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Effect of photoperiod and KNO_3 concentration on the induction and development of potato (*Solanum tuberosum*) microtuber in vitro

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Abstract. *Choirunnisa JP, Wardana R. 2021. Effect of photoperiod and KNO_3 concentration on the induction and development of potato (*Solanum tuberosum*) microtuber in vitro. Cell Biol Dev 5: 70-75.* Potato (*Solanum tuberosum* L.) is a carbohydrate source plant that was developed as an alternative to food diversification. The availability of quality potato seeds is very limited due to the high attack rate of bacteria and viruses. The demand for potato seeds can be fulfilled by developing potato microtuber through the application of plant tissue culture. The purpose of this research was to determine the response of potato microtuber formation with different photoperiods and KNO_3 concentrations. This study design uses a factorial Completely Randomized Design (CRD) with 5 replicates. The first factor is 3 levels of photoperiod (8 hours/day, 12 hours/day, 16 hours/day). The second factor is 3 levels of KNO_3 (1900 mg/L, 2850 mg/L, 3800 mg/L). The study was conducted by observing the age of microtubers initiation, number of shoots, number of roots, number of microtubers, the diameter of microtubers, and wet weight of microtubers were analyzed using SPSS. The results showed that the combination treatment of 8 hours/day photoperiod and KNO_3 concentration of 3800 mg/L could accelerate the initiation of microtubers at 8 DAP (Days After Planting). The concentration of 3800 mg/L KNO_3 can increase the number of roots and microtubers, the diameter of microtubers, and the wet weight of microtubers. The highest diameter of microtubers (17.89 mm) and the highest wet weight of microtubers (278.81 mg) were found in the photoperiod of 16 hours/day. This study concludes that the higher concentration of KNO_3 and the longer photoperiod could be used for the induction and development of potato microtubers, while the short photoperiod could be used to accelerate microtubers initiation

Keywords: KNO_3, microtubers, photoperiod, potato

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the food crops in Indonesia and can be a top priority developed by the public as an alternative to food diversification (Nurjanah 2017). In addition, the development of potatoes as a staple in the snack food industry makes the need for potatoes in Indonesia continues to increase (Aminudin et al. 2014). However, potato production in Indonesia in 2014-2016 decreased from 1247818 tons/ha to 1213041 tons/ha (BPS 2016).

The availability of high-quality potato seeds is very limited; this is one of the factors that caused a decrease in potato production in Indonesia. Potato seeds cannot be fulfilled if only using conventional propagation. Cultivation of potato seeds in conventional propagation needs a long time, namely 100 days, so potato seeds cannot be fulfilled quickly. Tissue culture techniques can produce seeds in a short time with many quantities and are pathogens free (Sagala et al. 2012). Application of tissue culture techniques with potato plants can produce microtubers to support the need for quality seeds. The use of microtubers has several advantages; for example, if the microtubers are transferred to non-aseptic media, they are more resistant and tolerant, and the generated microtubers are uniform and disease-free (Morais et al. 2018).

The factor that can affect the formation of microtubers is photoperiod and nutrient increase in the culture media (Dixit et al. 2011). Culture media nutrients can be increased in-stock solution B of KNO_3 because KNO_3 can increase the size of potato microtubers. KNO_3 in culture media can cause photosynthetic translocation to be more fluent and more quickly into the tubers if the amount of photosynthate produced and absorbed by tubers increases more than the larger potato microtubers (Tessema et al. 2017). The increase in KNO_3 can increase 12% of carbohydrates used to increase potato tubers' weight by 33% (Haddad et al. 2016). In addition, the photoperiod length can increase the intensity of light received by explants to accelerate the process of forming microtubers and affect tuber initiation and the early growth stimulation of microtubers (Sambeka et al. 2012). According to Yasmin et al. (2011), a photoperiod of 16 hours can produce faster induction of microtubers with an average number of microtubers of 1.15.

The purpose of this research was to obtain the optimal concentration of KNO_3 and the right photoperiod for the formation of potato microtubers in a faster time and to produce a size large of microtubers so that they can be used as quality seeds pathogen-free.

MATERIALS AND METHODS

Experimental design

This study design uses a factorial Completely Randomized Design (CRD) with two factors. The first factor is 3 levels of photoperiod, namely 8 hours/day (P1), 12 hours/day (P2), and 16 hours/day (P3). The second factor is 3 levels of KNO_3, namely1900 mg/L (K1), 2850 mg/L (K2), and 3800 mg/L (K3), so it had 9 treatment combinations with 5 replicates. The research was conducted from September 2018 to January 2019 at the Plant Tissue Culture Laboratory, State Polytechnic of Jember, Indonesia.

Field experimental procedure

Making treatment media

The media used are MS media (Murashige and Skoog) which are made according to the Gunawan (1998) stages with a slight modification, for example, the increasing KNO_3 concentration in the stock solution B. The make of MS media can be done by making stock solutions A to H according to the composition of the MS base media, pipetting stock solution A to H according to the standard and put into a glass beaker, then adding the stock solution B (KNO_3) according to treatment and adding the 5 ppm/L BAP, 0.1 ppm/L IAA, 80 g/l sugar and 400 ml aquadest. The mixed-media solution was shaken, the pH was measured to 5.8, then distilled water was added to 1000 ml. The treatment media and 8 g/l jelly powder were put into a pan and cooked on the stove by stirring the solution until it boiled. The media solution was poured into 25 ml culture bottles each, and the bottles were closed with bottle caps. The media was sterilized using an autoclave for 20 minutes at a temperature of 121°C and a pressure of 17.5 psi; then, the media was placed on a culture rack.

Selection of explants

Explants were taken from MS0 media from the tissue culture laboratory and selected with the provisions of a uniform explant age of 12 WAP (Week After Planting) and the same explant stem size. The explants used were two plantlet stem segments with removing the tips and roots.

Subculture

Subculture begins by spraying tools and materials with 70% alcohol, then put into LAFC (Laminar Air Flow Cabinet). The dissecting set was sterilized by heating the bunsen and taking explants from culture bottles using a dissecting set. After the explants were taken, they were placed on a Petri dish, and the explants were cut into single-book micro cuttings with a segment length of 1-2 cm; the tips and roots of the explant were cut using a scalpel. The explants that had been cut were put into the treatment medium in a horizontal position with as many as 2 explants in each bottle; then, the bottles were closed and covered with plastic wrap. The bottles from the subcultures were stored for 3 months on a shelf in the incubation room with a temperature of ± 22°C, RH in a tissue culture room of 55%, and photoperiod time according to treatment. The irradiation on each incubation rack used 2 TL lamps and was set using an automatic timer for 8 hours/day, 12 hours/day, and 16 hours/day.

Observation

Observed variables in this study included the age of microtubers (day-) initiation was observed every day, number of shoots, number of roots, number of microtubers, the diameter of microtubers, and wet weight of microtubers observed at harvest with the age of explants 12 WAP.

Data analysis

Data were analyzed using SPSS (Statistical Product and Service Solution) with Analysis of Variance (ANOVA) and further tested with a Duncans Multiple Range Test (DMRT) level of 5%.

RESULTS AND DISCUSSION

Potato explants at high concentrations of KNO_3 can increase the number of roots, the number of microtubers, the diameter of microtubers, wet weight of microtubers and accelerate the initiation of microtubers. In contrast, explants at low concentrations of KNO_3 can increase the number of shoots. In addition, the long photoperiod can increase the number of shoots, number of roots, number of microtubers, the diameter of microtubers, and weight of microtubers, while the shorter photoperiod can accelerate the initiation of microtubers.

Number of shoots

The parameter of shoot number is a positive indicator in the formation of microtubers; more shoots can potentially form more microtubers. Each explant can grow axillary buds in several nodes or nodes, which can encourage the formation of micro shoots and tubers; according to the statement of Ni'mah et al. (2012), shoots at each node can encourage the formation of microtubers in vitro. The result of the number of shoots analysis is presented in Figure 1. Based on Figure 1, there was no interaction between photoperiod and the addition of KNO_3 to the number of shoots. The longer photoperiod of 16 hours/day could increase the number of shoots by 29% at the concentration of 1,900 mg/L KNO_3, 43% at the concentration of 2,850 mg/L KNO_3, and 30% at the concentration of 3,800 mg/L KNO_3. On the other hand, the highest concentration of 3,800 mg/L KNO_3 can reduce the number of shoots by 33% at various photoperiods.

The longer photoperiod can increase the number of shoots (Figure 1) because the longer photoperiod can increase the photosynthetic process and the results of photosynthesis, namely carbohydrates in explants. Photosynthate results can be used as an energy source for metabolic processes in forming new plant organs such as shoots. Martin et al. (2013) stated that vegetative plant growth, such as shoot growth, was more quickly formed in the 16 hours/day photoperiod due to the increase in photosynthesis, and photosynthesis results in the form of carbohydrates. Aside from that, adding exogenous plant hormones or plant growth regulators of cytokinins such as BAP on culture media can also stimulate shoot growth. BAP is a cytokinin that can affect the number of shoots because it is most active in cell division (Suparaini et al.

2013). Increasing the concentration of KNO_3 can produce a small number of shoots because giving too much K can inhibit N uptake for cell division and plant organ formation. K nutrients too much can be toxic to plants; for example, disrupting the N nutrients absorption has a role in the synthesis of amino acids, cell division, and formation of cells, tissues, and organs of plants (Salli et al. 2016).

Number of roots

The number of roots can be used to indicate plants' ability to absorb nutrients and nutrients. The greatest number of roots can affect the wider range of nutrient absorption so that the roots from the culture medium absorb more nutrients. The nutrients needed by plants can be used as nutrition to increase the plant metabolic processes for plant growth and development (Sarif et al. 2015). The result of the number of roots analyses is presented in Figure 2. Based on Figure 2, there was no interaction between the photoperiod and the addition of KNO_3 to the number of roots. The photoperiod of 16 hours/day could increase the number of roots of potato explants by 27-47% at various concentrations of KNO_3, while the concentration of 3800 mg/L KNO_3 resulted in the highest average number of roots by 19 in the 16 hours/day photoperiod.

The number of roots increased in the longer photoperiod with the higher KNO_3 concentrations (Figure 2) due to the longer photoperiod can increase the synthesis of auxin for root formation. Auxin is a plant hormone that can induce root formation and cell elongation (Lestari 2011). According to Pratiwi et al. (2015), the amount of auxin can increase in long photoperiods for promoting root formation. In addition, the increased concentration of KNO_3 can increase the nutrient content of K and N. The nutrients of K can increase root turgor pressure to absorb nutrients in the media, and the nutrients of N can stimulate the formation of plant vegetative organs so that these two nutrients can support faster root growth. The K nutrients in KNO_3 have a role in stimulating protein formation, increasing root turgor pressure, and stimulating root growth and development (Wahyudi et al. 2015). KNO_3 also contains nitrogen, which is important in plant physiological processes, stimulating root growth and stimulating the growth of plant vegetative organs such as stems and leaves (Leghari et al. 2016). Therefore, the increase of KNO_3 in this research can support root growth and maximize the number of roots.

Age of microtubers initiation

Microtubers are formed at the shoot tip or stolons tip and leaf axils with the characteristics of measuring 1 mm and having yellow, dark green, light green, and white colors. The initiation of microtubers can be influenced by the explant type, the culture medium used, plant hormones, sucrose concentration, and the culture environment (temperature and photoperiod) (Elfiani 2013). Faster formation of potato microtubers in vitro is initiated with swelling of stolons tip growing from the leaf axils due to the growth medium and environmental factors (Nugroho 2013). The result of the age of microtubers initiation analysis is presented in Table 1. Based on Table 1, there was an interaction between photoperiod and the addition of KNO_3 to the age of microtubers initiation. The photoperiod of 8 hours/day with a KNO_3 concentration of 3,800 mg/L resulted in faster initiation of microtubers at 8 DAP, while the photoperiod of 16 hours/day with KNO_3 concentration of 1900 mg/L as a combination treatment with the longest initiation of microtubers at 11 DAP. The age of microtubers initiation at concentration of 3,800 mg/L KNO_3 with 8 hours/day photoperiod was significantly different with 16 hours/day photoperiod.

Table 1. Age of microtubers initiation (DAP) of potato explants cv granola kembang at various photoperiod and KNO_3 concentrations

Photoperiod	KNO_3 concentrations		
	1900 mg/L	2850 mg/L	3800 mg/L
8 hours/day	8.67 ab	8.08 a	8.00 a
12 hours/day	9.78 c	9.08 b	8.75 ab
16 hours/day	10.67 d	10.33 cd	9.83 c

Note: The numbers followed by the different lowercase letters in each column and line showed a significant difference in DMRT level of 5%

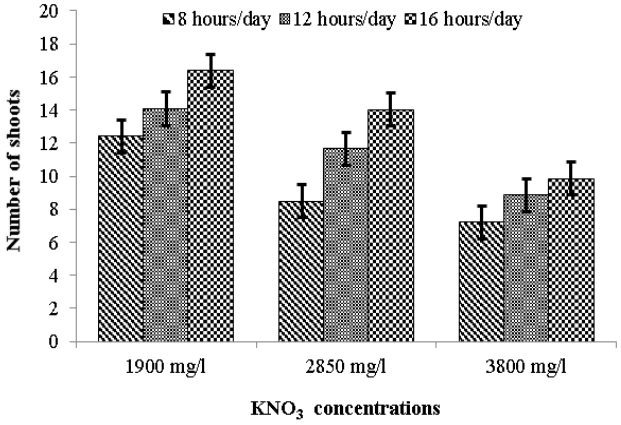

Figure 1. The number of shoots of potato explants cv. granola kembang at various photoperiod and KNO_3 concentrations

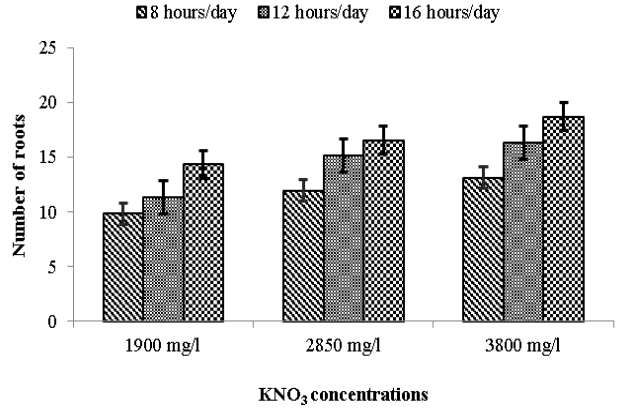

Figure 2. The number of roots of potato explants cv. granola kembang at various photoperiod and KNO_3 concentrations

Microtuber initiation is faster in the shorter photoperiod with higher KNO_3 concentrations (Table 1); this is due to the shorter photoperiod can cause explants to lack light and can produce plant hormones of abscisic acid (ABA) for initiation microtubers. Plants can produce ABA in response to environmental stresses (Verslues 2016), such as the shorter photoperiod for the initiation of microtubers. ABA hormone can inhibit growth and photosynthesis, resulting in carbohydrates being translocated and stored in the energy reserve storage organ, such as tubers. The highest concentration of KNO_3 can accelerate the microtubers' initiation because potassium has a role in accelerating the process of starch synthesis to be stored in storage organs (tubers). According to Utomo and Suprianto (2019), KNO_3 contains 45-46% potassium. Therefore, it has a role in plant physiological processes, such as synthesizing simple sugars, starches, and proteins and accelerating the translocation of carbohydrates to storage organs (tubers). Tubers are storage organs for food or energy reserves such as starch and protein and function as users (sinks) in sucrose metabolism (Turesson et al. 2014).

Number of microtubers

The number of microtubers can be used as an indicator to determine environmental conditions and suitable media for tubers in vitro. Growing environmental conditions and the media used can encourage microtubers initiation and affect the number of micro tubers formed (Hasni et al. 2014). The result of the number of microtubers analyses is presented in Figure 3. Based on Figure 3, there was no interaction between the photoperiod and the addition of KNO_3 to the number of microtubers. The photoperiod of 16 hours/day at the concentration of 3,800 mg/L KNO_3 can increase the number of microtubers by > 30% compared to the photoperiod of 8 hours/day. The concentration of 1,900 mg/L KNO_3 resulted in the maximum number of microtubers by 11-15 micro tubers. The number of microtubers increased by 45% at the KNO_3 concentration of 3,800 mg/L compared to the KNO_3 concentration of 1,900 mg/L.

The number of microtubers increased in the longer photoperiod. At the same time, the higher concentration of KNO_3 can also increase the number of microtubers (Figure 3) because the longer photoperiod can increase the rate of photosynthesis and produce more carbohydrates for the formation of microtubers. The formation of microtubers results from the assimilation process (the rate of photosynthesis) from light as an energy source (Ferreira and Sonnewald 2012). According to (Puangbut et al. 2015), the longer photoperiod can accelerate assimilation and affect the balance and availability of carbohydrates for the initiation and growth of tuber. The increase in KNO_3 concentration can increase the number of microtubers because potassium can increase root growth for nutrient absorption in the media, and adequate plant nutrients can increase the rate of photosynthesis for the formation of microtubers. Potassium can increase the rate of photosynthesis, assimilate yields, and translocate carbohydrates for tuber formation (Fatmawati et al. 2018). One of the products of the photosynthesis of fructans is also needed for tuber formation (Luo et al. 2018).

Diameter of microtubers

The diameter of microtubers can be an indicator of assimilating yields that are translocated to the formation and development of microtubers. The results have been more assimilated and will be translocated to storage organs such as microtubers, which can support the development of microtubers by increasing their size of microtubers. According to Kloosterman et al. (2008), the formation of potato microtubers consists of three stages: induction, initiation, and development of microtubers. The development of potato microtubers also has three stages, namely stalled stolon elongation, subapical swelling in the stolon area, and changes in the diameter of microtubers (Jova et al. 2005). The result of the diameter of the microtubers analysis is presented in Table 2. Based on Table 2, there was an interaction between the photoperiod and the addition of KNO_3 to the diameter of microtubers. The photoperiod of 16 hours/day with a KNO_3 concentration of 3,800 mg/L resulted in the highest microtuber diameter of 17.89 mm, while the photoperiod of 8 hours/day with a KNO_3 concentration of 1,900 mg/L as the lowest diameter of microtuber was 9.23 mm. The concentration of 3,800 mg/L KNO_3 with 16 hours/day photoperiod resulted in significantly different diameters of microtubers with 8 hours/day and 12 hours/day photoperiods. The longer photoperiod and the higher KNO_3 concentration can increase by 48% microtubers diameter.

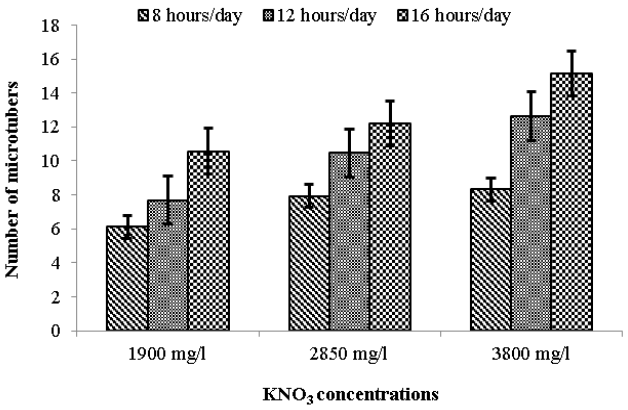

Figure 3. The number of microtubers of potato explants cv. granola kembang at various photoperiod and KNO_3 concentrations

Table 2. The diameter of microtubers of potato explants cv granola kembang at various photoperiod and KNO_3 concentrations

Photoperiod	KNO_3 concentrations		
	1900 mg/L	2850 mg/L	3800 mg/L
8 hours/day	9.23 a	10.42 ab	12.45 c
12 hours/day	11.17 b	12.32 c	14.24 d
16 hours/day	13.10 cd	15.80 e	17.89 f

Note: The numbers followed by the different lowercase letters in each column and line showed a significant difference in DMRT level of 5%

Table 3. The wet weight of microtubers of potato explants cv granola kembang at various photoperiod and KNO_3 concentrations

Photoperiod	KNO_3 concentrations		
	1900 mg/L	2850 mg/L	3800 mg/L
8 hours/day	112.43 a	165.24 ab	199.79 b
12 hours/day	154.36 ab	208.65 b	259.82 cd
16 hours/day	238.45 c	263.88 cd	278.81 d

Note: The numbers followed by the different lowercase letters in each column and line showed a significant difference in DMRT level of 5%

The longer photoperiod and the increased KNO_3 concentration can increase the diameter of microtubers (Table 2); the longer photoperiod can increase the photosynthesis process and accelerate the metabolic process by producing carbohydrates for tuber formation. At the same time, the short photoperiod can inhibit the absorption of water and nutrients in the media for the formation of explant generative organs. According to Golembeski et al. (2014), a short photoperiod on the tissue surface can inhibit the activity of phenolic compounds and cause inhibition of the absorption of water and chemical compounds from the media and the inhibiting of the photosynthesis process. The small size of potato microtubers due to the decreased photosynthesis process and the photosynthate results cannot be distributed optimally in forming generative organs such as tubers (Craze et al. 2018). The increasing KNO_3 concentration can also increase the diameter of microtubers. According to Miao et al. (2016), the process of forming microtubers needs to assimilate the results of carbohydrates. Potassium with sufficient photoperiod can combine CO_2 and water to form sugar that will be converted into ATP (Adenosine Triphosphate) to increase the photosynthesis process (Sulistiani, 2020). In light reactions, ATP is produced from photosynthesis for energy sources in the dark reactions to produce glucose and carbohydrates to form plant organs (Strand et al. 2017).

Wet weight of microtubers

The wet weight of microtubers can be an indicator of assimilating results that have successfully been translocated into tubers. The more assimilates were successfully translocated to the tubers, so the size of the microtubers has bigger. The larger size of microtubers can affect the weight of microtubers due to cell enlargement and cell division continuously so that it can support the development of microtubers (Suh et al. 2014). The result of the wet weight of microtubers analysis is presented in Table 3. Based on Table 3, there was an interaction between the photoperiod and the addition of KNO_3 to the wet weight of microtubers. The photoperiod of 16 hours/day with a KNO_3 concentration of 3,800 mg/L resulted in the highest wet weight of microtubers by 278.81 mg, while the lowest wet weight of microtubers (112.43 mg) was found in the photoperiod of 8 hours/day with KNO_3 concentration of 1900 mg/L. The wet weight of microtubers at 16 hours/day was significantly different with 8 hours/day photoperiod at a KNO_3 concentration of 3,800 mg/L. The increase of KNO_3 concentration from 1900 mg/L to 3,800 mg/L resulted in the wet weight of microtubers being significantly different at each level of the photoperiod.

The wet weight of microtubers increased with the longer photoperiod with the higher KNO_3 concentration (Table 3); this is due to the assimilate results being much more in the long photoperiod with the addition of potassium due to from faster opening and closing of the stomata. The light received by plants for photosynthesis is more when the stomata open and close faster, increasing the photosynthesis process and producing more assimilation. The nutrient K regulates the opening and closing of plant stomata, which can affect the process of receiving light for photosynthesis (Singh et al. 2014). The give of sufficient photoperiod can also suppress the work of auxin to prevent etiolation (cell elongation) in plants (Motallebi et al. 2013). The inhibited cell elongation can result in plants not being etiolated, so assimilated results can be focused on plant growth and development, such as development into microtubers. That is supported by Sarlikioti et al. (2011), that if the production of assimilation is higher, it will be more focused on the development of microtubers. According to Wattimena (1995), the standard as a microtuber propagule has a dry matter percentage of> 14%, a diameter of microtuber by > 5 mm and wet weight of microtubers by > 100 mg/tuber. This research resulted in microtuber wet weight by > 100 mg, so it has fulfilled the microtuber propagule standard.

This study concluded that the initiation of microtubers was faster at the age of 8 DAP with a treatment combination of 8 hours/day photoperiod and KNO_3 concentration of 3,800 mg/L. The photoperiod of 16 hours/day and the concentration of 3,800 mg/L KNO_3 can decrease by 33% shoots number but increase by 47% roots number, 60% microtubers number, 48% microtubers diameter, and 60% microtubers wet weight. The higher concentration of KNO_3 and the longer photoperiod can be used to increase the induction and development of potato microtubers. The short photoperiod can be used to accelerate the initiation of microtubers.

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