Effect of Added PPM on Murashige and Skoog Media for Shallot Meristematic Proliferation

A K Karjadi* and N Gunaeni
Indonesia Vegetable Research Institute /IVEGRI
Jl. Tangkuban Perahu no. 517 Lembang – Bandung

Email: asihkk@yahoo.com

Abstract
Shallot (Allium ascolonicum L) are plants that belong to genus Allium sp that were propagated vegetatively through bulbs. In the developed countries, shallot seed production can be produced through in vitro/micropropagation. The activity observed the effect of explant source and added of PPM (Plant Preservative Mixture) on Murashige and Skoog media in the growth and development of explants (Shoot tip, meristematic). The activity has been conducted in the tissue culture laboratory of IVEGRI from January to April 2019. As an explant bulb of shallot cv. Bima Brebes were infected OYDV or SYSV that has been tested by serology DAS ELISA. Media composition was MS with supplement sucrose 30 g L⁻¹+ IAA 2 mg L⁻¹ + Kinetin 2 mg L⁻¹ + GA3 0.01 mg L⁻¹ + Myo-inositol 100 mg L⁻¹ + gel rite 2 g L⁻¹, pH 5.7. The treatment were PPM concentration (0, 0.25, 0.50, 0.75, 1 mL L⁻¹) and the explants: shoot tip (A), meristematic (B). Results of the experiment on visual observation were carried out randomly on growth and development, serology test of OYDV, and SYSV of the plantlets. On visual observation obtained MS media composition with 1 ml L⁻¹ PPM the percentage of proliferation, there were no different from other concentrations of PPM and proliferation ≥ 60% for all media composition. DAS ELISA test for plantlet infected OYDV and SYSV for both explants was 47.37% - 69.33%.

1. Introduction
Shallot (Allium ascolonicum L) is one of the Allium species that is propagated vegetatively through bulbs. In the developed countries the production of onion seeds could be through in vitro/micropropagation. [1,2]. The plant tissue culture propagation is known as a technique for growing cells, tissue organs into plants in artificial media which are carried out aseptically. The basic principle in tissue culture was the cell theory by Scheiden and Schwann (1839-1939), that cell is the smallest biological unit capable to reproduce and perform living activities [3,4].

Growth and development of explant in vitro/micropropagation is influenced by several complex factors e.g. (a) genetic factors, (b) nutrient media: macro, micro and carbohydrate elements, (c) physical factors: light, temperature, pH media, the concentration of O2 and CO2, (d) organic acids, growth regulators, amino acids and vitamins. Based on references [5-7] plant propagation by tissue culture is influenced by the response of cultivar, genotypes, type of explant, explant treatment, and media composition.

PPM (Plant Preservative Mixture) is a solution formulation that can prevent contamination or reduce contamination caused by bacteria and fungi in culture [8,9]. Shallot propagation using tissue culture techniques is influenced by several factors i.e media composition, genotype, source of explant and treatment of explant materials [10-13].
The aim of this research was to observed the effect of explant source and additional PPM on MS media in growth and development of explant (shoot tip, meristematic). Our proposed hypothesis was that the explant type and additional PPM would increase the growth and development of explant and reduce the percentage of contamination.

2. Material and Methods
The activity was conducted in tissue culture laboratory on January until April 2019, the explant materials were shallot bulbs cv. Bima Brebes infected Onion Yellow Dwarf Virus (OYDV) or Shallot Yellow Strip Virus (SYSV). Media composition [14]: MS + MS vitamins + Sucrose 30 g L\(^{-1}\) + IAA 2 mg L\(^{-1}\) + Kinetin 2 mg L\(^{-1}\) + GA3 0.01 mg L\(^{-1}\) + Myo-inositol 100 mg L\(^{-1}\) + gel rite 2 g L\(^{-1}\) and pH 5.7. The treatments were explant source shoot tip (A), meristem (B) and concentration of PPM (0, 0.25, 0.5, 0.75, 1 ml L\(^{-1}\)).

The experiment was carried out through the following steps:

a) Shallot bulbs infected with OYDV as well as SYSV which had been tested with DAS ELISA were pulled for shoots seclusion which then dipped into alcohol 70% and soaked for 15 minutes in chlorox solution 25%. The shoot was rinsed with a sterile aquadest 3-5 times transferred to a sterile petri dish.

b) Explant inoculation was carried out in a sterile environment in the laminar air flow cabinet (LAFC). The culture was placed in a test 20 x 150 mm with 8-10 ml media. And incubate in the culture room with a temperature of 22-24 °C, photoperiod 16 hours light, 8 hours dark.

c) Each treatment consisted of 20 test tubes, thus, total culture 200 test tubes: 100 meristem cultures and 100 shoot tip cultures. The observation was carried out visually on 10 cultures which randomly selected for the growth and development of shallot plantlets. Incidence of viral diseases from plantlets with serological DAS ELISA were tested for OYDV dan SYSV.

3. Result and Discussion
Visual observation on the treatment of media and explant shallots cv. Bima Brebes at 4 to 6 Week after planting (WAP). In Figure 1, the percentage of the proliferation of ≥ 60% in explant shoot tip (A) and meristem (B). The growth and development of explants in tissue culture is influenced by a various highly complex factors e.g. (a) genetic factor, (b) nutrition in the media: macro, micro, and carbohydrate source, (c) environmental factor: light, temperature, pH of media, the concentration of CO\(_2\) and O\(_2\), (d) plant growth regulators, amino acids and vitamins. The success of plant proliferation is also influenced by culture response (genotype), explant type, explant treatment, and media composition used [5-7]. According to [8], the origin of explant was an important role in the success and closely related to the ability of regeneration or proliferation.

Note: WAP = Week After Planting. A= shoot tip, B= meristem ; Media composition I = MS +PPM 0 ml l\(^{-1}\); II = MS +PPM 0.25 ml l\(^{-1}\); III = MS + PPM 0.50 ml l\(^{-1}\); IV = MS + PPM 0.75 ml l\(^{-1}\); V= MS + PPM 1 ml l\(^{-1}\)

Figure 1. Percentage of explant proliferation
Percentage of contamination, growth, and development, the treatment of additional PPM (Plant Preservation Mixture) in the media showed no significant effects of explant growth and development. The addition of PPM in growing media with high concentration will reduce the percentage of contamination. In visually percent contamination, on media II, with PPM 0.25 ml/L explant meristem (B), shoot tip (A) give the same percentage. The small size explant will be incapable to grow and develop in media.

Contamination is generally caused by fungi and bacteria, additional treatment of PPM (Plant Preservation Mixture), visually gave low percentage contamination. In other words, the increasing concentration of PPM in MS media will reduce the percentage of contamination caused by bacteria or fungi. Mostly percentage of contamination explant shoot tip (A) higher than explant meristem (B).

According [15], this contamination is also influenced by several factors such as the technique of planting, the growing environment of explant. The contamination is a major obstacle, the source of contamination is generally carried from explant sources, unfavorable planting techniques, or the environment in the culture room when incubating plants. The average percentage of contamination culture up to 6 WAP less than 30%.

Selecting contaminant-free explants is a very important step, the contaminated could be fungi or bacteria. If contaminants are not removed in growth media that is contain sugar, vitamins, mineral, it will grow faster. The explants that are covered in contaminants will eventually die or not develop as a direct result of fungal, bacteria, or indirect attack due to toxic compounds produced by the fungus or bacteria [16-17].

Visual observation of the average number of plantlet roots showed that explant shoot tip (A) has more roots than explant meristem (B). According to [18] and [19] to increase the root number of shallot plantlets, it can be done with a subculture in the same media composition. Subculture can make the explant easier to produce roots, but sometimes also cause a decrease in the ability to regenerate and proliferation.

Visual observation on the average number of plantlets leaves showed that the explant shoot tip (A) will have a higher number of leaves in other words the number of leaves from explant shoot tip (A) better than meristem (B). The additional PPM in MS Media with high concentration give the effect of high numbers of leaves.

Plant propagation through tissue culture response of the explant depends on the component of culture condition that is media composition, type of explant (variety, size, the origin of explant). It is often to combined two or more of these components that are applied simultaneously or partially to increase the response of explant [2,20,21].

According to [22] and [23], in vitro propagation have several advantages (1) only needs small
materials (2) the environment grows in vitro is aseptic and under control (3) high-velocity propagation (4) can produce disease-free seed from a parent have been infected pathogen (5) for producing require small spaced.

Note: WAP = Week After Planting, A= shoot tip, B= meristem; Media composition I = MS + PPM 0 ml L$^{-1}$; II = MS + PPM 0.25 ml L$^{-1}$; III = MS + PPM 0.50 ml L$^{-1}$; IV = MS + PPM 0.75 ml L$^{-1}$; V = MS + PPM 1 ml L$^{-1}$.

**Figure 3.** Average number of roots number of shallot plantlet.

**Figure 4.** The average number of leaves from a shallot plantlet on 4,6 WAP

**Table 1.** The results of virus test with DAS ELISA method

| Media| No. culture| No. infected cultures OYDV| No. infected cultures SYSV| % infected| No. culture| No. infected cultures OYDV| No. infected cultures SYSV| % infected |
|------|------------|---------------------------|---------------------------|-----------|------------|---------------------------|---------------------------|-----------|
| I    | 13         | 5                         | 4                         | 9         | 16         | 6                         | 3                         | 9         | 56.25     |
| II   | 12         | 3                         | 4                         | 7         | 15         | 5                         | 4                         | 9         | 60        |
| III  | 16         | 4                         | 5                         | 9         | 18         | 6                         | 6                         | 12        | 66.67     |
| IV   | 18         | 5                         | 5                         | 10        | 18         | 7                         | 7                         | 11        | 61.11     |
| V    | 19         | 4                         | 5                         | 9         | 19         | 5                         | 5                         | 11        | 57.89     |

Note: OYDV = Onion Yellow Dwarf virus; SYSV = Shallot Yellow Strip Virus; Media composition I = MS + PPM 0 ml L$^{-1}$; II = MS + PPM 0.25 ml L$^{-1}$; III = MS + PPM 0.50 ml L$^{-1}$; IV = MS + PPM 0.75 ml L$^{-1}$; V = MS + PPM 1 ml L$^{-1}$

The results of [24], there is a group of common viral diseases affecting plants *Allium* group
originated from Carlavirus, Potty-virus, and Alexi-virus. In Indonesia, onion viruses include SLV (Shallot Latent Virus), OYDV (onion Yellow Dwarf Virus), SYSV (Shallot Yellow Strip Virus). According to [25] reported the incidence of tuber-borne viral diseases in onions and detected OYDV, SYSV, and combined OYDV and SYSV infections.

The combined infection of several viruses is a phenomenon that is often found. Viral disease infections in plants that are propagated vegetatively will accumulate from one generation to the next. Tuber-borne viruses can cause stunted growth, it is believed that the virus develops together with plant growth.

The results of virus testing with the DAS ELISA on growing plantlets were found 47.37% to 69.33% (Table 1). This result could be said that the shoot tip (A) growth better than meristem (B). And the percentage of infected with shoot tip (A) is smaller than meristem (B), the growth of explant shoot tip may be better than meristem. Viral disease has been detected indicates that explant or meristem tissue implantation was not optimum for eliminating viral diseases so that when explants are planted in the regeneration media the virus particles are still carried away [26].

4. Conclusion

The results of the experiment showed that treatment of media composition with additional PPM 1 ml/l was no different on percentage proliferation on explain meristem (B) and shoot tip (A), and was more than 60%. MS media added PPM could be a reduced percentage of contamination caused by fungi or bacteria. Visual Observation number of roots leaves explant shoot tip (A) higher than explant meristem (B). The results of the DAS Elisa test showed that plantlets infected OYDV and SYSV in a range between 47.37% to 69.33%.

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