ISSN: 2963-2536 (media online)
Proceedings of Malikussaleh International Conference on Multidisciplinary Studies (MICoMS)
Organized by center of Excellence of Technology Natural Polymer and Recycle Plastics
International Conference Proceedings 00035 (2024) DOI: https://doi.org/10.29103/micoms.v4.2024

Effect of Photoperiod and Kinetin Concentration on In Vitro Propagation of Potato (Solanum tuberosum L.) 'Granola' as Bioplastic Raw Material

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Abstract. Potato is a horticulture plant that contains complex nutrition like high carbo and phosphor. Low potato productivity can be caused by the use of low-quality (degenerate) seed potatoes that are conventionally propagated by the farming community. Tissue culture techniques can be an effective solution to produce healthy and disease-free seed potatoes. This study aimed to examine the combination treatment between photoperiod (P) and Kinetin (K) on the growth of granola potato micro cuttings in vitro. This research was conducted at the Plant Tissue Culture Laboratory, Faculty of Agriculture, Malikussaleh University, from February to April 2022. This study used a split-plot design in a completely randomized design. The main plot was photoperiod (0 hours ligth and 24 hours light). The sub plot was concentration of kinetin (0; 2; 4 ppm). The resut showed that there was an interaction between the treatment of photoperiod and kinetin concentration on the growth of 'Granola' potato micro cuttings on the variables of shoot growth percentage, number of shoots, number of leaves, and number of roots. The best treatment was 24 hours of light + 4 ppm kinetin.

Keywords. Bud, explant, hormone, planlet.

1. Introduction

Potato (Solanum tuberosum L.) is a horticultural commodity that is widely used in fulfilling food needs. This plant is one of the sources of carbohydrates that support food diversification programmes [1] and contains high levels of vitamin C, phosphorus, and calcium [2]. Potatoes are in great demand from the household scale to the food industry, especially fast food.

Potatoes contain a lot of starch, so in addition to being used as a food ingredient, it can also be used as a raw material for making bioplastics. Bioplastics (biodegradable) are a type of plastic made from renewable materials. Raw materials for bioplastics can be obtained from starch, vegetable oils, microbiota. The availability of bioplastics is very abundant in nature and has a diversity of non-toxic structures. This renewable material has high biodegradability so it has the potential to be used as a bioplastic material [3]. Potato (Solanum tuberosum L.) is a horticultural commodity that is widely used in fulfilling food needs. This plant is one of the sources of carbohydrates that support food diversification programmes [1] and contains high levels of vitamin C, phosphorus, and calcium [2]. Potatoes are in great demand from the household scale to the food industry, especially fast food.

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structures. This renewable material has high biodegradability so it has the potential to be used as a bioplastic material [3].

The availability of potatoes in Indonesia still has to be increased, because it must fulfil the needs of food and bioplastic raw materials. One way to produce potato seeds in large quantities that produce healthy, disease-free and quality plants can be produced using tissue culture techniques. In addition, the culture technique is an effective solution to produce seeds in a short period of time and the use of small space [4].

Tissue culture is a plant propagation technique by separating plant parts such as leaves, roots or other organs, and isolating these plant parts in an aseptic environment so that they grow into complete plants [5]. Plant tissue culture systems can produce healthy, high quality, disease-free potato plants, especially those caused by bacteria and fungi. In addition, plant propagation in tissue culture can provide seed needs throughout the season [6]. Based on research by [7] that the use of tissue culture techniques in vitro can reproduce plants that come from a small number of parents, besides that [8] stated that in vitro plant propagation can obtain many seedlings in a short time.

The success of plant tissue culture is strongly influenced by many factors, including environmental conditions, media composition [9], and nutrients given to the planting media. Environmental conditions that greatly affect the growth of potato explants are the length of irradiation (photoperiod), while the type of growth regulator given to the media affects the composition of the media that can affect the growth of potato explants.

The photoperiod affects the process of plant growth and development. The quality, intensity, and duration of radiation received by plants have a major influence on various plant physiological processes in tissue culture [10]. The length of irradiation affects the process of plant organogenesis [11]. This is in accordance with the results of [11] in the 24-hour dark treatment showed the best results in the initiation period, the number of tubers, wet weight and dry weight of potato tubers. Research by [12] showed that the 18-hour dark treatment showed the highest internode elongation in chrysanthemum explants.

The success of in vitro plant propagation is also determined by the growth regulators used. The provision of growth regulators on MS media is necessary to support the growth and development of potato planlets [13]. The use of ZPT serves to stimulate explant growth [14]. Kinetin is a type of cytokinin that has the most potential to induce shoot growth in plants [15]. The hormone kinetin plays a role in shoot formation, stimulates cell division, callus proliferation, and encourages the proliferation of merisem tips. Therefore, without kinetin, shoot growth will be inhibited [16. The results of [17] stated that Kinetin treatment of 2.5 ppm slightly effect to improve tuberization in vitro.

Kinetin is a growth regulator that plays an active role in cell growth and callus proliferation. Kinetin is one type of cytokinin that is widely used in tissue culture. According to [18] kinetin has an influence in accelerating shoot induction. Based on [19], the multiplication of potato shoots in vitro by giving kinetin at a concentration of 3 ppm can trigger the growth of micro shoots, as well as shoot height [20] and shoot growth time [21].

The purpose of this study was to determine the effect of photoperiod and Kinetin concentration on the growth of ‘Granola’ potato explants. The success of this research is very useful for fulfilling the needs of food and food industry and bioplastics

2. Materials and Methods

This research was conducted in Tissue Culture Laboratory, Faculty of Agriculture, Universitas Malikussaleh, Aceh, Indonesia. The implementation of this research starts from Januari to April 2022.

The tools used are laminar air flow cabinet, autoclave, oven, analytical scales, hot plate, magnetic stirrer, pH meter, and planting tools. The materials used were sterile 'Granola' potato planlets from IP2TP Berastagi, North Sumatra, distilled water, sterile distilled water, 70% alcohol, sugar, agar, sterile opaque paper, plastic wrap, label paper, tissue, black mulch, MS (Murashige and Skoog) media, and Kinetin.

This study used a split-plot design arranged in a completely randomised design. The main plot was photoperiod which consisted of two levels 0 hours of light and 24 hours of light. The subplots were kinetin concentrations of 0 ppm, 2 ppm, and 4 ppm. The 24-hour light treatment was given by placing the culture bottle on a shelf illuminated by an 18-watt TL lamp. The 24-hour dark treatment was done by covering the culture rack using black plastic mulch.

Potato explants were sterile explants that were cut to a size of 1 cm and had 1 bud. The cut explants were planted in the planting medium according to the treatment in a horizontal position, 4 explants per culture bottle. Planted culture bottles were kept in an incubation room with 24-26 oC.

The observation data obtained were statistically analysed using analysis of variance (Anova). If the results obtained in the variance analysis were significantly different at the 5% level, then further tests were carried out using DMRT (Duncan's Multiple Range Test) at the 5% level.

3. Results and Discussion

Anova results showed that there was an interaction between photoperiod and kinetin concentration on the growth of potato explants. The average percentage of success and the percentage of shoot growth are listed in Table 1.

Table 1. Effect of photoperiod and kinetin concentration on the percentage of success and shoot growth of 'Granola' potato explants. [a]

Treatments		Culture success percentage (%)	Shoot growth percentage (%)
Photoperiod	**Kinetin concentration**		
0 jam	0 ppm	73.33 (8.51) a	80 (0.48) ab
0 jam	2 ppm	73.33 (8.52) a	90 (0.48) a
0 jam	4 ppm	72.50 (8.48) a	70 (0.47) ab
24 jam	0 ppm	70.00 (8.29) a	70 (0.47) ab
24 jam	2 ppm	77.50 (8.77) a	60 (0.47) b
24 jam	4 ppm	72.50 (8.48) a	90 (0.48) a

[a] Notes are referenced using alpha superscripts

[b] Data in the same column that are followed by the same letters do not differ significantly at the 5% level, according DMRT. The numbers in parentheses are the transformed data = Log(x+2).

The effect of the treatment factor was seen in the percentage of shoot growth. In the shoot growth phase, the treatment of 0 hours of light + 2 ppm kinetin and 24 hours of light + 4 ppm kinetin showed the largest percentage of shoot growth at 90%. The effect of the treatment factor was not seen in the variable culture success percentage.

In this study, the treatment of photoperiod and kinetin concentration had a significant effect on the percentage of shoot growth. [22] stated that a combination of photoperiod and hormone was needed for

organ formation. This is supported by the research of [23] that the interaction between light intensity and endogenous cytokinins directly affected shoot proliferation.

The treatment of photoperiod and kinetin concentration on the variable percentage of growth success was not significantly different. This is thought to be because the explants used are young tissues that are still actively dividing and already have endogenous hormones. This is supported by research by [24] that the use of shoot explants gives a high response and success. This is inversely proportional to the use of explants derived from old tissues where cell regeneration slows down [25]. In vitro micro cuttings have meristematic properties, namely their cells are still actively dividing with high regeneration compared to other plant tissues and good adaptability so that the level of contamination of explants is low [26]. Another factor that supports the success of the percentage of explant growth in this study is thought to be the MS media used already contains a complete composition for explant growth [16].

The effect of treatments was also seen in several other observation variables. The average observation results on the number of shoots, number of leaves, shoot length, and number of roots are listed in Table 2.

Effect of photoperiod and kinetin concentration on the number of shoots, number of leaves, shoot length, and number of roots of 'Granola' potato explants[a]

Treatments		Number of shoots	Number of leaves	Shoot length (cm)	Number of root
Photoperiod	**Kinetin concentration**				
0 hour	0 ppm	0,80 (0,44) ab	0,15 (0,38) b	4,86 (0,94) a	0,40 (0,50) b
0 hour	2 ppm	0,90 (0,46) ab	0,25 (0,39) b	4,45 (0,93) a	0,45 (0,50) b
0 hour	4 ppm	0,80 (0,44) ab	0,70 (0,42) b	5,55 (0,94) a	0,15 (0,48) b
24 hours	0 ppm	0,70 (0,43) bc	5,10 (0,62) a	4,14 (0,92) a	1,30 (0,54) a
24 hours	2 ppm	0,60 (0,41) c	1,30 (0,45) b	4,28 (0,94) a	0,40 (0,50) b
24 hours	4 ppm	0,95 (0,46) a	4,85 (0,61) a	4,45 (0,93) a	1,90 (0,55) a

[a] Notes are referenced using alpha superscripts

[b] Data in the same column that are followed by the same letters do not differ significantly at the 5% level, according DMRT. The numbers in parentheses are the transformed data = Log(x+2).

The effect of treatment factors was seen in the number of shoots, number of leaves, and number of roots. In the three observation variables, the treatment of 24 hours of light + 4 ppm kinetin showed the best results compared to other treatments. The effect of the treatment factor was not seen in the shoot length variable.

The treatment of photoperiod and kinetin concentration affected the variable number of shoots. The highest number of shoots was found in the treatment of 24 hours of light + kinetin 4 ppm with an average of 0.95 buds. An increase in kinetin concentration induced the bud system to form more branches [16]. According to [27] on the observation of Curcuma zedoaria shoot induction, the higher the kinetin administration correlated with the number of shoots formed but their growth became inhibited. This is supported by [16] that giving 3 ppm kinetin can spur shoot multiplication which encourages meristem cells in explants to divide and develop into shoots.

The treatment of photoperiod and kinetin concentration significantly influenced the variable number of leaves 8 weeks after planting. The highest number of leaves was found in the combination of 24 hours of light + kinetin 0 ppm treatment and 24 hours of light + kinetin 4 ppm treatment. Light is an

important environmental factor, and plays a role in plant growth and development, morphogenesis, metabolism, the amount of chlorophyll in cells, tissues and organs [28].

The highest number of roots was shown by the treatment of 24 hours of light + kinetin 4 ppm. Light is influential in determining the growth of explants, one of which is in the formation of root organs. This is supported by the opinion of [29] that environmental parameters such as the length of irradiation interacting with growth regulators have a significant effect on the development of explants.

The number of plant roots can indicate how far the plant is able to reach and absorb nutrients and nutrients, the greater the number of roots, the more nutrients the plant gets. Light can affect the number of roots because photosynthate is used by plants in the process of organogenesis or the formation of new organs such as roots. This is supported by the opinion of [30] that the results of the photosynthesis process in the form of sucrose are needed by plants to form plant organs. According to [31] the amount of light entering the plant area affects photosynthesis, low light intensity will result in a low photosynthesis rate.

4. Conclusions

There was an interaction between the treatment of photoperiod and kinetin concentration on the growth of 'Granola' potato micro cuttings on the variables of shoot growth percentage, number of shoots, number of leaves, and number of roots. The best treatment is 24 hours of light + 4 ppm kinetin.

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