The effect of explant size and addition of antiviral ribavirin on proliferation of meristematic potatoes (*Solanum tuberosum* L.)

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Abstract. Potato (*Solanum tuberosum* L.) belongs to Solanacearum genus which is vegetatively propagated through tubers or cuttings. Viral systemic diseases are an important disease that can be carried within seeds. Conventional techniques of tissue culture when combined with chemotherapy can eliminate systemic diseases, especially viruses. Research activity was carried out in the tissue culture laboratory of Indonesian Vegetable Research Institute (IVEGRI) from April until November 2018. The study was aimed to observe the effect of (1) antiviral Ribavirin concentrations (0; 5; 10 mg l\(^{-1}\)) added into MS medium supplemented with GA\(_3\), 0.15 mg l\(^{-1}\); kinetin, 0.1 mg l\(^{-1}\); Calcium panthothenate, 2 mg l\(^{-1}\); Myo inositol, 100 mg l\(^{-1}\); coconut water, 100 ml l\(^{-1}\); sucrose, 30 g l\(^{-1}\); agar, 6.5 g l\(^{-1}\); pH 5.7; and (2) genotypic difference of given varieties i.e. Granola, Median and Atlantic to the growth and development of the meristematic tissue (E1), shoot tip (E2). The results showed that growth percentage and development of meristematic shoot at 4 weeks after treatment was 50 – 80%, contamination rate was 20 – 50%; the percentage of normal growth was 40 – 50%, average number of shoots was 0.25 – 0.95, the number of nodes was 0.35 – 2.50, roots 0.20 – 0.93; while visual observation of antiviral Ribavirin on MS medium showed influences on growth where explants of shoot tip growth was better than meristem of the three varieties studied.

1. Introduction
Potato (*Solanum tuberosum* L.) is classified as a root tuber vegetable enriched with vitamin C and potassium. This tuberous vegetable becomes one of Indonesia’s prioritized commodities considering its role as the source of non-rice carbohydrates which has a potential function in food diversification programs.

The tissue culture technique is a method for isolating parts of a plant such as protoplasm, cells, a group of cells, tissues, organs and growing them in aseptic conditions so that these parts can develop and regenerate into complete plants again. The basic principle in tissue culture is the cell theory developed by Scheiden and Schwan (1939-1939), that the cell is the smallest unit that can carry out reproductive and growth activities [1]. The success of using the tissue culture method depends on the composition of the media used. Plant tissue culture media provides not only macro and/or micro-elements but also a source of carbohydrates which is generally in the form of sucrose. Its functions is
to replace carbons normally obtained from the atmosphere through photosynthesis. Better results will be obtained if the media is supplemented with vitamins, amino acids, and growth regulators [2, 3].

Meristem culture is the culture of plant tissue using meristematic tissue [4]. In meristem culture, the development of the tissue is intended to produce low-content virus infected or virus-free plants. This meristem culture method was developed to remove/eliminate one or more types of viruses. Its implementation can be combined with chemotherapy treatment using the antiviral Ribavirin. Antiviral Ribavirin is a chemical that can be added to aseptic growing media to inhibit virus development in plant tissue.

According to [5, 6], and [7], for vegetatively propagated plants, viral systemic disease is one of the important restrain that need to be resolved. One of the removal methods is called chemotherapy technique which is approached by the addition of antiviral Ribavirin in a meristem culture process. The combination of these techniques can increase the quality and quantity of seeds produced.

The research aimed to determine the effect of several concentrations of antiviral Ribavirin and explant size in MS medium on the growth and development of three potato varieties. Hypothetically, that the addition of antiviral Ribavirin to aseptic media will increase the growth, development, and improve the quality of potato plantlets.

2. Materials and methods

The research was conducted at the tissue culture laboratory of the Indonesia Vegetable Research Institute/IVEGRI from April to November 2018. The treatments included (1) antiviral Ribavirin concentrations (0, 5, 10 mg l$^{-1}$); (2) the size of the explant meristematic tissue size < 0.5 mm (E1) and shoot tip > 0.5 mm (E2); (3) different potato varieties i.e.: Granola, Median, and Atlantic. The growth media used MS (1962) was supplemented with GA$_3$ 0.15 mg l$^{-1}$, Kinetin 0.1 mg l$^{-1}$, Calcium pantothenate 2 mg l$^{-1}$, Myo inositol 100 mg l$^{-1}$, coconut water 100 ml l$^{-1}$, sucrose 30 g l$^{-1}$, agar 6.5 g l$^{-1}$, pH 5.7 [8].

The experiment was carried out through the following steps:

a) Preparation of explant material. Sprouts of potato var. Granola, var. Median and var. Atlantic were dipped in 70% alcohol, and immersed in 25% Clorox solution for 15 minutes, rinsed again with sterile purified water for 3-5 times, and transferred to sterilized Petri dishes.

b) Explant planting. This step was carried out in a sterile environment in a laminar airflow cabinet (LAFC), in a 15 x 150 mm test tube with medium volume of 5 ml. The culture was incubated in the culture room with a temperature of 22-24 °C, photoperiod of 16 hours light, 8 hours dark.

c) There are 18 total treatments, 20 test tubes per treatment. Observation was carried out 10 weeks after treatment (WAT) for the following parameters: (1) percentage of surviving explants, (2) percentage of contaminated plants, (3) number of shoots initiated, (4) number of nodes and roots, and (5) percentage of normal and abnormal plants.

3. Results and discussion

The visual observation on growth and development of the explants from the three potato varieties with the addition of antiviral Ribavirin were obtained 10 Weeks after treatment.
In the terms of explant growth percentage of meristem tissue (E1) and shoot tip (E2) among the given varieties (Granola, Median, Atlantic), the size of explant meristem (E1) and antiviral concentration of Ribavirin 5 mg l\(^{-1}\) affected on decreasing percentage of growth, the higher concentration of antiviral Ribavirin resulted in a lower percentage of growth.

According to Geier [9, 10], explant selection in tissue culture were an important role in the success and closely related to the ability to regenerate, the goal of propagation. The success of the development and application of tissue culture in plants with specific targets is influenced by the composition of the growing media, the compatibility of the explants, \textit{i.e.}, the genotype, and the type of explant [11, 12, 13, 14].

Several studies have stated that explant selection in tissue culture were an important role in success. The choice of explant and the composition of the aseptic growing media is closely related to the ability to regenerate [15] and the targets of the propagation to be achieved [10, 16].

Percentage growth observation showed that the addition of antiviral Ribavirin in MS media (1962) reduced the percentage of surviving explant meristem (E1) for all three varieties. The success of the application of tissue culture in plants for various purposes is influenced by the media component, \textit{i.e.}, the genotype and the type of explant [12, 14].

Meristematic tissue growth, explant physiological conditions affect the proliferation process. The failure of explants to differentiate is due to non-totipotent cells of the explants [17].

The percentage of contaminated explants, the addition of antiviral Ribavirin increased the proportion of contamination from the explants of meristem tissue (E1) when compared to shoot tip explants (E2). Contamination is generally caused by bacteria or fungi. The source of contaminants
usually was carried within the explant materials. The surface sterilization of the explant material is not sufficient to remove the source of contaminants [18, 19, 20].

In plant propagation activities using tissue culture techniques, explant material or plant donors that are free from contaminant sources are things that must be considered and are very important for growing tissue aseptically. If the source of contaminants is not removed from plant media containing sugars, vitamins, and mineral sources of contaminants, the contamination will occur rapidly. Completely contaminated explants eventually will die or not develop, directly resulted from the infection of fungi, bacteria, or indirectly toxicities by the compounds produced by the contaminant agents [21, 22].

![Figure 3. Percentage of explants with normal and abnormal growth at age 10 weeks after treatment.](image)

The percentage of abnormal explants, the result showed that a higher concentration of antiviral Ribavirin produced a higher number of abnormal explants of meristem tissue (E1) in all given varieties.

The success of conventional natural propagation was affected by cultivar/variety, explant type, explant treatment/explant donor treatment, and composition of the growth media [9, 11, 23].

Visual observation showed an abnormal proportion of treatment with high concentrations of antiviral Ribavirin. It showed a higher abnormality percentage and meristem explants (E1) abnormality percentage for Median and Atlantic varieties. According to [15], the origin of the explants, the composition of the media plays an important role in the belief and type of the explants, the composition of media is closely related to the ability of the explants to proliferate.

Unconventional propagation/in vitro provides an alternative in eliminating systemic diseases, especially viruses through the meristematic tissue culture technique. This technique can be approached with antiviral Ribavirin in addition to the aseptic media.
Figure 4. The average number of shoots growth at the age of 10 weeks after treatment.

The average number of shoots growth per plantlet is generally only one per plantlet. Some explants do not form buds, only grow and turns green. Visual observation showed the addition of antiviral Ribavirin 5 and 10 mg/l and varieties did not give a different effect, it can be seen that the combination treatment of varieties, the size of the explants, and the composition of media affect the average number of shoots, where the shoot tip explant gave a better result than the meristem. The addition of the antiviral Ribavirin inhibits the growth of the shoot.

The formation of shoots and growth in vitro culture was an effect of various factors [24], the type and intensity of light in the incubation room, besides that also various very complex factors, i.e., genetic factors (a) genetic factors (b) nutrition: air, macro, micro and carbohydrate sources (c) physical factors of light, temperature, media pH, concentrations of O₂ and CO₂ (d) growth regulators, amino acids and vitamins.

Figure 5. The average number of roots at the age of 10 weeks after treatment.

The observation of the average number of roots, the higher the antiviral concentration of Ribavirin, effect of ability to root for both meristem explants (E1) and shoot tips (E2) of the three varieties. Root growth from explant treatment, media composition, the ratio between auxin and cytokinin added to medium or the endogenous plant growth substance content of the explants [20].

To stimulate root growth, auxins concentration are needed higher than cytokinin. Smaller explant size will reduce its durability when cultured. Where each type of plant and plant organ has an optimal explant size for cultures in order to grow into a plant.
According to [25] and [26], root growth of explants can be increased by sub-culturing explants on the same medium. Besides changing the rooted explants to be easier to root, subculturing will cause a decrease in the ability of shoot regeneration and growth.

Success in tissue culture techniques is influenced by the response of the cultivar (genotype), the type of explant, and the composition of the media [9, 12]. According to [11] the success of the development and application of tissue culture in many plants for various purposes is strongly influenced by the culture media and the compatibility level of the explants planted.

![Figure 6](image-url)  
**Figure 6.** The average number of nodes at age 10 weeks after treatment.

Observation on the number of nodes per plantlet of the three potato varieties showed that the number of explants from shoot tip was higher than that of explants from meristem tissue. The addition of antiviral Ribavirin visually decreased the number of nodes per explant. From all the three potato varieties, the higher concentration of antiviral Ribavirin affected the number of nodes per explant.

In plant propagation through tissue culture, explant response varies depending on the culture component (media composition, elements added to the growing medium), type of explant (cultivar, size, source of the explant). Often a combination of two or more of these components that are applied simultaneously or partially is required to increase the response of the explant culture [19], [10], [27].

Tissue culture techniques are increasingly popular as an alternative to vegetative propagation plants. This technique includes the asexual propagation method with the main objective of multiplying plants that have superior properties. The success of this in vitro propagation depends on the ability of plant regeneration in aseptic growing media and controlled in vitro [11], [23].

### 4. Conclusion
The visual observations showed that the explant meristem growth and shoot tip of three potato varieties (Granola, Median, Atlantic) in MS medium were influenced by the size of explants and concentration of antiviral Ribavirin. Explant meristem percentage growth was smaller than of the shoot tip. The high concentration of antiviral Ribavirin resulted in a low percentage of growth and increased the percentage of abnormal explants.

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