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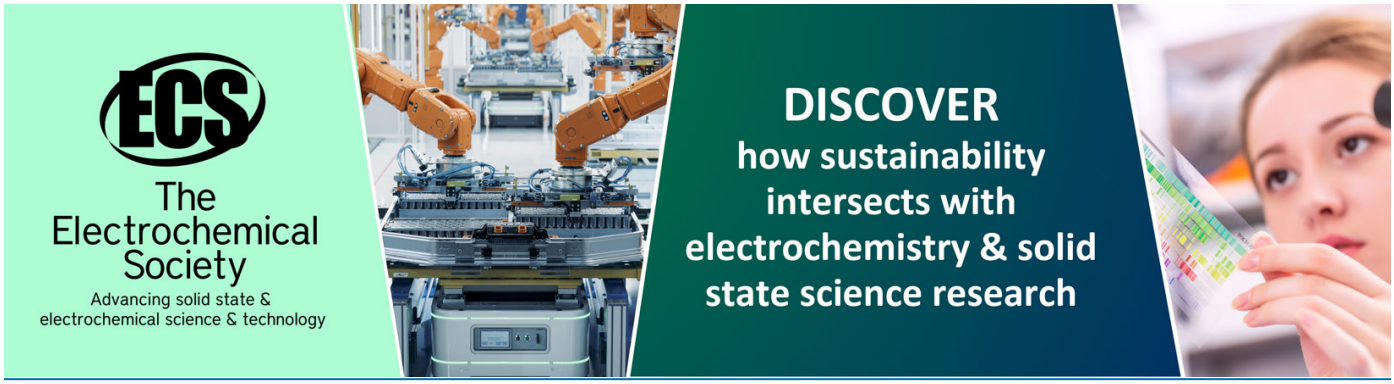

Shoots and Roots Induction of Garlic on Different Composition of Plant Growth Regulators and Photoperiod

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Abstract. Plant Growth Regulators and photoperiods will affect callus morphogenesis from garlic bulbils. The study aimed to obtain the best interaction between media composition and photoperiod for shoots and root induction of the garlic callus. The research hastwo-stage. The first stage consisted of two factors. The first factor is the combination of PGR forshoots induction which consists of three combinations, i.e., 0.1 ppm NAA + 1 ppm BAP, 2 ppmNAA + 2 ppm BAP, and 0.4 ppm 2,4-D + 2 ppm kinetin. The second factor is photoperiod whichconsists of 10, 16, and 22-h light conditions. The second stage is the root induction in different PGRs (2 ppm IBA and 0.2 ppm NAA + 2.25 ppm BA) under continuous dark conditions. The results of the first stage showed that the PGR produced the most shoots was 0.1 ppm NAA + 1 ppm BAP. After four weeks, the frequency of an explant emerging during the shoot reached 64%on media 0.1 ppm NAA + 1 ppm BAP. The treatment MS media supplemented with 0.1 ppm NAA + 1 ppm BAP at 16-h photoperiods increased the growth of shoots per explant in 11 -15 shoots and produced the most extended shoot length. The result of the second stage showed thatthe number of roots on 2 ppm IBA is the same as NAA 0,2 ppm + BA 2,25 ppm, but the treatment2 ppm IBA has the highest root number in three months.

1. Introduction

Garlic (*Allium sativum* L.) is one of the agricultural commodities needed by people in Indonesia. This plant is used as a spice and herbal medicine. As a spice, garlic is widely used as a flavouring for various foods. Clinically, garlic has been evaluated for its benefits in various ways, including as a treatment for hypertension, hypercholesterolemia, diabetes, rheumatoid arthritis, and fever or as a preventive drug for atherosclerosis and an inhibitor of tumour growth. Garlic has pharmacological potential as an antibacterial, antihypertensive, and antithrombotic agent [1].

In 2019, garlic production was only 88.82 thousand tons, while garlic consumption reached 560.69 thousand tons. Because production is insufficient, 90% of the national garlic needs come from importing around 472.92 thousand tons in 2019 [2]. Therefore, it is necessary to increase theproduction of garlic. One of the efforts to increase garlic production is expanding the area for plantinggarlic. It needs the availability of seeds in large quantities.

In Indonesia, most garlic cannot produce seeds, so the propagation of this plant is done vegetatively using bulbs. The bulbs used are generally tubers from the previous harvest. Conventional vegetative propagation is ineffective because one clove can only develop or produce one plant [3]. In addition, garlic propagation has a long dormancy period, which is 5-6 months after harvest, so it takes a long time to produce propagules (seed bulb). This results in one-year planting only can be cultivated once or twice

depending on the garlic used. Alternative use of other planting materials, such as seeds, is tough to obtain because garlic cannot flower naturally, which causes little genetic variation.

Another problem is that many of the elite garlic cultivars are often infected by viruses; therefore, invitro regeneration by shoot or root meristem is the only option to obtain virus-free garlic plants. The advantage of the in vitro plant propagation system is that it can produce large quantities of plant seeds quickly, is free from pests and diseases (systemic and non-systemic), is the same as the parent, and does not depend on the season. Developing microseed tubers from tissue culture from flower buds is a big challenge and market opportunity for farmers and investors in developing garlic cultivation.

The growth medium formulation is a significant factor in influencing shoot and root induction. Murashige and Skoog (MS) medium is the most commonly used medium in tissue culture and has a complete nutrient composition compared to other mediums [4]. According to Warnita [5], the medium is widely used for potato tissue culture to form micro shoots and potato tubers. In addition to thegrowth regulator medium (PGR), it also affects the growth of cultured anthers. Auxin is one PGR thatplays a role in callus formation, cell suspension, and root growth. Other types of PGR that also play anessential role are cytokinin, which affects cell division, shoots' proliferation, root growth inhibition, and tuber induction [6]. The interaction of the two PGRs simultaneously on a specific medium can affect the growth and morphogenesis of plant tissue [4].

Research on the in vitro propagation of garlic has been widely published. The formation of shootsin garlic culture is strongly influenced by the concentration and composition of the medium's Plant Growth Regulator (PGR). Wiendi *et al*. [7] obtained the best media for the induction of adventitious shoots of garlic cv. Lumbu Putih was treated with 2.0 ppm Kinetin and 0.4 ppm 2,4-D media. Suh and Park [8] performed the culture of garlic bulb explants from Fusion and Nando cultivarson MS media with 2.0 ppm NAA and 2.0 ppm BAP added to produce the highest shoot number. Fauziah and Widoretno [9] achieved the highest number of shoots on 0.1 ppm NAA and2,0 ppm BAP. When the shoots are transferred to a culture medium with the addition of 10 ppm BAP, the shoots will be elongated. Differences in the variety and origin of explants will give different responses to cultural explants and growing environmental conditions. Therefore, we must choose the medium to shoot multiplication and root formation of garlic from callus bulbil.

Other factors affect the growth and proliferation of plant tissue in liquid culture, such as light conditions [10]. The duration of irradiation (Photoperiod) in plant tissue culture reflects the periodicity needs of the plant concerned in the field. Photoperiod mechanisms include the detectionof the light signal in the leaves, the entrainment of circadian rhythms, and the production of a mobile signal which is transmitted throughout the plant White light from a fluorescent lamp with an intensityof 3000 – 10,000 lux can induce rooting and plantlet preparation before acclimatization for tissue culture needs [11]. The light and dark periods are generally set in the range of 8-16 hours of light and 16-8 hours of darkness, depending on the variety of explants being cultured. Wattimena *et al*. (1992) suggested that the duration of irradiation was 16 hours per day [4]. Dinarti [12] gave a 24-hour irradiation time at 2500 lumens with a room temperature of 22ºC capable of inducing shoots from tuber explants stored for four months, as many as 3.8 shoots at six weeks after planting. Kim *et al.* [13] found 29 bulblets were developed from each explant cultured under dark, whereas 21 bulblets developed in the explants cultured under 16-h light/8-h dark conditions [13]. Bulblet weight was significantly increased in dark-treated cultures, and a 10-fold increase in bulblet weight was also observed. Research related to photoperiod and the composition of medium for shoot multiplication androot induction needs to be carried out as an alternative to supplying garlic seeds of the Sangga Sembalun variety from in vitro.

2. Material and Methods

2.1. Material

Callus from Garlic CV. Sangga Sembalun was regenerated from the meristem of bulbil. Using medium Murashige and Skoog Media (MS), sucrose, BAP, Kinetin, NAA, 2,4-D, NaOH, HCl, NaClO,aqua dest, alcohol, 70% and 96%, agar plant TC. The equipment includes lamps (fluorescent lamps on 2000 lux) and lighting time regulators.

2.2. Methods

The research has two-stage. The purpose of the first stage is shoot induction. The first stage consisted of two factors. The first factor is combination of PGR on MS medium for shoots induction, consisting of three combination levels, i.e., 0.1 ppm NAA + 1 ppm BAP (A1), 2 ppm NAA + 2 ppm BAP (A2), and 0.4 ppm 2,4-D + 2 ppm kinetin (A3). Each treatment combination was repeated four times so there were 36 experimental units. Groupings are based on different planting days. The second factor is photoperiod, consisting of three light conditions (10 hours, 16 hours, and 22 hours). The second stage is the root induction of shootlet from the first stage in different PGRs (2 ppm IBA and 0.2 ppm NAA + 2.25 ppm BA) under continuous dark conditions. Observational data obtained were processed using graphs and bar charts.

The lighting source used in this experiment is LED. LED lamps used have a power of 15 watts, an aluminum body as a cooler (heat sink), and length of the lamp 30 cm and wide is 5 cm. LED lights are safer if exposed directly to explants. LED lights with different colors and wavelengths, namely red LED lights with a wavelength of 660 nm, blue LED lights with a wavelength of 430-460 nm and whiteLED lights.

3. Result and Discussion

3.1. Ability Forming the Shoots

Shoots proliferation were observed in all medium composition (MS medium + 0.1 ppm NAA + 1 ppm BAP, MS + 2 ppm NAA + 2 ppm BAP and MS + 0.4 ppm 2,4-D + 2 ppm kinetin) and three conditions of photoperiod (10, 16 and 22-h light condition).

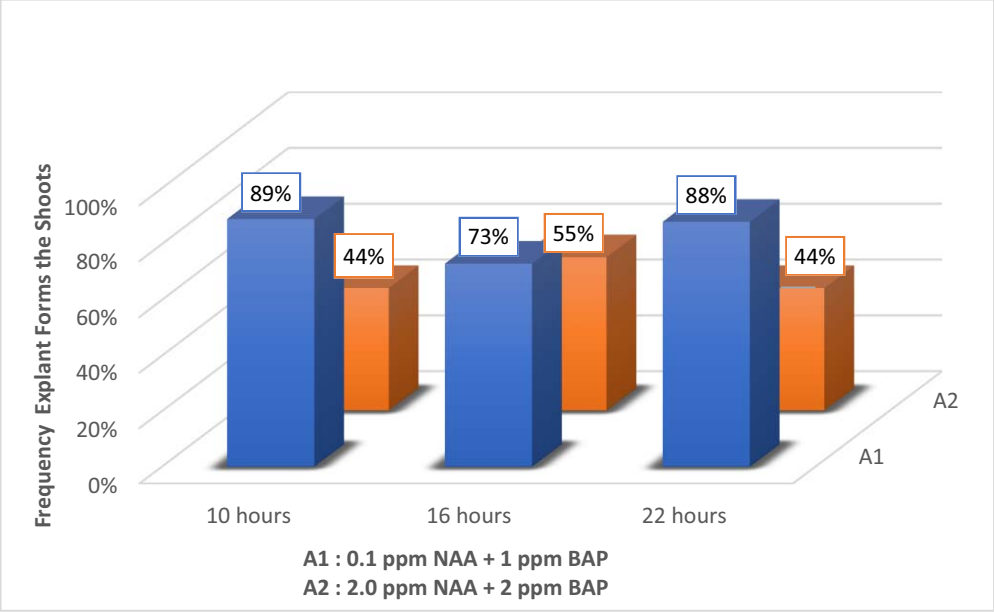

Figure 1. Frequency explant forms the shoots after 8 weeks of culture

Figure 1. shows that the media produced the higher shoots was MS medium supplemented with 0.1 ppmNAA + 1 ppm BAP. Otherwise, MS medium + 0.4 ppm 2, 4-D + 2 ppm kinetin does not produce any single shoots after 8 weeks. That indicates that if the concentration of BAP, which is a cytokinin is higher than NAA, which is an auxin type, the development of plant organs will lead to budding. When the media used contains auxin and cytokinin with a higher concentration, but the ratio of auxin to cytokinin is the same (A2 medium), it causes the ability of explants to produce shoots tends to decrease. Watimena stated that the ratio of cytokinin and auxins influenced shoots and root morphogenesis [4]. Ahigh cytokinin-auxin ratio will encourage shoot formation. According to Fereol *et al*., auxin inhibits shoot growth, while combining high cytokinin concentrations with low auxin is essential in forming shoots and leaves [14]. In tissue culture, these two PGR groups have been shown to support tissue growth when used at the right concentration. In vitro culture, most plants require cytokinin for shoots and leaf formation, whereas auxins are inhibitory [15].

MS medium + 0.4 ppm 2,4-D + 2 ppm kinetin could not form the shoots because 2,4-D is a strong auxin while kinetin (cytokinin groups) is low adequate. 2,4-D as an auxin dominates cell activity,and cells are more encouraged to form callus than shoots. 2,4-D is generally used for callus induction compared to shoot induction; 2,4-D is also an auxin group that can inhibit plant growth in high concentrations [4]. Ramasami [16] stated that BAP has the same structure as kinetin but is more effective than kinetin because it has a benzyl group. Generally, plants respond better to BAP than kinetin, so BAP is more effective for producing shoots in vitro.

3.2. Speed of Shoots Emerging

The speed of emergence of the shoots can be judged by how long the callus explant can produce the highest number of shoots. Of course, the best explant performance is in a short time. This speed is important in tissue culture because associated with the time needed to produce seed tubers. The faster forming the shoots, it means that the time to produce tubers will a short time. The longer the in vitro phase required, the greater the possibility that both explants and media will be exposed to contaminants.

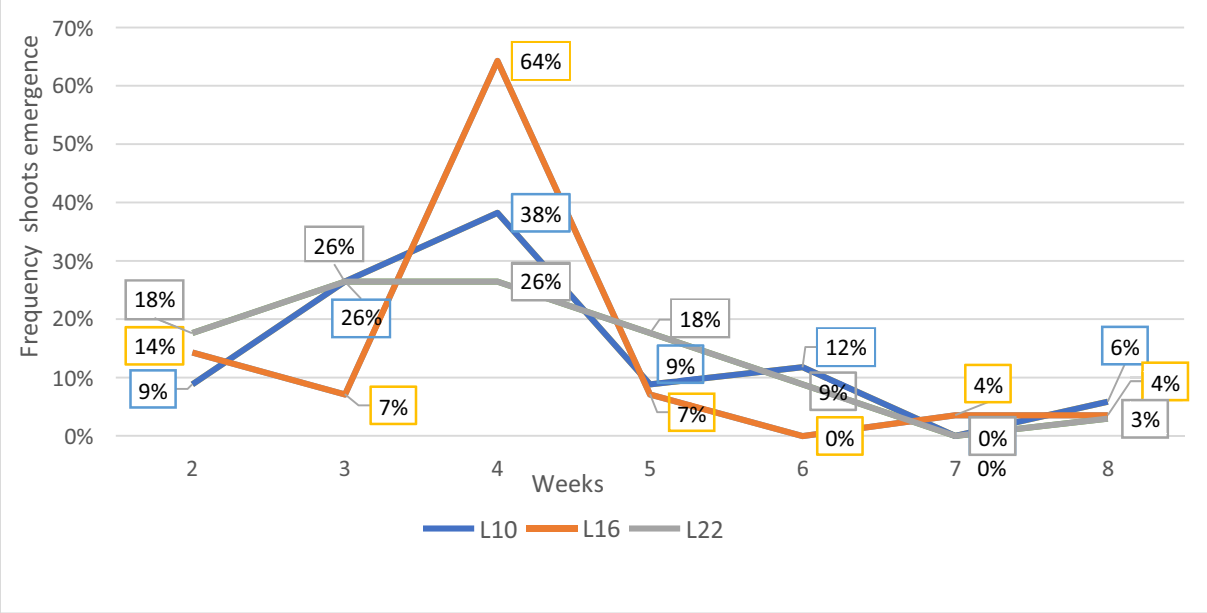

Figure 2. Frequency explant that is emerging the shoots from 2- 8 weeks on medium MS supplemented with 0.1 ppm NAA + 1 ppm BAP (A1) with different photoperiod

Figure 2. shows that the rate of shoot emergence each week is different. In the second week of culture, the emergence of shoots was still lo

w in each treatment. After that time, we found that 64% of explant forms the shoots in the fourth week on 16-hours of light, while at 10 and 22 hours of light treatments only formed shoots of about 38% and 26%, respectively. In the following weeks, shoots appear less and less. It means that treating MS medium supplemented with 0.1 ppm NAA + 1 ppm BAPon 16-h light brings callus differentiation into shoots more quickly and drastically.

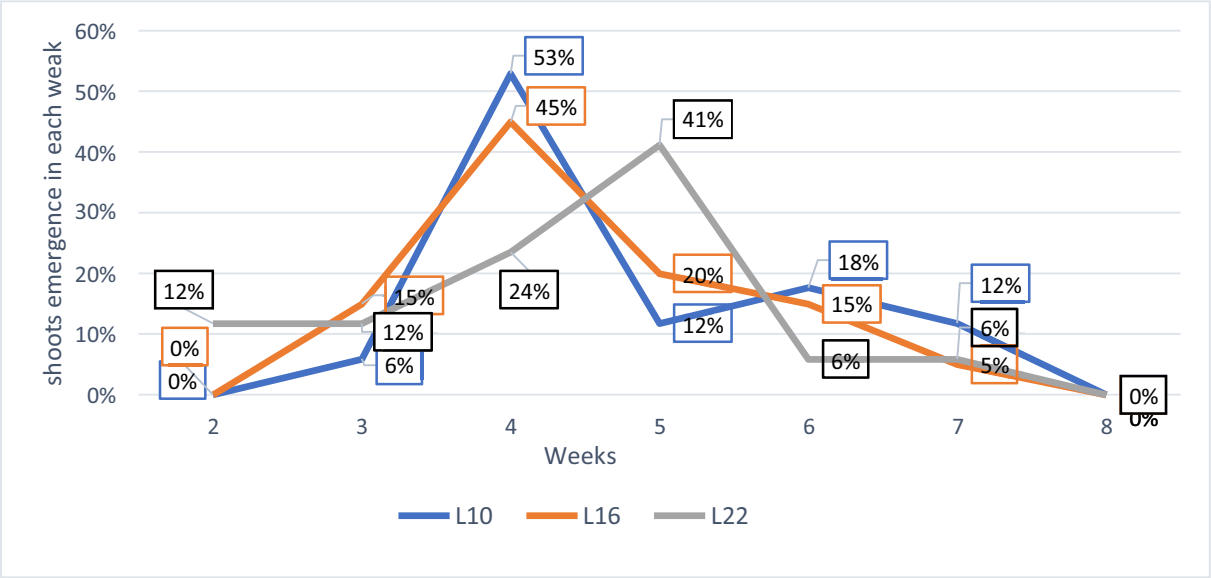

Figure 3. Frequency explant that is emerging the shoots from 2- 8 weeks on medium MS supplemented with 2 ppm NAA + 2 ppm BAP with different photoperiod

Figure 3. shows the pattern of shoot emergence in the treatment of MS media supplemented with 2 ppm NAA + 2 ppm BAP almost the same as the treatment of MS media + 0.1 ppm NAA + 1 ppm BAP (Figure 2.). That shows the highest shoot emergence in the fourth week. The shoots at 10-h did not differ much from the 16-h photoperiod treatment, respectively, 53% and 45%. Meanwhile, at 22-h light, it produced more shoots in the fifth week (41%). Figure 2 and Figure 3 indicate differencesin plant response to photoperiod that influence the pattern of shoot formation due to different media compositions. Photoperiod in plant tissue culture reflects the periodicity needs of the plant concerned in the field. The quality of light affects the direction of tissue differentiation [11]. Wattimena [4] suggested that irradiation was 16 hours per day. However, Dinarti [12] gave a 24-h light at 2500 lumens with a room temperature of 22ºC, which induced 3.8 shoots in six weeks from the callus of the shallot that had been stored for four months. Kim *et al.* [13] found that light intensity and temperature affected shoot proliferation and bulblet formation. The best shoots proliferation occurred on an intensity of light 50 µmol m^{-2} s^{-1} and a temperature 25ºC.

3.3. The frequency of the number of shoots per explant in the eighth week

The number of shoots is affected by the media type and the irradiation length. Axis X in Figure 4 shows that the number of shoots produced from each callus explant in all treatment combinations wasvaried. Media MS supplemented with 0.1 ppm NAA + 1 ppm BAP on 10-h photoperiod produces shootsranging from 1 to 25. That media on 16-h photoperiod produces 1 to 28 shoots, and 22-h can produce 1 to 25 shoots per explant.

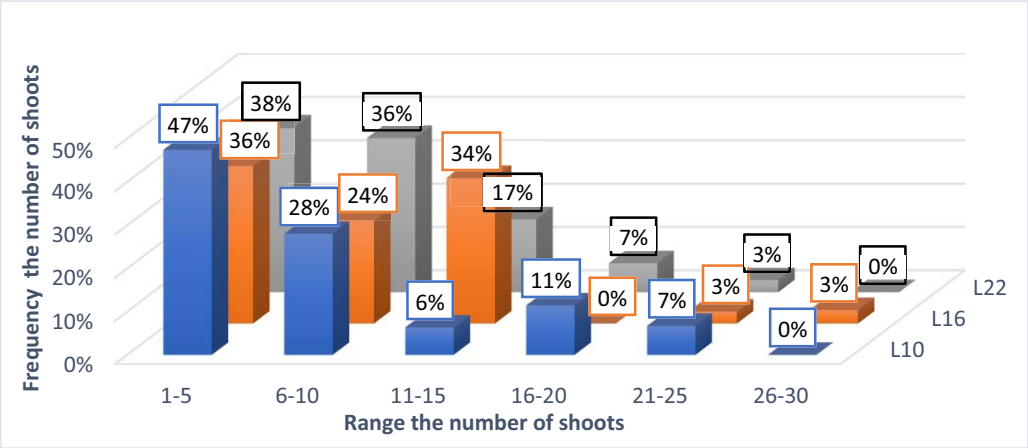

Figure 4. Percentage of the number of explant forms shoots in a specific range from the treatment of MS media supplemented with 0.1 ppm NAA + 1 ppm BAP at various photoperiods in the eighth week

Figure 4. shows that on medium 0.1 ppm NAA + 1 ppm BAP at 10-h of light condition, about 47% of explants were able to grow 1-5 shoots, about 28% of explants were able to produce 6-10 shoots, and explants can produce 11-15 shoot only 6%. While at 16-h photoperiod, the explants that produced 11 - 15shoots were around 34%. It means that the treatment of MS media + 0.1 ppm NAA + 1 ppm BAP at 16-h of irradiation increased the growth of shoots per explant. In treatment medium + 0.1 ppm NAA + 1 ppm BAP and 22-h light condition, the explant that produced shoots in the range 11-15 was less than treatment 16 hours irradiation but higher than treatment 10 hours irradiation. BAP is one of the cytokinin groups which are more stable, cheaper, and more effective than kinetin. BAP encourages callus growth and, at the same time, stimulates the emergence of shoots from the formed callus [17]. Dinarti [12] found the least number of shoots formed because cytokinin was not added to the shallot micro tuber media (MS + vitamin B5 + 150 g L -1 sucrose.

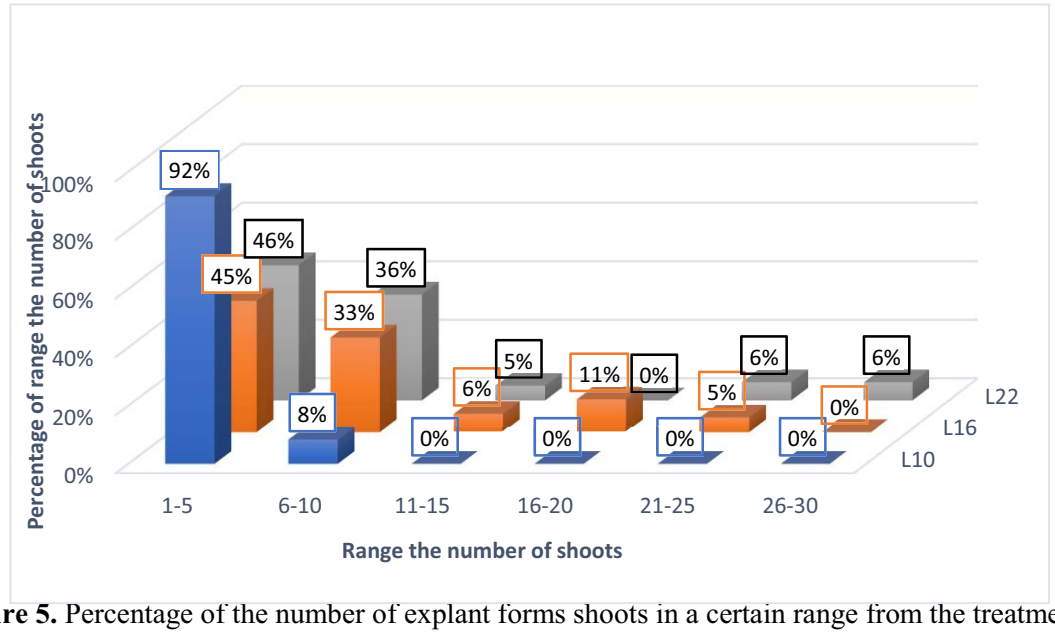

Figure 5. Percentage of the number of explant forms shoots in a certain range from the treatment of MS medium supplemented with 2 ppm NAA + 2 ppm BAP at various photoperiod in the eighth week

Axis X on Figure 5. shows that the number of shoots produced from each callus explant in medium MS supplemented with 2 ppm NAA + 2 ppm BAP on all photoperiod treatments was varied. That medium on 10-h photoperiods produces shoots ranging from 1 to 10 shoots. On 16-h photoperiods, produce 1 to 25 shoots. On 22-h can range from 1 to 30 shoots. It means that long light conditions stimulate higher shoots per explant than a short day. Figure 5. also shows that more explant on 10-h photoperiod and medium MS supplemented with 2 ppm NAA + 2 ppm BAP treatment only could grow 1-5 shoots (92%). At the same media on 16-h and 22-h light conditions, the number of shoots in the range 1-5 was only 45% and 46%, respectively, but the number of shoots per explant in range 6-10 was increased than others by around 33% and 36% respectively. All treatment shows that the explant with 11-15 shoots only 0 – 6%.

Figure 4 and Figure 5 illustrate how the ability of each medium containing different PGRcompounds responds differently at different photoperiods. Medium MS supplemented with 0.1 ppm NAA + 1 ppm BAP on 16-h photoperiods treatment encouraged the formation of shoots in the range of11 -15 fruit which reached 33.68% after eight weeks of culture. However, in medium 2 ppm NAA + 2 ppm BAP on 16-h photoperiods, the percentage of explants capable of producing shoots in the range of11-15 was not up to 10%. This phenomenon shows best irradiation time to increase shoot growth is 16-h photoperiod, and the medium must be MS media supplemented with 0.1 ppm NAA + 1 ppm BAP. Giving irradiation for 10 hours tends to reduce the ability of explants to grow more shoots per explant. According to Wattimena [4], the light and dark period are generally set in the range of 8-16 hours of light and 16-8 hours of darkness, depending on the variety of explants cultured recommended giving 16hours of irradiation per day. According to Badriah *et al*. [18], cytokinin affect shoots initiation. The typeof cytokinin most often used is 6-Benzyl Amino Purine (BAP) because of its high effectiveness [11].

3.4. Shoots length after 4 weeks (cm)

Shoots induction was observed in all types of medium composition (MS medium supplemented with 0.1 ppm NAA + 1 ppm BAP, MS + 2 ppm NAA + 2 ppm BAP and MS + 0.4 ppm 2,4-D + 2 ppm kinetin) and three types of photoperiods (10, 16 and 22-h light condition).

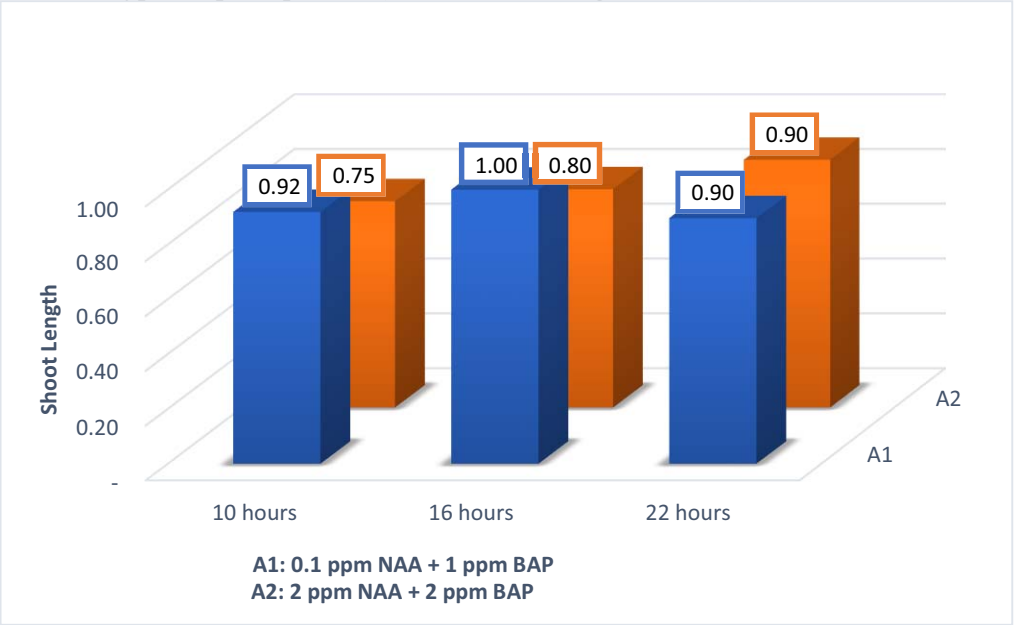

Figure 6. Shoots length after 4 weeks

Figure 6. shows that if the concentration of cytokinin is higher than auxin in media so the shoot tends to be longer than a medium with the same auxin and cytokinin concentration. MS medium supplemented with 0.1 ppm NAA + 1 ppm BAP with 16-h of irradiation produces more extended shoot length than the other treatments. Low auxin concentrations will stimulate cell enlargement and

elongation after cell division stimulated by cytokinin. However, if the concentration ofauxin used is too high, as in medium 2 ppm NAA + 2 ppm BAP, it will cause inhibition of cell elongation. The higher concentration of auxin, the higher the ethylene concentration produced, inhibiting auxin activity in cell elongation but increasing cell dilation [19]. Cytokinin stimulates cell division but inhibits lengthwise stem growth. In this treatment, this happens on shoots treated with medium 2 ppm NAA + 2 ppm BAP. Cytokinin/BAP in that medium inhibits the cell elongation process by auxin/NAA [20].

3.5. Percentage of the number of roots in dark condition.

Figure 7. shows that percentage of the number of roots on the treatment MS media + 2 ppm IBA is the same as NAA 0.2 ppm + BA 2.25 ppm, around 90 – 91%. Both media contains auxin (IBA and NAA). Auxins are compounds that positively influence cell enlargement, bud formation and root initiation. They also promote the production of other hormones and in conjunction with cytokinin, theycontrol the growth of stems, roots, and fruits, and convert stems into flowers [21].

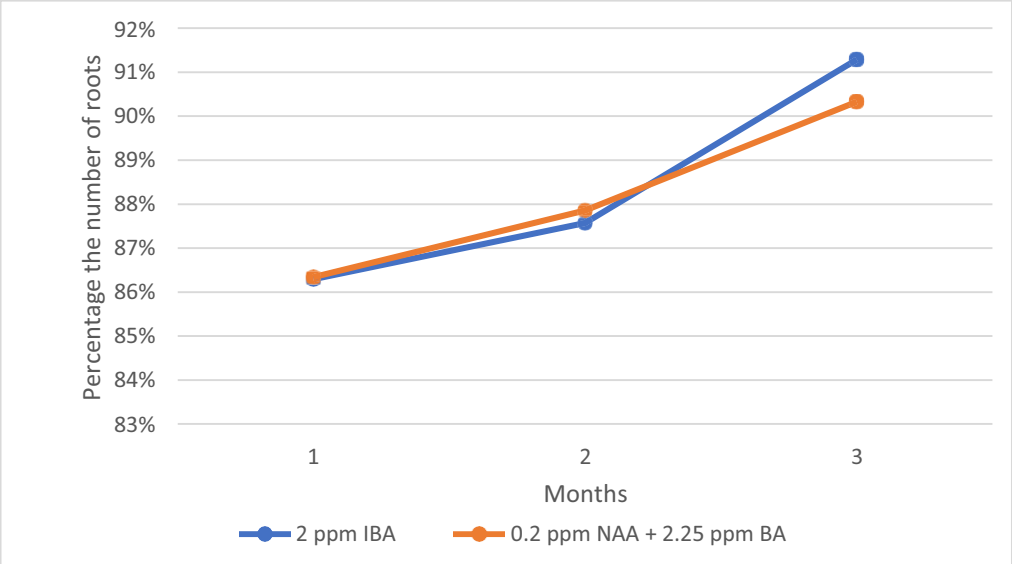

Figure 7. Percentage of the number of explant forms roots from the treatment of MS media supplemented with 2 ppm IBA and NAA 0.2 ppm + BA 2.25 ppm in dark conditions until three months

By changing the flexibility of the cell wall, auxin affects cell elongation. They encourage the division of cambium, a subtype of meristem cells, and the differentiation of secondary xylem in stems. Auxins stimulate lateral root formation and growth and restrict the growth of buds lower down the stems (apical dominance). When taking plant cuttings, auxins, particularly 1- Naphthaleneacetic acid (NAA) and Indole- 3-butyric acid (IBA), are frequently used to promote root growth. Phototropism, geotropism, the growth of downward-growing roots in response to gravity, flower production, fruit set, and growth are all biological bending. NAA and IBA are frequently used as active ingredients in treatments that promote root development in plant cuttings. Auxins generate a proton outflow out of the cell, enhance plasma current, and make the cell wall more flexible. Figure 7 demonstrates how auxins affect the membrane's ion pumps, regulate certain enzymes' activity, and speed up transcription [22].

3.6. The number of roots on three months

The type of growth regulator strongly influences the number of roots. The treatment of MS media + 2 ppm IBA has a number of roots higher than NAA 0.2 ppm + BA 2.25 ppm, the higher concentration of auxin (2 ppm IBA) stimulates root formation. Otherwise, the combination between low auxin and high cytokinin concentration (NAA 0.2 ppm + BA 2.25 ppm) cannot stimulate root formation in three months.

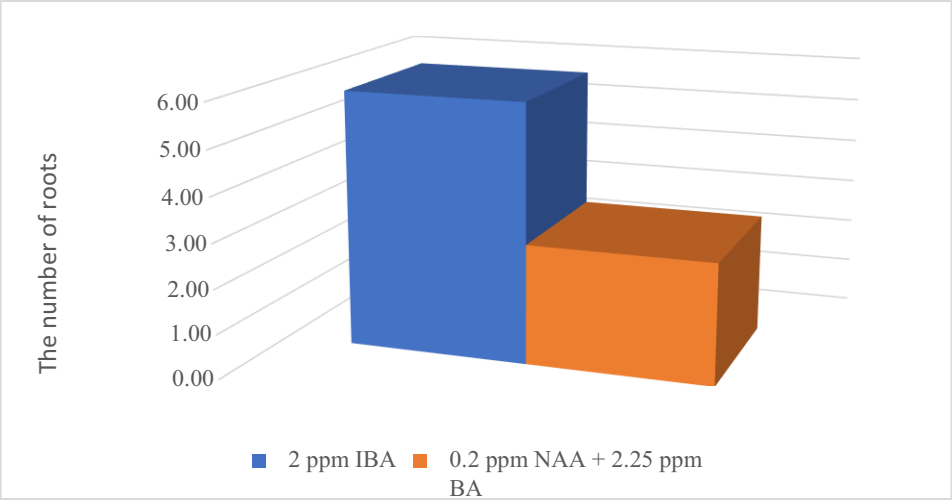

Figure 8. The number of roots from the treatment of MS media + 2 ppm IBA and NAA 0.2 ppm + BA 2.25 ppm in dark conditions in three months

Auxins are explicitly transported through the membrane. In vitro, auxin affects the release of calcium ions from the vacuole and selectively binds tonoplasts. A proton carrier (S) that produces symport and an auxin anion carrier (AC) that is an active antiport carrier have both been identified as different import and export carriers. Asymmetrical distribution of these auxin carriers within the membrane has also been shown to exist. [22].

4. Conclusion

In conclusion, the in vitro multiplication of garlic and root induction need special conditions. The results of the first stage of the study showed that the media that produced the most shoots was MS + 0.1ppm NAA + 1 ppm BAP media. MS medium supplemented with 0.4 ppm 2,4-D + 2 ppm kinetin failedto induction the shoots. After four weeks, the highest number of explants that formed the shoots appeared at 16-h photoperiod, reaching 64% explant on 0.1 ppm NAA + 1 ppm BAP and all of the photoperiod. The treatment of MS media supplemented with 0.1 ppm NAA + 1 ppm BAP at 16-h photoperiods increased the growth of shoots per explant in the range of 11 -15 shoots, reaching about 34%. Medium MS + 0.1 ppm NAA + 1 ppm BAP with 16-h photoperiods produced the most extendedshoot length. From the above results, it can be concluded that the best media for shoot induction fromthe callus of garlic bulbil was MS medium supplemented with 0.1 ppm NAA + 1 ppm BAP at 16-h photoperiod. The result of the second stage showed that the percentage of the number of roots on the treatment 2 ppm IBA is the same as NAA 0,2 ppm + BA 2,25 ppm in dark conditions, around 90 – 91%but the treatment 2 ppm IBA has the highest root number in three months.

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