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The Effect Of Phytohormone Picloram And Bap On Shallot Meristematic Proliferation

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Abstract.

Shallot (*Allium ascolonicum* L) is one of *Allium* species that is vegetatively propagated. In vitro/micropropagation has been carried out for virus free-seed production. The research was conducted at the Vegetable Research Institute Tissue Culture Laboratory started from August until December 2018. The research goal was to observe the effect of phytohormon picloram and BAP on the growth of meristematic cv. Maja. Totally 9 (nine) treatments were resulted from modified composition e.g. basic MS media (1962) + MS vitamins + sucrose 30 g / l + IAA 2 mg / l + kinetin 2 mg / l + GA_3 0.01 mg / l + Myo inositol 100 mg / l + CaP 2 mg / l + gel rite 2 g / l, pH 5.7, combined with phytohormon picloram (0,1,2 mg / l) and BAP (0,1,2 mg / l). Parameters observation showed that (1) percentage of contamination until 8 WAP was 30- 50 %, generally caused by fungi or bacteria, (2) percentage of visual proliferation was between 65 - 100%, (3) explant growth in M1 to M9 media was one shoot per explant. DAS ELISA detected that tested plants were infected with OYDV, SYSV 36.36% - 53.85%.

Keywords: Shallot (*Allium ascolonicum* L), picloram, BAP, MS Media.

1. Introduction

Shallot (*Allium ascolonicum* L), is one of Allium species that is propagated vegetatively through bulbs. In developed countries onion seeds have been produced through in vitro / micropropagation or unconventional either for the purpose of improving quality or simply for plant propagation (Abo El Nill, 1977; Kamstaityte and Stanys 2004, Bittner *et al* , 1989).

Plant tissue culture unconventional propagation, carried out in aseptic artificial media. The basic principle of tissue culture is cell theory proposed by Scheiden and Schwann (1839 - 1939) that cells are the smallest biological unit capable to reproduce and perform living activities (Ayabe and Sumi, 1998; Gabriela *et al* , 2001)

Unconventional propagation/ tissue culture is known as a technique for growing cells, tissues, organs into plants in artificial media which is carried out aseptically. The growth media used in tissue culture techniques consists of macro, micro elements, amino acids, vitamins and other orginal supplements such as carbohydrate sources, growth regulators (Gamborg *et al* ,

1976; Seif *et al* , 2011). Onion propagation using tissue culture techniques is influenced by several factors, eg. the composition of the media, genotype from explant source, original explant/donor explant, and explant source treatment (Buiteveld, *et al* 1994; Eady *et al* , 1998; Zheng *et al* 1998).

The aims of this experiment was to observe the effect of phytohormone picloram and BAP in MS media on the growth of meristematic tissue of cv .Maja. Submitted hypothesis was that by adding BAP, picloram on MS medium will increase the growth and development of the meristematic tissue.

2. **Material and Methods**

The research was conducted in the tissue culture laboratory of IVEGRI from August to December 2018 using shoot tip material (meristematic tissue with some primordia leaves) from cv. Maja (local variety) were used as material.

There were totally 9 (nine) treatments made from combination between modified composition e.g basic MS (1962) + MS vitamins + sucrose 30 g / l + IAA 2 mg / l + K inetin 2 mg / l + GA_3 0.01 mg / l + Myo inositol 100 mg / l + CaP 2 mg / l + gel rite 2 g / l, pH 5.7 combined with phytohormone picloram (0, 1.2 mg / l), BAP (0.1.2 mg / l),

Table . 1 The media composition of the treatment .

Treatment	Picloram mg / l	BAP mg / l
M1	0	0
M2	0	1
M3	0	2
M4	1	0
M5	1	1
M6	1	2
M7	2	0
M8	2	1
M9	2	2

The experiment was carried out through following steps:

a). The OYDV as well as SYSV infected explants which had been tested with DAS ELISA were peeled for shoots seclution which then dipped in 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.

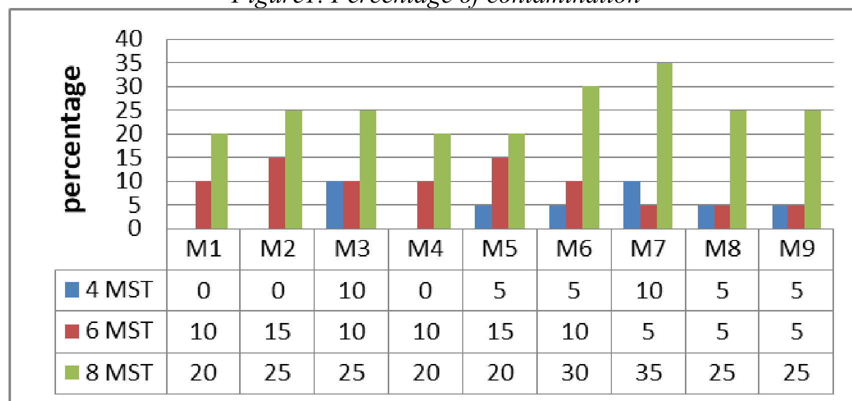
b).Explant culture/inoculation was carried out in a sterile environment in the laminar airflow cabinet (LAFC). Culture ~~is~~ was placed in a test tube 20 x 150 mm with ~~a~~ 8-10 ml media . Culture was incubated in the culture room with temperature of 22-24 ^{o}C, photoperiode 16 hours light, 8 hours dark.

c). Each treatment consisted of 20 tubes, the total number of cultures were 180 test tubes. Visual observations were made for (1) % explant growth (2) average number of leaves, (3) % normal and abnormal plants, (4) average number of roots, (5) incidence of viral diseases from plantlets with DAS ELISA tested for OYDV and SYSV viruses.

3. **Results and discussion**

The results of visual observation on the treatment of planting media and explant shallots of cv Maja at the 4,6,8 WAP as follows:

Figure1. Percentage of contamination



Note: MST = Weeks after planting/WAP

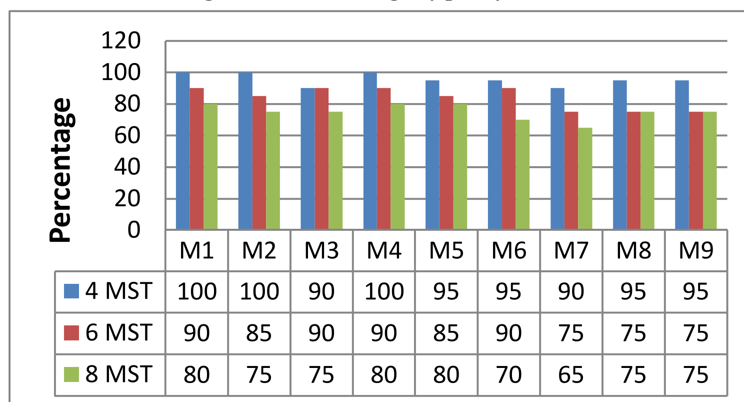
Figure 1 showed that the total contamination was 30-50% of the total culture. Contamination is generally caused by bacteria and fungi. Sources of contaminants ould be resulted from the explant source, surface sterilization, or insufficient explant material/donor explant (Haque *et al,* 1997; Roksana *et al,* 2002).

Free contaminant multiplication through inconventional /plant tissue culture is a very important step, in other words, contamination is a constrain in tissue culture technique.

If the contaminants are not removed from the donor explant, when the explant was grown on media which contain sugar, vitamin and mineral sources, the contaminants will grow and develop rapidly. Explant covered either fungal contaminants or bacteria will stop to grow ~~or~~ and finally died as ~~a~~ direct result of fungus attacks, bacteria or indirectly due to toxic compounds produced by fungi or bacteria (Armini, 1992; Naik and Chandra, 1993).

According to Gunawan (1987), the source of contaminants is generally carried out from explant material either on the surface or in the tissue explant (endogenous). It can also be caused by poor planting techniques, inadequate environment in the culture room at the time of incubation. Of all the sources of contaminations, the most difficult to overcome is the source of contaminants originated from explants.

Figure 2 . Percentage of proliferation



	M1	M2	M3	M4	M5	M6	M7	M8	M9
4 MST	100	100	90	100	95	95	90	95	95
6 MST	90	85	90	90	85	90	75	75	75
8 MST	80	75	75	80	80	70	65	75	75

Note: MST = Weeks after planting/WAP

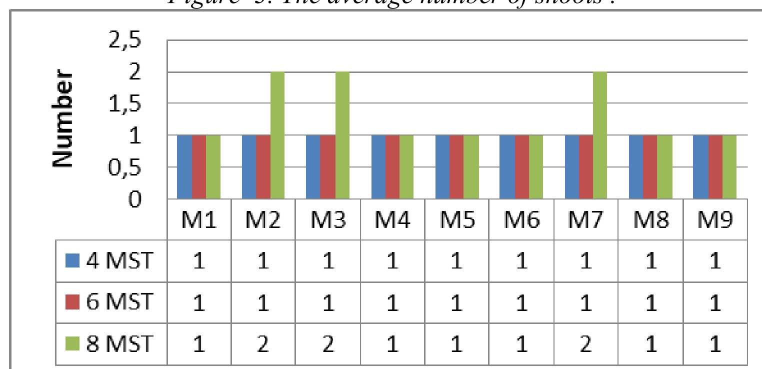
The percentage of growth and development of shallot explant shoot tip visually observed at the age of 4-8 WAP, was between 65-100% . At the time of 4 WAP in the media M1, M2, M4 grew 100% but decreased at the age of 8 WAP.

The successful application of tissue culture in propagation plants by various goal is strongly influenced by the composition of growth media, genotype and the type of explant (George, 2008; Geier 1990 ; Hamidah *et al* 1977, Khar *et al* , 2005).

According to George and Sherington (1984) propagation of plants in vitro has several advantages including (1) the plant material used is smaller so it does not damage the parent tree, (2) the environment grows from aseptic and controlled explants, (3) high propagation speed, (4) can produce disease-free plantlet from a parent that already contains internal pathogens and (5) requires a relatively small place to produce large amounts of plantlets.

In the shoot growth treatment of shallot cv.Maja, media MS with BAP treatment (M4, M5) and without the addition of hormones (BAP or picloram) M1 formed 80% proliferation at the 8 WAP. In the addition of picloram (auxin), percentage of proliferation is generally lower compared to BAP (cytokinin) addition. It can be considered that the addition of picloram (auxin) decreases the percentage of proliferation. In addition to the composition of chosing media explant in tissue culture were important role in the successfulin proliferation (Geier, 1990). –Moreover the selection explant is closely related to the ability explant regenerate (Theng, 1997). Also the purpose of propagation will be achieved (Chen *et al* , 1997; Kamstaity and Staney, 2004)

Figure 3. The average number of shoots .



	M1	M2	M3	M4	M5	M6	M7	M8	M9
4 MST	1	1	1	1	1	1	1	1	1
6 MST	1	1	1	1	1	1	1	1	1
8 MST	1	2	2	1	1	1	2	1	1

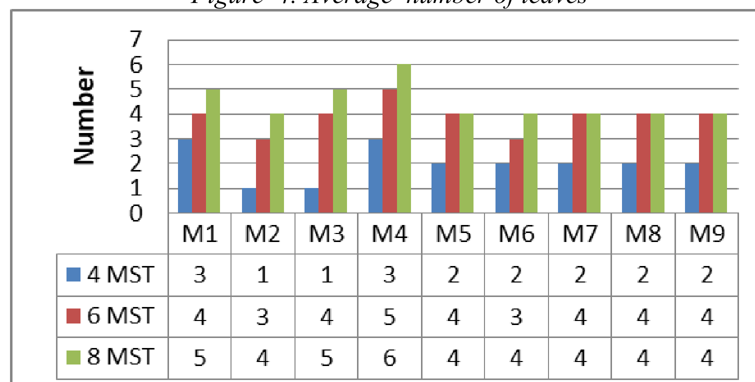
Note: MST = Weeks after planting/WAP

The average number of shoots at 4 – 8 WAP was generally only one shoot per explant except M2, M3 and M7, where the growing media added ZPT picloram (1-2 mg / l), with the exception of M7 media with the addition of BAP 2 mg / l. Visual observations showed that generally the addition of picloram (auxin) will induce a growing shoot.

The formation of shoots on in vitro culture is influenced by various factors (Shen et *al* , 2008), including the type and intensity of light in the culture room / incubation room. In addition, the growth and development of plants /shoot in vitro could be influenced by a variety of very complex factors, a.i (a) genetic factors, (b) nutrition: macro, micro elements, carbohydrate sources, growth regulators added to the media, (c) factors physical: light, temperature, pH of the media, concentration of O_2 and CO_2.

Generally, each genotipe can be offered in different responses to explant and media formulations (Haque an Manfield, 2004; Luc and Bridgen, 1996). The use of explants, basic media, explant treatment, growing environment and proper regeneration systems are thought to increase plant multiplication.

Figure 4. Average number of leaves



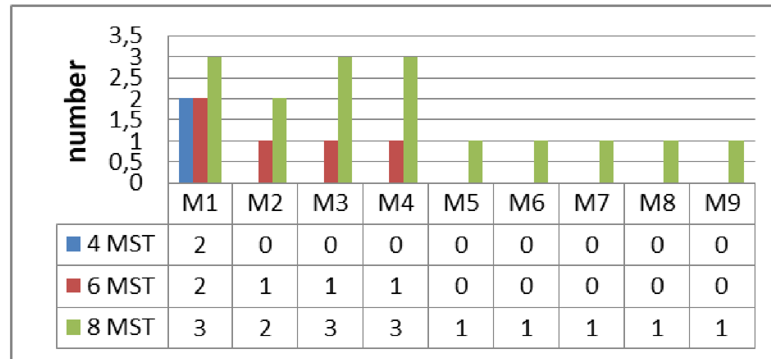
	M1	M2	M3	M4	M5	M6	M7	M8	M9
4 MST	3	1	1	3	2	2	2	2	2
6 MST	4	3	4	5	4	3	4	4	4
8 MST	5	4	5	6	4	4	4	4	4

Note: MST = Weeks after planting/WAP

On visual observation number of plantlets leaves , the addition of BAP or picloram in the growing medium did not effect on the average number of leaves. In plant propagation through

tissue culture techniques, the response of the explant planted in the growing media will have variation. It depends on several component, culture conditions (media composition, elements added in the growing media), explant type (cultivar, size, origin of explant). The combination of two or more components are applied, simultaneously or partially necessary to improve the response of the explant (Roksana *et al* , 2002; Kamstaity and Stanys , 2004; Kapoor *et al* , 2011).

Figure 5. Average number of roots



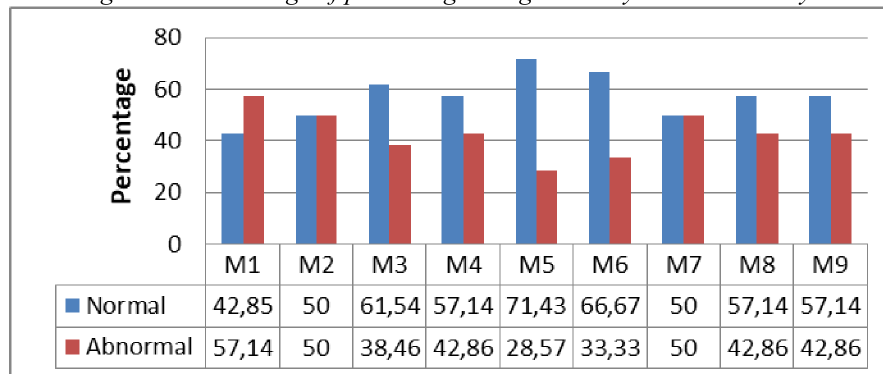
	M1	M2	M3	M4	M5	M6	M7	M8	M9
4 MST	2	0	0	0	0	0	0	0	0
6 MST	2	1	1	1	0	0	0	0	0
8 MST	3	2	3	3	1	1	1	1	1

Note: MST = Weeks after planting/WAP

Data showed that the number of roots at 4 - 8 WAP, treatment of M1 without the addition of ZPT, was the same as the media M3 and M4, which is added auxin / picloram 2 mg / l (M3) and BAP 1 mg / l (M4) . According to Welander (1985) and Noit an *et al* , (1992), treatment for increasing root number of explant can be done by sub-culture of explant on the same medium. With several times of sub culture the explant will be more easily rooted, but this can also cause a decrease in the ability to regenerate and plantlet growth.

Success in the propagation technique is influenced by the response of the varieties (genotype), the type of explant and the composition of the media used (Geier 1990; Hamidah *et al* , 1997). According to George *et al* (2008), the successful development and aplication on plant tissue culture with various objectives depend on media composition and level of conformity with explant material being planted. It is also dependent on the regeneration ability of plants in a in vitro growing media .

Figure 6. Percentage of plantlets growing normally and abnormally



	M1	M2	M3	M4	M5	M6	M7	M8	M9
Normal	42,85	50	61,54	57,14	71,43	66,67	50	57,14	57,14
Abnormal	57,14	50	38,46	42,86	28,57	33,33	50	42,86	42,86

Note: MST = Weeks after planting/WAP

Figure 6 showed that normal and abnormal growths of explant shoot tip shallot cv.Maja. The percentage of normal growth ~~is~~ was always higher than abnormal in the media added by picloram (auxin) a.i M3, M5, M6, M8, M9. In case of addition of BAP and picloram with the same concentration between BAP and picloram, percentage of abnormal and normal were 50%.

Some of the main factors that are key to success in in vitro culture are (a) explant sources (b) growing media and added of ZPT, (c) physical environment and (d) regeneration system (Wattimena *et al* , 2011; Dinarti *et al* , 2008; Dugassa and Feyssa, 2011). Moreover plant genotypes also influence the success of in vitro culture. Generally each genotype responds differently to treatment and the explant media formulation (Haque and M ansfield 2004 ; Luc and Bridgen 1996). The use of explants, media, explant origin treatment, growing environment and proper regeneration systems are thought to increase plant multiplication .

Table 2. Results of serology tests DAS ELISA plantlet var. Maja

Media	Amount of Culture	Number of infected cultures		Total	
		OYDV	SYSV	Number of infected	% infected
M1	9	1	3	4	4/9 = 44.44
M2	10	2	3	5	5/10 = 50
M3	12	3	2	5	5/12 = 41.67
M4	11	4	1	5	5/11 = 45.45
M5	14	4	1	5	5/14 = 35.71
M6	13	5	2	7	7/13 = 53.85
M7	10	3	2	5	5/10 = 50
M8	11	2	2	4	4/11 = 36.36
M9	11	2	2	4	4/11 = 36.36

Description: OYDV = Onion Yellow Dwarf virus, SYSV = Shallot Yellow Strip virus

Efforts for virus diseases elimination on different types of plants have been successfully performed using several methods, such as meristem culture, heat therapy / thermotherapy or using antiviral Ribavirin / Chemotherapy followed by propagation through tissue culture techniques (Tan *et al* , 2010). The success of elimination can also be influenced by several things

such as the size of the explant (Ashnayi *et al* , 2012, Hu *et al,* 2012) virus concentration in plant tissue (Pramesh and Baranwal, 2015), plant genotypes, types of ZPT used in tissue culture and methods elimination (Bhojwani and Datu, 2013).

Virus-free plants can be interpreted with negative detection of certain viruses. The most common group of viruses that attack the *Allium* is derived from Carla - V irus, Potty virus and Allexi - virus. The main viruses in onion plants include OYDV (Onion Yellow Dwarf virus), SYSV (Shallot YellowStrips virus) and LYSV (Leeks Yellow Strips virus) (Diekmann, 1997) . The results of research by Gunaeni *et al* (2011), the serology DAS Elisa method in Indonesia detected 3 types virus that is OYDV, SYSV and LYSV. A compound of some viral infection is a phenomenon that is often found in diseases caused by virus.

In table 2. the DAS ELISA test results, the percentage of plantlets infected with OYDV and SYSV 35, 71 - 50%. And according to Zaitlin and Palukautis (2000), the virus was still detected. This indicates that planting of shoot tip shallots has not been able to eliminate the virus which means particles virus have been still regenerate.

4.Conclusion

The results of experiment :

The percentage of contamination 30 to 50% is generally caused by fungi or bacteria. Based on visual observation, the percentage of proliferation is between 65-100%, on 4 to 8 WAP where the leaves and roots have been formed.In average, there was only one shoot grown per explant from all teatment media .The highest percentage of abnormal was found in media M1 and the lowest was found in M5.The results of DAS ELISA test showed that plantlets were infected OYDV and SYSVin a range between 36.36 % to 53.85%.

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