In addition, most of the substances used in the disinfection of samples today have adverse effects on human health. It is really necessary to find a new type of disinfectant that is safe for health, effective in sample disinfection and has a stimulating effect on the samples.

Silver and silver salts have widely been used in medical disinfection because of their antifungal and antibacterial properties without affecting the health and proliferation of epidermal tissues [3,12]. On the other hand, silver ions also play a vital role in influence somatic embryogenesis, shoot formation and rooting, positively influencing physiological processes including morphogenesis of the explant [4]. Therefore, silver ions have been used in plant tissue sample to stimulate explants as well as limit the number of infected samples [6]. However, silver ions are always accompanied by cations that exist in the form of salts such as silver nitrate, silver thiosulphate. This affects the absorption and disinfection efficiency of silver ions.

To deal with the situation, nanotechnology was born with outstanding features such as increasing the efficiency of surface contact, so ions can easily adhere to penetrate into microbial or plant cells, easier to transport in plants to help them be quickly absorbed and give higher efficiency, which promises to bring outstanding success in the field of plant tissue culture [7,11]. Electrolytic nanosilver is one of the friendly materials without chemical residues. Preparation of silver nanoparticle solution by electrochemical method using low voltage and without using chemical protectant is a very new research direction [2, 9]. Silver nanoparticles have an average size of 5-20 nm, good solution stability through zeta index > ±40mV the effectiveness of killing typical bacteria in the laboratory is over 99.99% at a concentration of 10ppm after 3 minutes of exposure, proving that when silver nanoparticles have no protection, the antibacterial effect is very high. Many studies that prove silver nanoparticles have the ability to disinfect have been carried out, but there is no systematic and complete study on the effects of electrolytic nanosilver in the disinfection and morphogenesis of silver nanoparticles explants from ex vitro to in vitro stages of *Bletilla striata*.

2. MATERIALS AND METHODS

2.1. Material

The electrolytic nanosilver solution made by the Institute of Environmental Technology according to the studied procedure [2, 9].

Samples of *Bletilla striata* are provided by the Institute of Agricultural Biology (The length of in vitro shoots are 3.0 - 3.5 cm)

Culture medium: use medium like that of Murashige and Skoog (1962) - MS supplemented with 30 g/L surcrose, 8.5g/L agar, pH 5.8. The entire medium was autoclaved at 121°C, 1 atm pressure in 20 minutes, then divided into 500 mL glass flasks with 100 mL of medium.

$Ca(ClO)_2$ và $HgCl_2$ Laboratory chemicals made in China with purity > 99.9%

2.2.Research Methods

The electrolytic nanosilver solution was used modern physical methods to evaluate the properties: The silver concentration in the solution was determined by an Atomic absorption spectrometer (AAS) at the Institute of Chemistry (VAST). Hitachi UH-5300 UV-Vis spectrometer for elemental silver determination and SZ-100 zeta potentiometer to determine the stability of silver nanosolutions at the Institute of Environmental Technology (VAST)

Study on the effect of nanosilver on the ability to disinfect the original sample: the stem of the mature plant was cut into segments containing the sleeping eyes, which are/were washed cleanly

with soap, under the running water. The sample was then further treated with 70º alcohol in 30 seconds, further disinfected with an electrochemical silver nano solution with different concentrations: 50 mg/L, 75 mg/L and 100 mg/L in 20, 30 and 40 minutes, the control is $HgCl_2$ 0.1%(w/w) in 5 minutes and $Ca(OCl)_2$ 10%(w/w) in 10 minutes. The sample was washed again with sterile distilled water and added to the starting medium (MS + 1 mg/L BA + 8.5 g/L agar). After 10, 30 days of culturing in the laboratory, the parameters of survival and disease-free samples were monitored.

2.3. Experimental setup and data processing

The experiment was arranged in a completely randomized design, each recipe was repeated 3 times, with 5 sprouts each time.

The experiment was placed in a microclimate at the Institute of Environmental Technology with fixed lighting conditions of 2,500 lux, optical cycle 12h/day, temperature 25 ± 2ºC. The data obtained in the experiments were analyzed and processed by *Excel 2016* and *IRRISTAT 5.0* statistical software.

3.RESULTS AND DISCUSSION

3.1. The resuls evaluate the properties of electrolytic nanosilver using low voltage

The research procedure published in the previous volume [2,9] the prepared electrolytic nanosilver will be evaluated for use in disinfection experiments. The results of the evaluation are presented in Figures 1 and 2 below

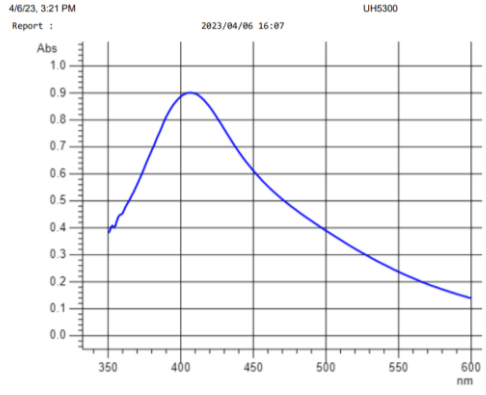

Fig. 1. Maximum wavelength image of electrolytic nanosilver

In the UV-Vis measurement results, it was found that the electrolytic nanosilver sample has an adsorption wavelength in the region of 410 nm, a sharp adsorption peak and high adsorption intensity. This result shows that the electrolytic nanosilver are small in size and quite uniform. Besides, in Fig. 2, the average zeta potential of the samples reached the average value above 40mV, demonstrating good sample stability. The results of analysis of silver content by AAS obtained 100 mg/L as stock solution for use in disinfection experiments.

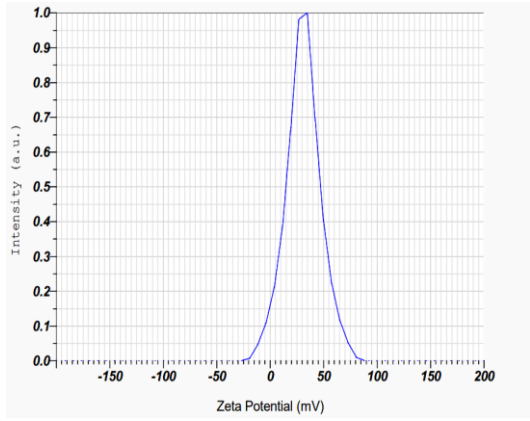

Fig. 2. Average zeta potential of the electrolytic nanosilver sample

3.2.Effect of electrolytic nanosilver concentration on survival rate

To evaluate the effectiveness of using electrolytic nanosilver to disinfect *Bletilla striata* stem samples on the survival rate, the study used electrochemical silver nano solution with different concentrations: 50 mg/L, 75mg/L and 100 mg/L; Common disinfectants $Ca(ClO)_2$ 10% (w/w) and $HgCl_2$ 0.1% (w/w), were controllably used and obtained the results as Table 1.

The results in Table 1 show that after 30 minutes of disinfection with electrolytic nanosilver, the best effect was achieved with the survival rate of 77.1% with the concentration of 100 mg/L and the survival rate of 76.1% with a concentration of 75 mg/L. Thus, soaking the sample in a high concentration of nano silver solution for a long time also affects the survival of the cells. For examples, if samples are disinfected in 40 minutes, they may die (turned brown-black) or unable to regenerate cells. This led to a lower

survival rate compared to other samples with short disinfection times. However, in the formulations with short disinfection time (20-30 minutes), the percentage of sample contamination was greater than in the formulations with long disinfection time. Compared with some other studies that used silver nanoparticles prepared by the Institute of Environmental Technology as research results of Dong Huy Gioi [4] the concentration of silver nanoparticle solution is most suitable for disinfection of *Phalaenopsis* is 125 ppm, 45 minutes treatment time for a survival rate of 72.13% or research results of Phan Thi Thu Hien, the samples of *philodendron selloum* were disinfected with silver nanoparticle at 150 ppm for 40 minutes for survival rate is 71.27% [6]. This study in which used silver nanoparticles with lower concentration and shorter time because we used electrolytic nanosilver by using low voltage method and without chemical protection is a very new research direction for nanoparticles with larger surface area, increased contact capacity [9].

Table 1. Effect of electrolytic nanosilver concentration on survival rate after 10 days

Sanitize chemical	Concen-tration	Disinfection time (minutes)	Survival rate after 10 days (%)
Electrolytic nanosilver	50 mg/L	20	$43,5^{g}$
		30	$58,2^{f}$
		40	$60,3^{e}$
	75 mg/L	20	$72,1^{bc}$
		30	$76,1^{ab}$
		40	73.3^{b}
	100 mg /L	20	$73,8^{b}$
		30	$77,1^{a}$
		40	$69,2^{cd}$
$HgCl_2$	0,1 %	5	$72,3^{c}$
$Ca(ClO)_2$	10 %	10	$73,6^{b}$

Note: Different letters (a,b,c,...) in the same column represent significant difference at α = 0.05 level

3.3.Effect of electrolytic nanosilver concentration on the rate of shoots

Ending the process of monitoring the survival rate of samples after 10 days, the shoot growth of the samples after 30 days of disinfection was followed up. The monitoring results are processed and presented in Table 2.

Table 2. Effect of electrolytic nanosilver concentration on the rate of shoot-producing samples after 30 days

Sanitize chemical	Concen-tration	Disinfection time (minutes)	Proportion of samples producing shoots after 30 days (%)
Electrolytic nanosilver	50mg/L	20	$54,87^{g}$
		30	$57,24^{f}$
		40	$58,34^{e}$
	75mg/L	20	$64,17^{cd}$
		30	$69,34^{a}$
		40	$67,29^{b}$
	100 mg/L	20	$65,25^{c}$
		30	$67,13^{b}$
		40	$63,23^{d}$
$HgCl_2$	0,1 %	5	$52,37^{h}$
$Ca(ClO)_2$	10 %	10	$53,61^{g}$

Note: Different letters (a,b,c,...) in the same column represent significant difference at α = 0.05 level

Fig. 3. Proportion of shoot generation after 30 days of culture

Based on the results presented in Table 2 and Figure 3, it was shown that the shoot growth rate after 30 days in the formula using electrolytic nanosilver with concentrations of 100 mg/L and 75 mg/L after soaking in 30 minutes was the best with the highest percentage shoot generation nearly 70%. In addition, electrolytic nanosilver is known as a biostimulant, which was effective on the ability to produce shoots and root and stem elongation [8]. Besides, the two control substances, $Ca(OCl)_2$ and $HgCl_2$ gave an efficiency of over 50%. $Ca(ClO)_2$ disinfectants are

highly corrosive and this is also their bactericidal mechanism-corroding cell membranes; Therefore, plant tissues are very susceptible to damage when exposed to $Ca(ClO)_2$, $HgCl_2$, wich led to difficult regeneration of the explants and prolonged regeneration time [12]. As the result, it was found that with a disinfection time of 30 minutes, using electrolytic nanosilver at concentrations of 75 mg/L and 100 mg/L gave similar best results in terms of survival rate and shoot ability, so we decided to use electrolytic nanosilver at concentrations of 75 mg/L is the most reasonable because it will save costs by using lower concentration.

4.CONCLUSION

Using electrolytic nanosilver at a concentration of 75 mg/L disinfected *Bletilla striata* stem samples for 30 minutes for the most optimal disinfection effect, helping the survival rate and the survival rate of shoots to reach 76% and 69% with the use of two disinfectants $HgCl_2$ and $Ca(OCl)_2$. This is also the first study on the use of electrolytic nanosilver without chemical residues in the disinfection of samples that can replace toxic disinfectants.

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