Biosaintifika 10 (2) (2018) 379-387

Biosaintifika
Journal of Biology & Biology Education

http://journal.unnes.ac.id/nju/index.php/biosaintifika

Effect of IBA and BAP on Shoot Growth of Tawangmangu Tangerine (*Citrus reticulate*) by In-Vitro

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DOI: http://dx.doi.org/10.15294/biosaintifika.v10i2.14977

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History Article

Received 5 May 2018
Approved 12 June 2018
Published 30 August 2018

Keywords

IBA; BAP; Shoot Growth; Tawangmangu Tangerine

Abstract

Tawangmangu tangerine (*Citrus reticulate* Blanco subsp. tawangmangu) is one of prime local fruit from Tawangmangu region, Karanganyar, Central Java. This tangerine have good appearance, easy to peeled, sweet flavored fruit, and high production rate. However at 1984 Tawangmangu tangerine run into depreciation of population caused by *CVPD* (*Citrus Vein Phloem Degeneration*). As a result, this variety listed as endangeredThe effort of consevation by culture tissue technique can order to obtain prime seeds that free from virus or disease. This study was aimed to understand the effect of IBA and BAP in culture medium on growth of apical and lateral shoot of Tawangmangu tangerine. The treatment provided were IBA (0 ppm, 0.5 ppm, and 1 ppm) and BAP (0 ppm, 1 ppm and 2 ppm). The treatment in this research was using complete randomized factorial design in 9 different treatment. This research resulted that added combination of IBA and BAP affecting growth of Tawangmangu tangerine but the interaction of IBA and BAP did not happen. The highest shoot length occurs on treatment IBA 1 ppm+BAP 2 ppm while when the fastest shoot appears occurs on treatment IBA 0 ppm+ BAP 2 ppm and the highest shoot formed on treatment IBA 0.5 ppm+BAP 2 ppm. Benefit of the research Efforts to conserve Tawangmangu orange plants need to be done to maintain the diversity of germplasm and support the cultivation in a manner. One technique that can support the conservation of plants is by tissue culture techniques.

How to Cite

Sofian, A. A., Prihastanti, E., & Suedy, S. W. A. (2018). Effect of IBA and BAP on Shoot Growth of Tawangmangu Tangerine (*Citrus reticulate*) by In-Vitro. *Biosaintifika: Journal of Biology & Biology Education*, 10(2), 379-387.

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p-ISSN 2085-191X
e-ISSN 2338-7610

INTRODUCTION

Tawangmangu tangerine (*Citrus reticulata* subsptawangmangu) as alocally prime fruitthat from Tawangmangu region, Karanganyar, Central Java. The prime this Tawangmangu tangerine emboosed in Minister of Agricultural Decree Number: 456/Kpts/PD.210/9/2003 Strikethroughrelease Tawangmangu tangerine as Prime Varieties on 15 September 2003. The characteristic of prime is skin fruit easy to peel, have good appearance, thefruit flavor is sweet, and high production rate. This citrus runs into depreciation of population that caused *CVPD* in 1984. It was this variety have been endangered (Wahyuningsih, 2009).

The effort to conservation this plant need to do to keep germplasm diversity which exists. Culture tissue technique (*In Vitro*) can be an alternatif solution in propagation germ plasm which exists. Ahmed and Anjum (2010), state conservation by *in-vitro* can offer some benefit than conservation in field or *in-vivo* because aseptic system ensures plant stock that free from pathogen, reduce genetic erosion with optimal maintenance, save space, reduce maintenance cost, easily propagated in bulk, and make it easier to germplasm exchange.

Culture tissue technique basically depends on totipotency concept that is capability cell of plant to regeneration become a new individu in the proper and aceptic enviroment (Hussain *et al.*, 2012).The ability totipotency of cell, tissue or organ can be applicated on utilyzation type of explant.Lestari (2011), plant propagationby in-vitro method useshoots tip as one of technique micropropagation that do with cultured explant which have shoots meristem with the purpose to stimulate and propagate new shoots (axsillary and apical).Shoots formation by in vitro culture will determine the succesfull production of seeds that fast and lot, where to multiply buds is required addition plant growth regulator with the right concentration.

Gilbert (2006), cytokines and auxin as a plant promoting growth that many used in plant culture tissue. Cytokines have a role in buds formation, while auxin has a in dominancy apical that can suppress the outgrowth of axillary buds.

Harliana *et al.*, (2012), the result of research about organogenesis tangerine plant (*Citrus nobilis* Lour) with added many consentration of IAA and BAP shows that IAA 1.0 ppm and BAP 1.0 ppm gave the best result againts the number of leaves with an average 2.9 leaves, while when the best bud sappear occurs in the treatment 0.1 ppm IAA and 0.6 BAP with a value of 11 DAP (days after planting). Therefore research about the giving hormone IBA (Indole Butyric Acid) and BAP (Benzyl Amino Purine) done to know the effect of growth axillary and apical shoots Tawangmangu tangerine explant.

METHODS

This research was carried out at BSF Plants (Biology Structure and Function), Departement of Biology, Science and Mathematics Faculty, Diponegoro University. Reseach held on January – March 2017.

Tools used include : measuring cup, drop pipette, erlenmeyer, micropipette, petri dish, bekker glass, stirring bar, autoclave, Laminar air flow (LAF), disecting tools, culture bottle, methylated spirits lamp, analitycal scales, PH meter, incubator and oven. Materials used include : shoots Tawangmangu tangerine explant, ascorbic acid, HgCl, fungicide, antibiotic, alcohol (70% and 96%), MS (murashige and Skoog), myoinositol, sukrosa, methylated spirit, detergent, aquadest, filter paper, allumunium foil, IBA (Indole Butyric Acid) and BAP (Benzyl Amino Purine).

This research begin with taking explant in field at 10 am, and then proceed with sterilization explant used a fungicide, detergent, and HgCl. To minimize from browning soaked in ascorbic acid. Afterwards, explants cut out to separate the leaves from shoot buds, after that explant entered to laminar air flow and it sterilization again with antibiotic, HgCl and alcohol. The last explant cut and entered in culture bottle and observed for 21 days. The parameters of research are the appearance of buds (day after planting), amount of buds, length of buds (mm), and weight of buds (g).The treatment in this research was using complete randomized factorial design in 9 different treatment and 3 repetitions.As for the consentration of growth regulators is A0 (0 ppm IBA) A1 (0.5 ppm IBA), A2 (1 ppm IBA), and B0 (0 ppm BAP), B1 (0.5 ppm BAP), B2 (2 ppm BAP). Here is the treatment of a combination from growth regulators (IBA and BAP) :

A0B0 : 0 ppm IBA + 0 ppm BAP
A0B1 : 0 ppm IBA + 0.5 ppm BAP
A0B2 : 0 ppm IBA + 2 ppm BAP
A1B0 : 0.5 ppm IBA + 0 ppm BAP
A1B1 : 0.5 ppm IBA + 0.5 ppm BAP
A1B2 : 0.5 ppm IBA + 2 ppm BAP
A2B0 : 1 ppm IBA + 0 ppm BAP
A2B1 : 1 ppm IBA + 0.5 ppm BAP
A2B2 : 1 ppm IBA + 2 ppm BAP

RESULT AND DISCUSSION

Shoot appear and Shoot Amount

Shoots appear counted with units of days after planting (DAP) while the number of shoots amount by counting all shoots that grow on the explant. Based on analysis of variance IBA (Indole Butyric Acid) and BAP (Benzyl Amino Purine) added on media is notsignificant effect towards shoot appear. The result of the average value when the shoot appears after 21 days observation shows that there is a tendency on BAP 2 ppm giving in media capable to accelerate induction buds.

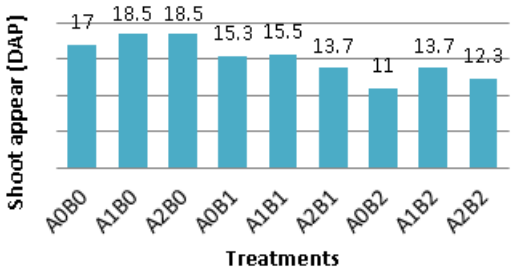

Figure 1. Histogram average days shoot appear of Tawangmangu tangerine orange after 21 days planting.

The best treatment of shoot appears contained in the treatment A0B2 (0 ppm IBA +2 ppm BAP) where have the value rate of shoot appear for 11 day after planting.Another thing that can be seen based one the above dated (Figure 1) is that at all treatment BAP 2 ppm has the average time of shots emerging fastest both one treatment A0B2 (1 ppm IBA + 0 ppm BAP), A1B2 (1 ppm IBA + 0.5 ppm BAP) and A2B2 (1 ppm IBA + 2 ppm BAP). It was shown shown that the BAP awarding in media with 2 ppm concentration can stimulate shoot formation fastest than with concentration under 2 ppm. The BAP with highest concentration allegedly capable to accelerate formation of the shoot on explant. The BAP has a role in cell division process where the cells on meristem tissueespecially apical and lateral shoot physiology activity which has an impact on the appearance of buds quickly. Santoso (2012), high level of BAP hormone exogenously on specifik level concentration, has changed the rate of concentration cytokines endogen so the ratio of cytokines auxin in the plant more increasing that cause faster shoot formation.

BAP (Benzyl Amino Purine) included in a plant growth promoting hormone group cytokines which gives effect topoliferation buds and axillary shoots formation (Soelaiman and Ernawati, 2013). Addition of cytokines at a certain consentration can encourage cells regeneration and tissue which work in cell division cycle, the higher consentration is given then regeneration potential of the cell will also increase (Hardarani, 2014). The availability of IBA in the media does not have a significant effect on shoot induction time, giving IBA is possible only to assist the performance of BAP in the elongation of stem cells. Dewi (2008), states that IBA is auxin groups that play a role in encouraging the elongation of buds that are developing.

Basically the addition of auxin and cytokines is very beneficial in explaining primarily in the explain that has a network is still young (meristematis). With the auxin and cytokines, it is possible to interact between auxin and cytokines given. But it all depends on the chemical conditions explant where many endogenous hormone content in the explant that certainly can inhibit the growth of explant itself. Karjadi and Buchory (2008), states that the growth of meristem tissue is influenced by the presence of auks in and cytokines in the media. In the absence of auxin or cytokines on either combination or individual media may be a less favorable factor for the explant. Thus giving exogenous hormone is a bit important for the growth of apical, lateral, or callus shoots on the explant. Biswas*et al.*, (2010), states that regardless of the hormone auksin indigenous cytokines, the presence of the hormones auxin and cytokines side by side can trigger the formation of callus or directly on organogensis process on tissue culture techniques. Auxin has a wide range of effects on plant growth and morphogenesis. A natural auxin of higher plants is involved in regulating cell elongation, cell division, and differentiation. Cytokinin can promote cell enlargement in certain tissues.

The results of research on the number of shoots amount the average data of each treatment as Figure 2.

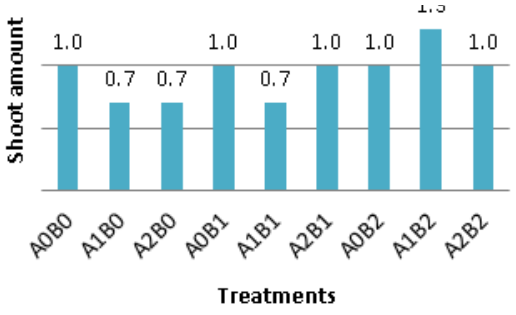

Figure 2. Histogram average amount of shoot Tawangmangu tangerine orange after 21 days planting.

Based on the Figure 2 that shows the treatment A1B2 (0.5 ppm IBA + 2 ppm BAP) is the treatment with the largest number of shoot. It shows that provision of IBA 0.5 ppm and BAP 2 ppm exsogenousin medium capable to induction the shoot on explant Tawangmangu tangerine orange optimally, so the number of shoots that formed more than the other treatments. with an average value growing at 1.3 times greater than other treatments. The smallest number of shoots occurred in treatment A1B0 A2B0 and A1B1 with the average shoot number of 0.7. Almost all treatments are capable of producing shoots, as for the overall percentage of the total number of explants bud is 85.18%.Bella *et al.*, (2016), mention that the ability of explants in improving the multiplication of shoot other than genotype influenced also influenced by the administration exogenous auxin and cytokinin concentration. The administration of plant growth promoting in both auxin and cytokinin is given only to a certain concentration level or to certain species only.

The giving BAP on the media tend to be able to affect the induction of shoots on planted explant, the higher the concentration of BAP that given the greater the number of explants that can sprout, it is shown in Figure 2 on the provision of BAP 2 ppm capable of producing the best buds. Manurung (2007), the percentage of shoot formation is increasing with increasing of BAP concentration. The absence of BAP on the media resulted in inhibition of shoot formation in explant, so that bud initiation time is also inhibited. Hardarani (2011), stated that BAP treatment on in vitro culture media has an effect on shoot time and explant the ability to induce bud formation.

The presence of cytokinin (BAP) is very important role in the process of shoot formation, from the average result of the outflow buds are affirmed that almost all treatments given cytokinin (BAP) have a significant growth buds compared with no cytokinin (BAP) (Figure 2). Syahid and Kristina (2007), based on preliminary trial results have shown that callus derived from the treatment of 2.4-D 1.0 mg / l + Kinetin 0.1 mg / l and the origin of 2.4-D 1.0 mg / l + Kinetin 0.3 mg / l was unable to provide a growth response on the control medium (without Benzyl Adenine / BA). Without giving cytokines (BA) into the regeneration medium, callus is unable to regenerate to form new shoots. Until the age of four weeks there is no formation of shoot nodules and eventually the callus turns brown and dies. While in all treatments with the administration of cytokine (BA), all callus is able to regenerate well form buds and leaf buds. It can be said here that the regeneration process requires a cytokines intake which in this case is obtained from BA at various concentrations.

The appearance of most shoots at A1B2 (0.5 ppm IBA + 2 ppm BAP) treatment was thought to be due to the role of the IBA and BAP, which worked optimally at the concentration of 0.5 ppm IBA and 2 ppm of BAP. Bella *et al.*, (2016), states that the ability explant in increasing the multiplication of buds in addition influenced by genotype also influenced by the giving of concentrations of cytokines and auxin exogenous. Djumat (2014) states that each genotype or tissue in vitro culture has a different response to the absorption of growth regulators in the medium and contains different growth regulators. Therefore, the administration of growth regulators is only required at a certain concentration level or on certain spesies only. Goerge *et al.*, (2008), cytokiness stimulate protein synthesis and play an active role in cell cycle control, the presence of cytokines with auxin spur cell division and regulate morphogenesis. This is indicated by the highest number of shoots found in A1B2 treatment (IBA 0.5 ppm + BAP 2 ppm) in the Figure 2.

Of all the planted explants showed a tendency to produce lateral shoots compared to apical shoots, this is caused by the selection of explant and the concentration of growth regulating agent given. Dewi (2008), states that auxin and cytokiness play a role in the regulation of apical dominance in which both play an antagonistic role. In this case, physiologically in an auxin plant, transported under the canopy of the terminal shoot directly inhibits the growth of axillary buds, on the other hand the cytokines that enters from the roots into the plant canopy system will counteract the auxin work by hinting the axillary buds that begin to grow. Based on the above, the concentration of IBA and BAP exogenously influences the growth of apical or lateral shoots. Harliana (2012), mentioned the emergence of direct lateral shoots or organogenesis in explant can be caused by the eye buds on the armpit of the leaves so it is more easily formed buds when sufficient concentration of cytokines hormone.

Shoots Length

Based on analysis of variance shows that the interaction of IBA and BAP has no significant effect on shoots length of Tawangmangu tangerine orange ($p > 0.05$). But on a single factor BAP has significant effect on shoots length Tawangmangu tangerine oranges($p < 0.05$).

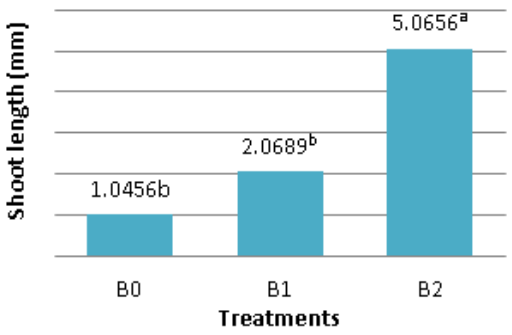

Figure 3. Histogram average shoot length of Tawangmangu tangerine orange on BAP addiction treatment after 21 days planted. Information: The numbers followed by the letters show that the difference is not really based on the DMRT (Duncan Multiple Range Test) at 95% confidence level. B0 (0 ppm BAP), B1 (0.5 ppm BAP), B2 (2 ppm BAP).

Further test results of DMRT on single factor of administration of BAP shows that treatment B2 (BAP 2 ppm) has the highest average shoot length with a value of 5.0656 mm compared with the treatment of B0 and B1. The data showed that the treatment of BAP 2 ppm (B2) was the best treatment for affecting the length of buds of Tawangmangu tangerine orange. The average value increase from B0 to B1 is 97.87% while the increase of B0 to B2 is 348.47%.

The presence of treatment of BAP on the media is very influential on physiological explant where with the existence of this BAP stimulates explant growth that triggers the growth of shoots on explant along with elongation of the shoot. The morphological appearance of the explants picture at the beginning and end of the observation are as Figure 4.

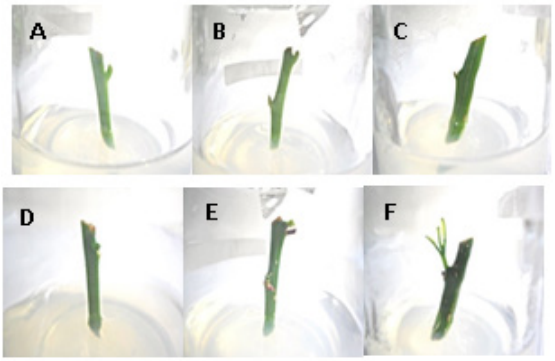

Figure 4. Morphology of Tawangmangu Tangerine orange buds on BAP administration factor after 21 days of planting.B0 (0 ppm BAP), B1 (0.5 ppm BAP), B2 (2 ppm BAP). Information:Figure A (B0), B (B1), and C (B2) are explant images at the beginning of planting. Figures D (B0), E (B1), and F (B2) are explanatory images at the end of the observation.

Cytokines in plants play an important role in the process of cell division that occurs in the cell cycle. In addition, there is the role of auxin although not significantly give a significant effect but with the auxin on the media can increase endogenous auxin on explant. (Purwanto, 2008). BAP plays an important role in the cycle of cell division that affects the performance of CDKs enzymes (Cyclin Dependent Kinase) in the end phase of G1 to S and phase G2 to phase M. The G1 is a phase where growth is an increase in the quantity of organelles and increased cytoplasmic volume. Once the G1 (GAP 1) phase is ready the cell enters the S phase (DNA synthesis) in which DNA synthesis results in DNA replication identical to the parent DNA. S phase is followed by the G2 phase (GAP 2) in which the cell begins preparing for further mitosis to the M phase (mitosis) which in this phase occurs nuclear division (chromosomal separation) and cytoplasmic separately. In addition, there is the role of the actin although it does not a significant effect, but with the presence of auxin in the medium may increase endogenous auxin in explant. This auxin plays a role in the proliferation of cells where auxin control the proton pump mechanism causing the cell cytoplasm to become more acidic, so that water easily enters the cells and the cells undergo magnification (Fatmawati, 2011).

In addition there is the role of auxin although not significantly give a significant effect but with the auxin on the media can increase endogenous auxin on explant. This auxin plays a role in the proliferation of cells in which the auxin controls the proton pump mechanism causing the cell cytoplasm to become more acidic so that water easily enters the cell and the cell undergoes an enlargement of size. Soelaiman and Ernawati (2013), the addition of BAP on the medium can increase the growth of plant height, the treatment of BAP (Benzyl Amino Purin) 2 mg/l and IAA (Indol Acetic Acid) 0 mg/l can increase the height of pepper curl (Capsicum annuum L) in the first week.

Cytokines (BAP) is involved in various growth processes and the growth of shoots, at the cellular level controlling many gene expression and secondary metabolite synthesis that triggers increased cell division, while auxins play a role in cell enlargement in triggering proton pumps to increase the amount of H^+ into the cell so that the cell cytoplasm becomes more acid that causes the loosening of polysaccharide bonds on the cell

wall. As a result water easily to osmosis into the cell and cause the cell to experience enlargement. This is what causes the extension of stem cells that grow (Kieber, 2002; Fatmawati, 2011). Dewi (2008), cytokines with auxin is able to stimulate cell division and affect the path of differentiation. When a network of stem parenchyma is cultured without the use of cytokines, it grows large but does not divide, but when cytokine is added with auxin it will be able to divide.

Growth of shoots on explant can be influenced by the presence of endogenous hormones, so giving the concentration of cytokines becomes an important factor in shoot formation. George *et al.*, (2008), the presence of endogenous and exogenous hormonal interactions must be balanced in this regard by administration of too high concentrations may inhibit the explant the growth process itself. Ngomou*et al.*, (2013), the low growth of explant form buds may be possible because the exploit is highly dependent on the endogenous growth regulator itself. The genetic characteristics of explant also affect the growth response, some explants that are used genetically may have slow growth, but there are also explants that have rapid growth this becomes one of the factors that affect the length of shoots produced (Samanhudi, 2007).

The formation of shoots and growth cannot be separated from the role of auxin and cytokinins that exist within the explant or added to the media. At a certain concentration level the presence of a high cytokine can spur the growth of shoots in the explant and inhibit the formation of roots and callus formation. Fitri*et al.*, (2012) suggests that administration of auxin and cytokines is performed simultaneously with comparable concentrations capable of spurring callus formation in explant. This applies to a nearly comparable concentration range, but in each species the plant has its own concentration range. Whereas at concentrations more or less able to cause the formation of axillary buds and inhibit adventitious shoots or trigger the formation of adventif shoots and inhibit shoots axiliary on explants grown on the treatment medium.

Wet Weight

Wet weight measurement is done by weighing the initial and final explant wet weight of the study. The data used is the difference between the final weight and the beginning of research. The result of variance analysis showed that IBA and BAP interaction did not have an effect on the wet weight of explant ($P > 0.05$), but in single factor giving significant effect to wet explant weight of Tawangmangu tangerine oranges ($P < 0.05$). As for the results of the analysis of data DMRT a further test is as Figure 5.

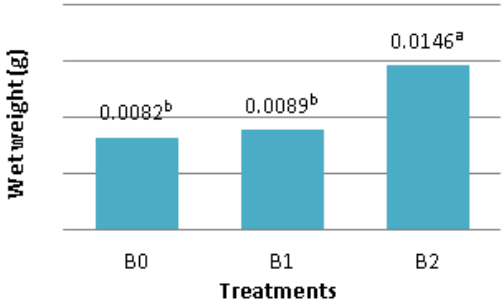

Figure 5. Histogram average wet weight of Tawangmangu Tangerine orange in factor of administration of BAP after 21 days planting. Information: The numbers followed by the letters show that the difference is not really based on the DMRT at 95% confidence level. B0 (0 ppm BAP), B1 (0.5 ppm BAP), B2 (2 ppm BAP)

The giving BAP in the media significantly affect the value of wet weight of Tawangmangu orange explants. B2 treatment (BAP 2 ppm) has the greatest average value compared to B0 and B1. Widiastoety (2014), mentioned that the provision of BAP on the media is able to stimulate cell division and able to induce the growth of buds in apical meristem and stem tissue. This has an impact on the occurrence of increased physiological activity resulting in increased mass and cell size that indirectly increase the weight of wetexplant. Tilaar *et al.*, (2015), administration of auxin in this case is NAA (Naphtalene Acetic Acid) alone on the media may inhibit the weight of explant chrysanthemum nodules because the auxin performance has a negative effect on shoot growth but a positive effect on root growth, the higher the NAA concentrations singly, the smaller the shoot weight.

The presence of auxin with cytokines stimulates cell division in meristem tissue where the presence of cytokines can control the turn of meristematic cells into de-differentiated cells or direct control of organogenesis (Moubayidin*et al.*, 2009). Thus, it is suspected that explant growth has an effect on wet weight with increased physiological activity caused by the presence of auxin (IBA) hormone which play a role in cell enlargement through proton pump mechanism and the presence of cytokines (BAP) which play a role in cell division (Fatmawati*et al.*, 2011). The plant by itself regulates the level of auxin and cytokinin requirements through the synthesis and conjugation of the growth regulator. The type and con-

centration of auxin and cytokinin or other growth regulators added to the culture medium are also important factors in influencing the synthesis of secondary metabolite materials, which in optimal conditions can increase the number, mass and volume of the cell resulting in the addition of wet weight to the explant (Siregar, 2010).

Sekhawat *et al.*, (2007), growth can be measured by the volume or weight associated with physiology. To find out the influence of IBA and BAP on each of the treatments can be seen from the average value of wet weight in each treatment. The average wet weightt of each treatment as follows:

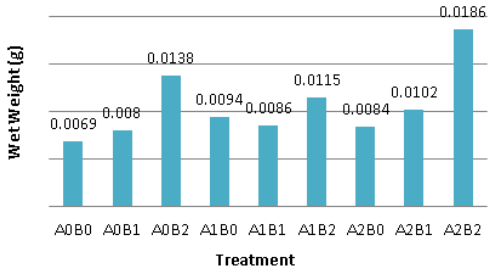

Figure 6. Histogram average wet weight of Tawangmangu tangerine orange in factor of administration of BAP and IBA after 21 days planting.

Provision of IBA and BAP basically play a role in the elongation and cell division that will affect the wet weight explant whether experiencing growth or not seen from the increase of explant weight. Samanhudi (2007), the wet weight of explants shows how cells of explant cells respond to treatment given to the media, so that the cell cell will divide until a certain time to adjust the treatment given. The best value of wet weight was found in the treatment of A2B2 (IBA 1 ppm + BAP 2 ppm) (Figure 6). This shows the tendency of wet weight influenced by IBA and BAP in the media, although the interaction of both did not significantly influence.

The highest wet weight is shown in the treatment of A2B2 (IBA 1 ppm + BAP 2 ppm) in Figure 6. It certainly proves that the administration of IBA and BAP affects the cellular activity in explant where there is a process of cell division and elongation which causes increased production of secondary metabolites and increased cell volume as a result of the physiological activity. Sekhawat *et al.*, (2007), growth can be measured by the volume or weight associated with the occurrence of physiological activity such as increased cell size, cell count and biochemical content. The provision of IBA and BAP affects cellular activity in explants where there is a process of cell division and elongation involving increased production of secondary metabolites and increased cell volume due to physiological activity. Saputro *et al.*, (2016), reported that every species of plant responded differently to the administration of growth regulators in the medium. In maize will affect shoot formation at concentration 4 ppm by auxin (2-4 D). Another case with species or other varieties have an appropriate concentration range in order to grow to form bud or callus.

BAP enhances regeneration by stimulating cell division within the tissues, the performance of this BAP through the cell division cycle by controlling the enzyme activity of CDKs (Cyclin Dependent Kinase) at the end of S, M and G phases (Hardarani, 2011). Auxins affect the elongation of cells in tissues by regulating the pumping of protons of plasma membranes, auxin also alters the rapid expression of genes that alter cells in the prolonged region, producing new proteins in which this protein is a transcription factor that can suppress or activate other gene expression (Dewi, 2008). From this whole process can increase cell metabolism, which affects the weight of explain.

Some factors that cause no effect of IBA and BAP interaction significantly on the growth of apical and lateral buds of tangerine Tawangmangu suspected to originate from the selection explant with unequal physical conditions. The origins of explant taking and the age of explant also have an influence on the growth process in the media. A good explant of course has a young age where there is a meristem network that has the ability to split and form a new network, it is related to the ability of cell totipotency in plants. Akin-idowu *et al.*, (2009), mentioned in several species, explants of various organs showed different growth and regeneration responses to the same treatment. Aryati (2015), each part of the plant has a different ability to regenerate in response to a variable, this is related to the physiological conditions both the type of explant itself and the age of explants used. Other than that many factors such as genotype,composition of the nutrient medium, and physical growth factors such as light, temperature, humidity,and endogenous supply of growth regulators are important for growth and development of explants.

CONCLUSION

Administration of IBA and BAP on the growth of apical and lateral buds of Tawangmangu tangerine did not significantly affect IBA and BAP interaction factors, but there was

a significant effect on the single factor of BAP administration on shoot length and wet weight. The best growth results on the growth of apical and lateral buds of Tawangmangu tangerine were found in A2B2 treatment (1 ppm IBA + 2 ppm BAP), the fastest shoots appeared in treatment A0B2 (IBA 0 ppm + BAP 2 ppm) with average shoot occurrence of 11 HST and buds Mostly in the treatment of A1B2 (IBA 0.5 ppm + BAP 2 ppm) with an average of 1.3 buds.

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