

The Effect Of Antiviral Ribavirin On Proliferation Of Garlic Cv. Lumbu Hijau, Cv.Lumbu Putih And Cv.Tawangmangu

Asih K. Karjadi, and Neni Gunaeni.

AARD - Indonesian Vegetable Research Institute

Jl. Tangkuban Perahu no. 517 Lembang - West Bandung

E-Mail: asihkk@yahoo.com

Abstract.

The garlic (*Allium sativum* L) belongs to the genus *Allium*, propagated in vegetative through bulb. In the plants propagated by the vegetative technique virus is an important disease. The tissue culture techniques in combination with chemotherapy can eliminate virus diseases. The experiment was carried out in the laboratory tissue culture of IVEGRI. The experiment aims to observe the effects of several antiviral Ribavirin concentrations in MS medium on growth and development Shoot tip cv. Lumbu Hijau, cv. Lumbu Putih, cv. Tawangmangu, to produce a virus-free plant. Media composition, were: R1 to R4 that is: MS + MS vits + sucrose 30 g / l + IAA 2 mg / l Kinetin + 2 mg / l + GA₃ 0:01 mg / l + gelgro 2 g / l + (Ribavirin 0, 5, 10, 15 mg / l), pH 5.7. The results of the experiment a) Culture contamination were caused by bacteria and fungi with a percentage of 25 to 55%. b). In high concentration of antiviral Ribavirin gave results on decreasing growth and development of three cultivar garlic. c). On visual observation cultivar and added of antiviral Ribavirin has no effect on the number of shoots. d). The added of antiviral Ribavirin and cultivar does not affect the growth of three cultivar garlic. e) The virus test by serological DAS ELISA, the percentage of infected plantlets were 54.55% to 100%.

Key word; Garlic (*Allium sativum* L); Antiviral Ribavirin; cultivar

1. Introduction

Plant tissue culture technique is a technique of growing parts of plants in the form of pieces of tissue or plant organs that are separated from the natural environment in an artificial medium. The basic principle in tissue culture is cell theory proposed by Scheiden and Schwann (1839 - 1939), that cells are the smallest biological unit that can carry out living, reproductive and growing activities (Ayabe and Sumi, 1998; Abo El- Nil, 1977 ; Gabriela *et al* , 2001).

Garlic (*Allium sativum* L) belonging to the genus *Allium* are propagated by vegetative through bulbs. In developed countries garlic propagation has been done in vitro / micropropagation or in conventional either for the purpose of improving quality or just propagation of plants (Abo - El-Nill, 1977; Moriconi, *et al* 1990).

In plants that are propagated vegetatively, viruses are one of the important diseases that need to be solved. According to Walkey (1987), infection with this systemic viral disease can reduce production between 25-50% of the number of cloves (clove), the

number and weight of bulbs can be reduced to 45%. Viruses that have infected will continue to develop to next generation. To eliminate the virus in plants with vegetative propagated chemotherapy treatment / addition of antiviral Ribavirin, heating or by planting meristematic tissue (tissue culture). This combination of techniques can improve the quality and quantity of seeds.

Propagation in conventional / tissue culture plants is known as a technique for growing cells, tissues, organs become perfect plants in artificial media conducted aseptically. Media composition that is used in the tissue culture technique consists of macro, micro elements, amino acid, vitamins and organic supplements other source of carbohydrates, plant growth regulators (Gamborg *et al*, 1976; Barandiara *et al*, 1999; Koch *et al*, 1995). Plant propagation of garlic using tissue culture techniques is influenced by several factors, a.i the composition of the growing media, genotype, explants / donor explants (Buiteved, *et al*, 1994; Eady *et al*, 1998, Zheng *et al*, 1998).

The aims of experiment to observe the effect of several antiviral Ribavirin concentration in media MS (1962) on the growth and development of the shoot tip of garlic cv. Lumbu Hijau, cv. Lumbu putih and cv. Tawangmangu. To produce disease-free plants by using tissue culture techniques combined with Chemotherapy (antiviral Ribavirin)

2. Materials and Methods

The research was conducted in tissue culture laboratory of IVEGRI, and plant materials (explants) that is used is a bulb of garlic cv. Lumbu Hijau, cv. Lumbu putih, and cv. Tawangmangu. which infected with the virus results of the DAS ELISA serology test.

Step of experiment as follows:

1. Sterilize explants

Garlic bulbs that have been broken the dormancy shelled and took that are in the garlic cloves. The shoots are washed with detergent solution and rinse with aquadest 2-3 times, then dip in 70% alcohol solution and soak in 25% chlorox solution for 15 minutes, rinse with sterile aquadest 3-5 times, transfer to sterile petri dishes.

2. Proliferation explants before treatment

Explant was grown in MS media + MS vits + sugar 30 g / l + IAA 2 mg / l + Kinetin 2 mg / l + GA₃ 0.01 mg / l + agar 0.65%, pH 5.7 - 5.8. The growth of explants is carried out for 2-3 weeks until the plantlets have 2-3 leaves.

Shoot tip / meristematic with some primordial leaves is carried out in a sterile laminar airflow cabinet (LAFB), and planted / inoculated in a 20 x 150 mm test tube with 8-10 ml of media. Cultures are incubated in culture room with temperature 22-24 °C, photo periode of 16 hours of light, 8 hours of dark.

3. Treatment of experiment,

After explants have been grown into plantlets with leaves 2-3 transferred to the treatment media,:

Media R1: MS + M S vits + sucrose 30 g / l + IAA 2 mg / l + Kinetin 2 mg / l + GA₃ 0.01 mg / l + gelgro 2 g / l + Ribavirin 0 mg / l, pH 5.7

Media R2: MS + M S vits + sucrose 30 g / l + IAA 2 mg / l + Kinetin 2 mg / l + GA₃ 0.01 mg / l + gelgro 2 g / l + Ribavirin 5 mg / l, pH 5.7

Media R3: MS + M S vits + sucrose 30 g / l + IAA 2 mg / l + Kinetin 2 mg / l + GA₃ 0.01 mg / l + gelgro 2 g / l + Ribavirin 10 mg / l, pH 5.7

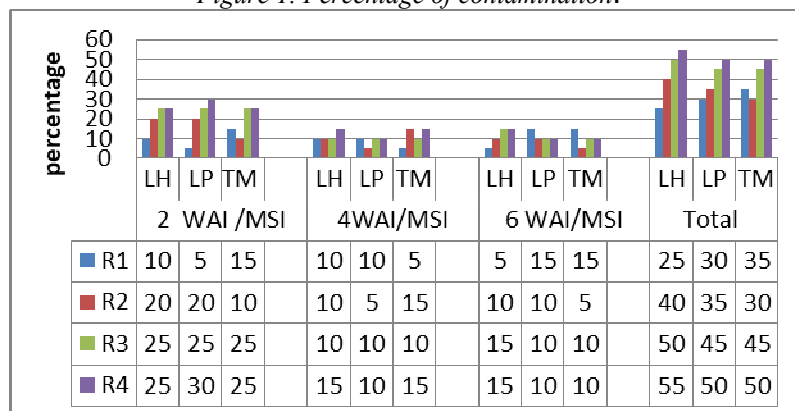
Media R4: MS + M S vits + sucrose 30 g / l + IAA 2 mg / l + Kinetin 2 mg / l + GA₃ 0.01 mg / l + gelgro 2 g / l + Ribavirin 15 mg / l, pH 5.7

Each treatment planted 20 test tubes 25 x 200 mm with 10 ml of treatment media. Observations were made visually on 10 tubes taken randomly on the growth and development of the garlic plantlet.

3. Results and Discussion

The observation of the growth and development plantlet in media with antiviral Ribavirin treatment in cv garlic culture. Lumbu Hijau (LH), cv. Lumbu Putih (LP) and cv. Tawangmangu (TM), is obtained:

Figure 1. Percentage of contamination.

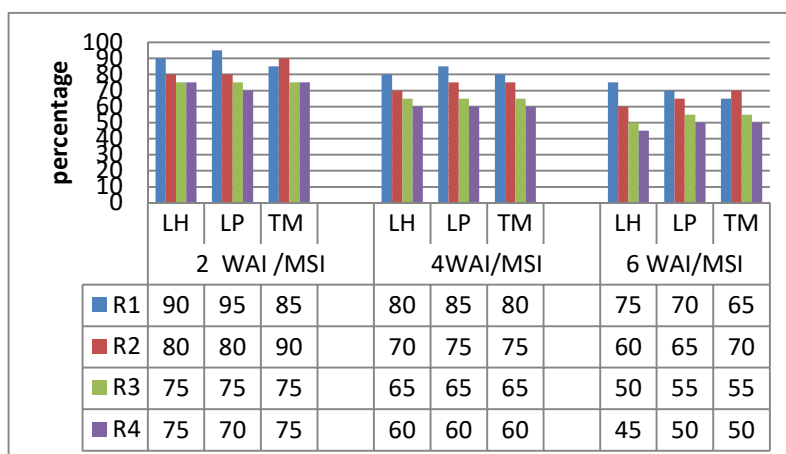


Note: R1 = Ribavirin 0 mg / l; R2 = Ribavirin 5 mg / l; R3 = Ribavirin 10 mg / l; R4 = Ribavirin 15 mg / l
 LH: cv. Lumbu Hijau; LP : cv. Lumbu Putih ; TM = cv. Tawang Mangu

On the figure the percentage of contamination in 2 to 6 WAI total contamination between 25 % to 55 % . Contamination generally caused by bacteria and fungi, antiviral Ribavirin treatment has no effect on percent contamination. It could be said that the source of contamination was carried by explants. The surface sterilization of explants material has not been sufficient to remove the source of contaminants on the surface of the explants source (Haque *et al* , 1997; Rokšana *et al* , 2002)

In tissue culture techniques plant material / explants that are free from contaminant sources is a very important step. Contaminants can be fungi and bacteria. If contaminants are not removed in growth media containing sugar, vitamins, mineral sources of contaminants will grow quickly. Explants that are covered with contaminants will eventually die or not develop, as a direct result of fungal, bacterial or indirect attacks due to toxic compounds produced by fungi, bacteria (Naik and Chandra, 1993; Badoni and Chamka, 2010).

Figure 2. Percentage of plantlets proliferation



Note: R1 = Ribavirin 0 mg / l; R2 = Ribavirin 5 mg / l; R3 = Ribavirin 10 mg / l; R4 = Ribavirin 15 mg / l
 LH: cv.Lum bu Green; LP : cv. Lumbu putih ; TM = cv. Tawang Mangu

Visually plantlet growth on 4 WAI , concentration of Ribavirin were affected. The higher concentration will give the lowest of growth and development on 3 cultivar of garlic.

The success of the development and application of tissue culture in many plants with various purposes is strongly influenced by the culture media and the level of compatibility with the planted explants, namely genotype and type of explants (George, 2008; Geier, 1990; Hamidah *et al*, 1977; Koch *et al* , 1995; Khar *et al*, 2005)

According to George and Sherington (1984), plant propagation in vitro has many advantages including (1) the plant material used is smaller so that it does not damage the parent tree, (2) aseptic and controlled invitro culture growing environment, (3) high propagation speed, (4) can produce disease-free seeds from a parent that already contains internal pathogens and (5) requires a relatively small place to produce large amounts of plantlets. Geier (1990), states that the selection of explants in tissue culture plays an important role in success, and the selection of explants is closely related to the ability of regeneration (Teng, 1997) as well as the purpose of the multiplication to be achieved (Chen *et. Al*, 1997; Kamstaiyte and Stanys, 2004)

Table 1. Average number of garlic plantlets.

Treatment	2 W AI / MSI			4 W AI / MSI			6 W AI / MSI		
	Cv.LH	CV LP	CV TM	CV LH	CV LP	CV TM	CV LH	CV LP	CV TM
R1	1 ± 0.38	1 ± 0.33	1 ± 0.32	1.29 ± 0.11	1.11 ± 0.37	1.2 ± 0.38	1.43 ± 0.16	1.22 ± 0.41	1.40 ± 0.44
R2	1 ± 0.45	1 ± 0.32	1 ± 0.32	1.40 ± 0.15	1.30 ± 0.41	1.2 ± 0.38	1.40 ± 0.15	1.40 ± 0.15	1.20 ± 0.38
R3	1 ± 0.38	1 ± 0.33	1 ± 0.32	1.51 ± 0.19	1.11 ± 0.37	1.2 ± 0.38	1.51 ± 0.19	1.11 ± 0.37	1.30 ± 0.41
R4	1 ± 0.41	1 ± 0.35	1 ± 0.32	1.67 ± 0.27	1.13 ± 0.39	1.2 ± 0.38	1.67 ± 0.27	1.13 ± 0.39	1.11 ± 0.35

Note: R1 = Ribavirin 0 mg / l; R2 = Ribavirin 5 mg / l; R3 = Ribavirin 10 mg / l; R4 = Ribavirin 15 mg / l
 LH: cv.Lumbu hijau ; LP : cvLumbu putih ; TM = cv. Tawang Mangu ; MSI/WAI: Weeks after inoculation

In the table 1, it can be seen that the average number of garlic shoots 3 cultivars plantlets is 1.0 to 1.67 per plantlet, it can be seen from one explant that only grows an average of 1 plantlet. Visual observations in general the addition of antiviral Ribavirin and cultivar did not give a different effect. The formation bud growth of plantlets in vitro culture is influenced by many factors (Shen *et al*, 2008), such is the type and intensity of light. In addition to the growth and development of plants in vitro culture is influenced also by many factors are very complex, namely (a) factors genes , (b) nutrition in a growing medium, water, elements of macro and micro as well as a source of carbohydrates, (c) factors physical: light, temperature, pH medium, the concentration

of O₂ and CO₂, (d) organic acids, plant growth regulator is added to the growing media, as well amino acids and vitamins.

Table 2. Average number of garlic plantlets.

Treatment	2 Week After Inoculation			4 Week After Inoculation			6 Week After Inoculation		
	Cv.LH	CV LP	CV TM	CV LH	CV LP	CV TM	CV LH	CV LP	CV TM
R1	4.0 ± 1.51	2.89 ± 0.96	3.5 ± 1.11	5.0 ± 1.89	3.44 ± 1.15	4.0 ± 1.27	5.0 ± 1.89	3.78 ± 1.26	4.30 ± 1.36
R2	3.6 ± 1.36	3.30 ± 1.04	3.3 ± 1.04	3.8 ± 0.68	3.90 ± 1.23	3.9 ± 1.23	3.90 ± 0.68	4.0 ± 1.27	4.0 ± 1.27
R3	4.14 ± 1.56	2.89 ± 0.96	3.0 ± 0.95	3.14 ± 1.18	3.33 ± 1.11	3.5 ± 1.10	3.42 ± 1.53	4.0 ± 1.33	4.20 ± 1.33
R4	3.33 ± 1.36	3.38 ± 1.19	2.9 ± 0.92	3.33 ± 1.36	3.75 ± 1.33	3.6 ± 1.14	4.0 ± 1.63	4.13 ± 1.46	4.1 ± 1.29

Note: R1 = Ribavirin 0 mg / l; R2 = Ribavirin 5 mg / l; R3 = Ribavirin 10 mg / l; R4 = Ribavirin 15 mg/l
 LH: cv.Lumbu Hijau; LP : cv. Lumbu putih ; TM = cv. Tawang Mangu , MSI/WAI : Weeks after inoculation

In the observation of the average number of leaves 3 cultivars garlic plantlet, number of leaves increases with increasing plantlet aged. The addition of antiviral Ribavirin visually did not affect the number of leaves. For the 3 cultivars garlic cultivar only the higher concentration of antiviral Ribavirin affected leaf growth and the development plantlet 3 garlic cultivars.

Propagation of plants through tissue culture, the response of explants varies depending on the components: culture conditions (media composition; elements added in the growing media), type of explants (cultivar, size, origin of explants). Often a combination of two or more components applied simultaneously or partially necessary for increasing response of explants (Roksana *et al* , 2002; Kamstaityte *et al* , 2004)

Table 3. Average number of plantlet roots of garlic.

Treatment	2 W AI / MSI			4 W AI / MSI			6 W AI / MSI		
	Cv.LH	CV LK	CV TM	CV LH	CV LK	CV TM	CV LH	CV LK	CV TM
R1	1.04 ± 0.43	1.44 ± 0.48	2.3 5 ± 0.73	1.57 ± 0.22	2.33 ± 0.78	3.2 1 ± 1.01	1.54 ± 0.43	2.89 ± 0.96	3.6 1 ± 1.14
R2	3.99 ± 1.51	2.65 ± 0.85	1.7 1 ± 0.54	3.8 ± 0.30	3.5 1 ± 1.11	2.5 1 ± 0.79	3.2 1 ± 0.45	4.2 1 ± 1.33	3.6 1 ± 1.14
R3	2.86 ± 1.26	1.24 ± 0.41	1.3 4 ± 0.41	2.86 ± 1.26	2.22 ± 0.74	2.39 ± 0.76	3.43 ± 1.29	2.99 ± 1.0	2.89 ± 0.92
R4	3.75 ± 1.63	2.48 ± 0.84	1.1 5 ± 0.35	3.85 ± 1.63	3.0 5 ± 1.06	2.21 ± 0.66	4.01 ± 1.63	3.51 ± 1.24	3.0 1 ± 0.95

Note: R1 = Ribavirin 0 mg / l; R2 = Ribavirin 5 mg / l; R3 = Ribavirin 10 mg / l; R4 = Ribavirin 15 mg / l
 LH: cv.Lumbu Hijau; LP : cv. Lumbu putih ; TM = cv. Tawang Mangu , MSI/WAI: Weeks after inoculation

Visual observation of the average number of roots, Ribavirin and cultivar treatments had no effect, roots grew in all treatments. According to Welander (1985) and Noiton *et al* (1992) to increase the root growth of explants can be done with explants sub culture on the same media. With the addition of sub-culture explants were difficult to rooted becomes easier to take root, but also led to a decrease in the ability of plantlet regeneration

Success in propagation techniques with tissue culture is influenced by the response of cultivars (genotypes), the type of explants and the composition of the media used (Geier, 1990; Hamidah *et al* , 1997). According to George *et al* (2008), the successful development and application of tissue culture in many plants for various purposes are deeply influenced by the culture and the level of compliance with the material explants were planted.

Tissue culture techniques are increasingly popular as an alternative propagation plant that is propagated vegetatively. This technique includes asexual propagation method with the main objective to make plants that have superior properties. The success of this in vitro propagation depends on the ability of plant regeneration in aseptic growing media in vitro.

Plant propagation in vitro provide an alternative in the elimination of viral diseases through meristematic culture combined with heat treatment or the addition of antiviral Ribavirin in the growing medium. In experiment was carried out planting meristematic tissue in the form of shoot tip or meristem tissue with few leaf primordia on MS medium with the addition several concentrations of antiviral Ribavirin.

Table 4 . Result of % infected virus with DAS ELISA test

Treatment		The amount of culture	Number of infected cultures		Total infected cultures	% of cultures infected
Media	Cultivar		OYDV	SYSV		
R1: 0 mg / l	cv. LH	15	7	6	13	86.67 (13/15)
	cv. LP	14	5	8	13	90.86 (13/14)
	cv. TM	13	6	7	13	100 (13/13)
R2: 5 mg / l	cv. LH	12	5	6	11	91.67 (11/12)
	cv. LP	13	5	5	10	76.92 (10/13)
	cv. TM	14	4	5	9	64.29 (9/14)
R3: 10 mg / l	cv. LH	10	3	4	7	70 (7/10)
	cv. LP	11	4	2	6	54.55 (6/11)
	cv. TM	11	3	4	7	63.64 (7/11)
R4: 15 mg / l	cv. LH	9	2	3	5	55.56 (5/9)
	cv. LP	10	3	4	7	70 (7/10)
	cv. TM	10	3	3	6	60 (6/10)

Note: R1 = Ribavirin 0 mg / l; R2 = Ribavirin 5 mg / l; R3 = Ribavirin 10 mg / l; R4 = Ribavirin 15 mg / l

OYDV = Onion Yellow Dwarf virus; SYSV = Yellow Strip Virus Shallots

LH: cv. Lum bu Green; LP : cv. Lumbu putih ; TM = cv. Tawang Mangu

According to the research of Diekmann (1997), the group of viruses that commonly attack onions comes from Carla - virus, Poty - virus and Alexi - virus. The main viruses in onion plants include SLV, OYDV, SYSV. In Indonesia Gunaeni *et al* (2011), reported the incidence of tuber-borne viral diseases in onions and detected infections of OYDV, SYSV and combined OYDV, SYSV. Mixed infection of several viruses is a phenomenon often found in diseases caused by viruses. A viral infection of the onion crop will be accumulated from one generation to the next. Seed borne virus that can cause plant growth , because of the virus could be develops with the growth of plants.

The results of detection of viral diseases in garlic plantlets (Table 4), seen the percentage of infected cultures 54.55% to 100%. From these results it can be said with antiviral Ribavirin treatment of infected plantlets percentage is still high. Viral disease is still detected, this indicates that the addition of antiviral Ribavirin is not optimal for eliminating the virus so that when explants are planted on regeneration media the virus particles are still carried on plantlets. (Zaitlin and P. Palukaitis, 2000).

4. Conclusion

The results of experiment :

The culture contamination is generally caused by bacteria and fungi with a percentage of 25 to 55 %. And the addition of high concentration antiviral Ribavirin in MS media, have the lowest percentage of growth and development for 3 cultivars with visually observation. Treatment antiviral Ribavirin has no effect on the number of shoots, leaves, roots for all three cultivars, (c v. Lumbu Hijau, cv. Lumbu Putih , cv. Tawangmangu). The results of virus testing using the DAS ELISA technique percentage of infected cultures were 54.55% to 100%.

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