Initiation of onion callus (*Allium wakegi* Araki) varieties of lembah palu at various light intensities

Maemunah¹, R Yusuf¹*, S Samudin¹, Yusran², Hawalina² and N S Rini³

¹Department of Agrotechnology Faculty of Agriculture, University of Tadulako
²Plant Biotechnology Laboratory Faculty of Agriculture, University of Tadulako
³Seed Technology Laboratory Faculty of Agriculture, University of Tadulako

*E-mail: ryusufus@yahoo.com

Abstract. Onion (*Allium wakegi* Araki) is a popular herb and vegetable commodity that can be used as a flavoring dish or traditional medicine. This study aims to determine the effect of light intensity for callus growth. This research was conducted at Plant Biotechnology Laboratory, Faculty of Agriculture, Tadulako University using Completely Random Design (RAL) with a single factor that is light intensity (A) consisting of 3 levels, i.e., 0-100 lux (A1), 300-800 lux (A2) and 1000-4000 lux (A3). The results showed that the onion cultured on the light intensity 1000-4000 lux accelerated the formation of onion callus varieties of lembahpalu. This callus began to form at 13.17 days after cultured. The light intensity 300-800 lux accelerated callus formation at a high percentage and embryonic callus cells. The percentage of callus formation reached 32.46%, resulting callus color was white up to 4 WAC (Week After Culture). The average color of white callus was found on 4 WAK with the average texture of crumb callus, while at the 6th and 8th WAC the color of callus was yellow.

1. Introduction

Red onion variety lembahpalu is one of the main commodities of Central Sulawesi and a raw material for the fried onion processing industry and has become a "local brand" of Palu. One of the uniqueness of this onion which distinguishes from other onions is that the tubers have a dense texture producing crispy and savory fried onions and the aroma does not change even though it is stored long in a closed container (Limbongan and Maskar, 2003). Based on data from the Department of Agriculture, Fisheries and Animal Husbandry of Palu City (2013), the productivity of onions of lembahpalu variety fluctuated with successive production data from 2007 to 2012. The uncertain condition of onion production in the lembahpaluvariety is caused by various factors. According to Setyowati et al., (2013), the red onion varieties of Lembahpalu that are widely cultivated in Indonesia cannot flower. As a result, the onion varieties of Lembahpalu have narrow genetic diversity. If planted continuously using the tubers can reduce the production of the onion itself.
Plant cultivation techniques using conventional methods in soil or sand media often face technical, environmental and time constraints, for example, the propagation of plants using seeds requires a relatively long time, and the results are often not like the parent plant. Other constraints that often arise caused by living bodies, such as disease pests and environmental stresses can interfere with the success of plant propagation in the field (Yusuf et al., 2012). The need for plant seeds in large quantities, quality, free of pests and diseases and the availability in a short time cannot be fulfilled by using conventional methods both generatively and vegetatively (Yuwono, 2012). In order to meet the needs of high onion seeds, tissue culture technology (in vitro) can be used as an alternative solution to the problem. The advantages of supplying seeds through in vitro culture are disease-free (especially viruses) and not dependent on the season. Tissue culture techniques are an efficient technique for plant clonal propagation. Tissue culture techniques also provide opportunities for the formation of individuals with superior character through induction of somaclonal variations or genetic engineering techniques (Kurniawan et al., 2016).

Light can affect plant development in vivo and in vitro. The state of a culture is influenced by photoperiodicity, quality, and intensity of light. Light affects the regulation of the production of metabolites in cell suspension cultures, including the production of primary metabolites such as enzymes, carbohydrates, lipids, and amino acids and secondary metabolites such as anthocyanin, carotenoids, polyphenols, volatile oil and terpenes (Seibert et al., 1980). Enzyme activity in the biosynthesis of cinamic acid, coumarin, lignin, flavone, flavonol, chalcone, and anthocyanin is significantly affected by light (Hahlbrock et al, 1980).

This study aims to determine the effect of light intensity for callus growth of red onion lembahpalu variety.

2. Material and method

This research was carried out at the Laboratory of Plant Biotechnology, Faculty of Agriculture, Tadulako University, Palu, started from February 2018 to June 2018. The tools used in this research were beaker, measuring cup, pipette, petri dish, culture bottle, tweezers, scalpel, stirring rod, SA 300 VA microm autoclave, 100-800 model oven, pH meter, laminar air flow cabinet / Biosafety cabinet model J-BSCV, AR1140 / C analytical scale, hand sprayer, Bunsen burner, hot plate cimarec 2, magnetic stirrer, shaker, refrigerator, plastic, aluminum foil, rubber band, funnel, filter paper, label paper, culture rack, and micropipette.

The material included plant explants onions (tubers) varieties of lembahpalu, MS media, vitamins, 2,4 D, BAP growth regulators, swallow globe brands, sucrose, 1 N NaOH, detergents, fungicides, bactericides, alcohol, chlorox, sterile aquades, and spritus. This study was arranged using a Completely Randomized Design (CRD) with a single factor treatment namely light intensity (A) consisting of 3 low levels (0-100 lux), medium (300-800 lux) and bright (1000-4000 lux). Each treatment was repeated six times so that there were 18 experimental units; each experimental unit was represented by two explants so that there were 36 explants. In order to find out the effect of the treatment, the data obtained in the analysis using variance. If the results of variance show a significant effect, it will be tested using Honestly Significant Difference (HSD) level of 5%.

The implementation of this study includes several stages of activity, namely the sterilization of tools and distilled water. The tools used in tissue culture must be sterile to prevent contamination. Glassware, including metal (scalpel and tweezers), was sterilized by autoclave at 121°C pressure of 17.5 psi for one hour. While other tools such as a flask, Erlenmeyer, beaker, and culture bottle were sterilized in an electric oven at 75°C for 2 x 24 hours after being washed. Laminar air flow cabinet was sprayed with 70% alcohol and then sterilized with ultraviolet light for 30 minutes. The tools used were also sprayed with 70% alcohol. The sterilization of distilled water was carried out by adding ± 150 ml
of distilled water into a bottle with a capacity of ± 250 ml. Then the bottles were closed tightly, then sterilized using an autoclave at 121°C and a pressure of 17.5 psi for 30 minutes.

The media used in this study were MS media, which was added 2.4-D 2.0 mg/l. The initial step in making the media was MS media stock solutions. Stock solutions are made according to MS media composition. The making of the media in this study was carried out by pipette MS stock solution according to the dose and put into a beaker. Then sugar, myoinositol, 2.4-D 2.0 mg / l, BAP 0.5 mg / l, sterile distilled water were added to reach the intended volume. Afterwards, the pH of the solution was set at 5.7-5.8 (if the pH is too low, 0.5 N NaOH will be added and if the pH is higher, 0.5 N. HCl will be added). As a compactor, 8 gram/l agar was added to media solution and heated using an electric heater (hot plate) while continuously being stirred until the solution became clear. The media was then put into each culture bottle with a volume of 25 ml. The media was sterilized by autoclaving at a temperature of 121°C pressure of 17.5 psi for 15 minutes.

For the sterilization of explants, the tubers were firstly stripped from the outer layer of skin, then washed with running water until clean. The washed tubers were then shaken using a shaker in a 10-minute detergent solution and rinse with running water. This procedure was repeated three times. Then the tubers were rinsed with sterile distilled water until the detergent foam was clean. After being washed with bulbous, onion detergent was soaked with bactericide (1.5 g / l) and fungicide solution (1.5g / l) for 24 hours, then transferred to a new sterile bottle. Bulbs that had been soaked were rinsed with sterile distilled water and then carried out the next sterilization stage. Explants were soaked in 15% chlorox solution for 15 minutes, 10% chlorox for 10 minutes and 5% chlorox solution for five minutes then rinsed with sterile distilled water for three times. The rinsed tubers were stored in the refrigerator in a 10% vitamin C solution for 24 hours. After 24 hours of removing the vitamin C solution, it was transferred to a petri dish. Afterwards, the explant was cut into pieces together, then planted in the media according to the treatment.

Tubers were planted in an upright position or fall by submerging some of the tubers into the media. The number of tubers grown in each medium was two tubers. Then the culture bottle was closed and labeled. Planted explants were placed in incubation room according to treatment with a temperature of ± 23°C. Chamber was kept clean, and the room temperature was maintained at a temperature of ± 23°C. The room was cleaned every day, and every two days, the culture rack was sprayed with 70% alcohol. Contaminated culture bottles were immediately removed from the culture room and autoclaved to prevent the spread of contamination to other explant bottles.

3. Result and discussion

Callus appeared. The result of the HSD test was 5%, showing that the treatment of high light intensity (1000-4000 lux) produced faster callus, with an average of 13.17 days after culture. It is in contrast to the treatment of moderate light intensity (300-800 lux) with the average 15.17 days after culture and low light intensity (0-100 lux) which resulted in slower callus growth, with an average of 16.00 days after culture.

Table 1. Callus appear of onions lembahpalu variety at Various Light Intensities

| Light Intensity | Average  | HSD 5% |
|----------------|---------|--------|
| Low (0-100)    | 16,00c  |        |
| Medium (300-800)| 15,17b  | 0,77   |
| High (1000-4000)| 13,17a  |        |

The average value followed by the same letter is not different from the HSD test at 5% level.
The presence of light does not always hamper the growth of plant culture in vitro; instead light is actually needed for optimal results. George and Sherrington (1984) stated that in most cultures, cells would be able to divide in bright conditions with external auxin in the media. Although light can damage auxin, the results of contrast tests show that light treatment can give the fastest callus appear. It shows that although callus plants can grow in low light conditions continuously, callus growth can be stimulated in bright conditions with certain light qualities.

Table 2. Average percentage of Callus Formation at 6 week after culture.

| Light Intensity | average | HSD 5% |
|----------------|---------|--------|
| Low (0-100)    | 25.49a  |        |
| Medium (300-800)| 32.46b  | 2.30   |
| High (1000-4000)| 24.11a  |        |

The number followed by the same letter in the column does not differ from the HSD test level at 5%.

There was an increase in the percentage of callus formation in red onion varieties of Lembahpalu on various light intensities. The increase was found in the treatment with moderate light intensity (300-800 lux) and low light intensity (0-100 lux).

When the 4WAC callus was still in the initial formation stage (young) and still experiencing an increase in size, explants from young tissue produced their own growth substances, and their cells were still actively dividing. The size of the plant reflected the increase in protoplasm, which occurred due to the increase in size and number of cells. Cell dividing speed can be influenced by the presence of certain types and concentrations of auxin depending on the plant, as well as other external factors such as light intensity and temperature (Trimulyono, et al. 2003).

Callus color showed that the average callus color formed on explants 4 WAC with various treatments of light intensity had almost the same color, namely white (score 1.00 - 1.17). The data were not presented here. Furthermore, the average callus color formed on explants 6 MSK with various treatments of light intensity underwent a change of callus color from white to yellowish white (score 2.17 - 2.50). All red onion calluses turned yellow to brown at 8 WAC (score 3.17-4.00). The average color change of the callus was the fastest color encountered in explants from giving bright light intensity (1000-4000 lux) at 4, 6 and 8 WAC which were different from explants placed on medium light intensity (300-800 lux) and dark (0-100 lux) where the color changed more slowly. When entering the 8 WAC, the average color of the callus experienced senescence (aging) which was indicated by the color of all callus to yellow, brownish yellow to brown.

Tissue browning because of the activity of copper-containing oxidase enzymes such as polyphenol oxidase and tyrosinase, which are released or synthesized and are available in oxidative conditions when the tissue is injured (Lerch, 1981).

HSD test results were 5%. Figure 1 shows that the treatment of medium-light intensity (300-800 lux) produces the highest average callus texture that is 1.67 but it is not different from dark light intensity (0-100 lux) with an average of 1.25 and bright light intensity (1000-4000 lux) which produces callus with the lowest average of 1.00.
Variations or differences in callus texture can be caused by plant type factors, media composition, growth-regulating substances, environmental conditions, and length of time. A callus that is textured crumbs (friable) to compact is formed due to the increased activity of cell division (Mardini, 2015). The presence of endogenous auxin hormones produced internally can stimulate crumb textured callus formation (Widyawati, 2010).

Watery callus generally has lost its cell division ability. Its metabolic activity is very low. The water content in the cell is so high that the type of callus is categorized as non-embryonic callus. The formation of watery and nonembryonic substances in this study was caused by the age of callus that was relatively old or mature.

Peterson and Smith (1991) stated that embryogenic callus is characterized by white to yellowish, shiny and crumbly callus colors which are easily separated into fragments, while non-embryogenic callus is characterized by a brownish yellow color, rather pale and watery so that it is difficult to separate. Callus which has a compact (hard) texture generally has a small cell size with dense cytoplasm, large cell nucleus and has many starch granules (carbohydrate content) Maemunah et al. 2019. Such cells have high regeneration potential. Conversely, calluses that have watery cells show low regeneration power or have even lost their regeneration (Bustami, 2011).

4. Conclusions

Plants that are cultured at high light intensity (1000-4000 lux) can be accelerated to callus formation. In such light intensity, a callus is formed at 13.17 days after culture. Medium-light intensity (300-800 lux) is good to get a high percentage of callus formation and embryonic callus cells.

Acknowledgments. This study was supported by a research grant from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

References

[1] Bustami, M. U. 2011. Penggunaan 2,4-D Untuk Induksi Kalus Kacang Tanah. Media Litbang Sulteng. Vol 4 (2) : 137-141.
[2] Dinas Pertanian, Perikanan dan Peternakan Kota Palu, 2013. Data luas lahan, luas tanam, luas panen, produksi dan produktivitas bawang merah varietas Lembah Palu tahun 2007-2012.
Palu, Sulawesi Tengah.

[3] George, E.F and Sherington, D.P. 1984. Plant Propagation by Tissue Culture Hand Book and Directory of Commercial Laboratories Exegetics Ltd. England.

[4] Kurniawan, A. D dan Widoretno, W. 2016. Regenerasi In Vitro Tanaman Bawang Merah Palu Di Sulawesi Tengah. Biotropika. VI 4 (1).

[5] Limbongan, J. dan Maskar. 2003. Potensi Pengembangan dan Ketersediaan Teknologi Bawang Merah Palu Di Sulawesi Tengah. J. Litbang Pertanian 22 (3): 103-108.

[6] Maemunah, R Yusuf, S Samudin, H Kasim, and Yusran. 2019. Optimization and regeneration of in vitro seedling of Shallot variety Lembah Palu in providing good quality seedling. IOP Conf. Ser.: Earth Environ. Sci 235 012051 doi:10.1088/1755-1315/235/1/012051

[7] Mardini, U. 2015. Pengaruh Kombinasi 2,4-D dan BAP Terhadap Induksi Kalus Eksplan Daun dan Batang Tanaman Binahong (Anrederacordifolia (Ten.) Steenis) Secara In Vitro. Skripsi. Fakultas Keguruan dan Ilmu Pendidikan. Universitas Muhammadiyah. Surakarta.

[8] Peterson, G and Smith, R. 1991. Effect of abscisic acid and callus size on regeneration of American and International rice varieties. Plant Cell Rep. Vol.24 (1):35-44.

[9] Seibert, P. G. Kadkade, In. Staba (Ed). 1980. Plant Tissue Culture as a Source of Biochemicals. CRC press. Boca Raton. Florida. USA. P. 123.

[10] Suryowati, M. Sulistyaningsih, E. Dan Purwantoro, A. 2013. Induksi poliploidi dengan kolkisina pada kultur meristem batang bawang wakegi (Allium x wakegi Araki) Ilmu Pertanian. Vol.16 (1): 58–76.

[11] Trimulyono, G. Solichatun dan Marliana, S. D. 2003. Pertumbuhan kalus dan kandungan minyak atsirinilam (Pogostemoscablin (Blanco) Bth.) dengan perlaku anasam a-naftalenasetat (NAA) dan kinetin. Jurnal Biofarmasi 2(1): 9-14.

[12] Widyawati, G. 2010. Pengaruh Variasi Konsentrasi NAA dan BAP Terhadap Induksi Kalus Jarak Pagar. Tesis. Universitas Sebelah Maret. Surakarta.

[13] Yuwono, T. 2012. Bioteknologi Pertanian. Gajah Mada University Press. Yogyakarta.

[14] Yusuf, R., P. Kristiansen and N warwick 2012 Potential Effect of Plant Growth Regulaotor in Two Seaweed Products. Acta Horticulture 958. Hal 133 – 138.