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INDUCTION OF IN VITRO CULTURE OF POTATO MICROTUBER BY USING ALAR AND DARK PHOTOPERIOD APPLICATION

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ABSTRACT

Providing virus free potato seeds in order to increase potato production in Indonesia could be carried out by using microtuber resulting from microcutting. The growth of single node microcutting potato can initiate microtuber formation when growth inhibiting substances such as alar in combination with dark photoperiod treatment is applied. This study was aimed to elucidate the effect of alar and dark photoperiod on the date of microtuber emergence and production. The experiment was arranged in a factorial Randomized Completely Block Design in which alar concentrations i.e. 0, 10^{-3}, 5 x 10^{-3}, and 10^{-2} mg/L, served as factor I, and dark photoperiods i.e. 16, 20, and 24 hrs/day, were used as factor II. Each treatment combination was replicated three times giving rise to 36 experimental units. Data were analyzed using ANOVA (F test) followed by Duncan Multiple Range Test (DMRT) when significant effect of the treatments existed. The results showed that alar and dark photoperiod affected individually on the date of microtuber emergence, while the best alar concentration to increase microtuber production was 10^{-3} mg/L with 10.67 microtubers/cutting. Dark period has no significant effect on the induction of potato microtuber.

Keywords: potato microtuber, alar, dark photo-period

INTRODUCTION

Most industrial countries have potato productivity of up to 40 ton/ha. This is much different from that in Indonesia, which is still relatively low i.e. between 14 and 16 ton/ha. One of the factors resulting in the low productivity is the use of low quality seeds. The seeds are frequently obtained from previous harvest, which are commonly not virus free (Gunarto, 2005; Sutarya *et al.*, 2006).

Virus free seeds to increase potato productivity in Indonesia can be produced from microtubers by means of *in vitro* culture. Microtubers usually result from microcuttings where their growth is stimulated by provision of certain ambient condition, either internal or external. Internal condition can be the contents of growth stimulating substances and allocation of carbohydrate metabolism product, while external condition includes photoperiod, temperature, humidity and nutrients.

One of the growth substances having significant effect on the growth of microcuttings to form stolon is gibberelins (GA). Jackson (1999) and Hajirezaei *et al.* (2000) stated that high GA content was found in microcuttings at long day. This will lead to optimum growth of microcuttings and promote stolon formation. GA, especially GA_3, is proved to cause transversal orientation of microtubules and microfibrils, so that cells at subapical stolon undergo longitudinal elongation. Consequently, stolon will also longitudinally elongate and microtubers are not formed.

To prevent longitudinal elongation of stolon, GA_3 content in stolon should be reduced, which can be carried out by applying GA_3 inhibiting substances, e.g. alar. As GA_3 content decreases, microtubules and microfibrils will have longitudinal orientation to cell axis, so that cells at subapical stolon will transversally elongate. This will lead to transversal growth of stolon causing initiation of micotuber formation.

On the other hands, potatoes are known as short day plant (SDP), so that dark photoperiod will affect tuber formation. Microtuber formation can be stimulated by means of eight-hour light period followed by 16-hour dark period treatment on single node microcutting. Such treatment should be applied to provide external condition as required by most SDPs. In such condition single node microcutting will change its growth direction from top meristem to lower meristem, including micotuber formation.

The effect of alar as growth inhibiting substance in a combination with dark photo-period on the initiation of potato microtuber formation and growth is still unknown. Compared to ancymidole and paclobutrazole, alar has some advantage as it can delay leaf senescense (Krishnamoorthy, 1981).

This study was aimed to know the effect of alar concentration and dark photoperiod on the date of microtuber emergence and production.

MATERIALS AND METHODS

The study was carried out from March to June 2007 in the Laboratory of Plant Physiology, Biology Faculty, Jenderal Soedirman University. The materials used were explants of potato cultivar Granola, Murashige and Skoog (MS) media with some modification, bacto agar, coconut milk, sucrose, alar, aquadest and absolute alcohol, while the main equipments were pH meter, hot plate and magnetic stirrer, autoclave, blender, culture bottles and laminar air flow.

Experimental method arranged in a factorial Randomized Bock Design was employed. Factor I was alar concentration (0, 10^{-3} , 5 x 10^{-3}, and 10^{-2} mg/L), while factor II was dark photoperiod (16, 20, and 24 hrs/day). Each treatment combination had three replications, so that 36 experimental units were obtained.

The observed parameters included the date of microtuber emergence, the number of microtubers per four explants (in one bottle), and fresh weight of microtuber per four explants. The data obtained were analyzed using ANOVA or F test followed by further analysis of *Duncan Multiple Range Test* (DMRT) when significant effect of treatment was found.

Modified MS media was made by taking stock solution corresponding to the respective concentration needed. Then 120 g sucrose, 100 mL coconut milk and distilled water up to 900 mL were added. The media pH was adjusted to 5.83 followed by addition of distilled water up to 1,000 mL. Agar of 9 g was added to the solution and heated to boil. This media containing agar was then poured into the bottle cultures as much as 60 mL per bottle and sterilized with autoclave of 2 psi for 30 minutes.

The explants to be planted were taken from tissue cultured potatoes. These were single node microcuttings of 1 to 2 cm with one leaf put aseptically into culture bottles containing steril media. Four single node microcuttings were put into each bottle.

After grown in the MS media without alar for three weeks, explants grew to be plants. These plants were then taken and put into MS media containing alar of the respective treatment. To adapt with alar in the media, these plants were grown in light condition for three weeks. Then, dark photoperiod treatments were applied by putting the culture bottles into storage racks. Observation was made every day until microtubers were formed. Soon after microtubers formed, the plants were moved from storage racks to culture room of 25^{o}C in light condition. These plants were grown up to harvest, in which observation was made daily since five weeks to eight weeks after alar application.

RESULTS AND DISCUSSION

Microtuber Formation

Microtuber formation was initiated since the plants were 30 days old. Nevertheless, Leclerc *et al.* (1994) reported that as many as 20% potato explants formed microtubers at 14 days after treatment. On the other hands, Garner and Blake (1989) said that potato explants formed microtubers about 30 days after treatment.

Some explants formed microtubers faster than others, i.e. only in about 21 days. However, these microtubers can grow again to form shoots (Figure 1). This occurred in treatments with no alar and dark photoperiod

of 16 hours. These microtubers can grow immediately after formed, because there is no growth inhibiting substances ABA in the tubers. On the other hands, tubers formed in plants with alar did not grow immediately. This is because alar inhibits geranyl-geranyl pyrophosphate (GGPP) conversion into cauren in the giberelic acid biosynthesis. Consequently, GGPP will accumulate and available for xanthoxin formation resulting in ABA, which leads to microtuber dormancy.

Kusmana and Sofiari (2007) described the shape of microtubers of potatoes Granola variety as a shortened oval. Two kinds of microtubers were formed in this study, which were white and green. Both were formed either at continuous dark treatment or at light treatment. Kusmana and Sofiari (2007) said that many lenticells were found at green microtubers with a relatively short depth of shoots. Oppositely, only few lenticells were found at white microtubers. Both types of microtubers can be used as seeds and grow well.

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Date of Microtuber Emergence

Anova of alar and dark photoperiod on the date of microtuber emergence showed no interaction effect between both treatments. Individually, however, both alar and dark photoperiod had significant effect on the date of microtuber emergence.

Further analysis on the effect of alar (Table 1) showed that microtubers emerged earlier at control than it did at other treatments. It seems that the presence of alar in media will tend to delay microtuber formation. Alar inhibits the growth of microtubers, because it promotes peroxidase and IAA oxidase activity leading to reduction of IAA contents. As IAA decreases, stolon growth will be inhibited and fewer microtubers are formed. Both alar concentration of 10^{-3} mg/L, $5x10^{-3}$ mg/L and 10^{-2} mg/L results in no different speed of microtuber emergence. The date of microtuber emergence with alar application ranged between 52.00 and 61.33 days.

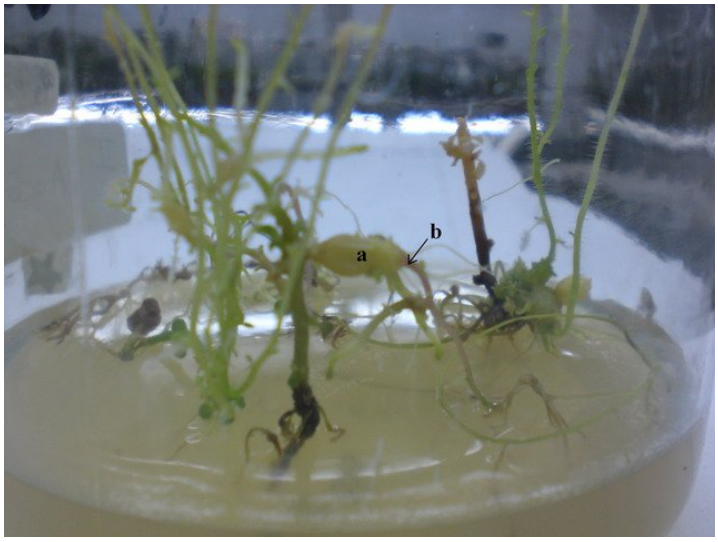

Figure 1. Microtuber capable of regrowing to produce nodes
(a) : growing microtuber (b) : stolon

Table 1. The effect of alar on the date of potato microtuber emergence, number and fresh weight of microtubers

Alar concentration (mg/L)	Date of potato microtuber emergence (days)	Number of microtubers	Fresh weight of microtubers (g)
0	46.33 b	5.22 c	0.174 b
10^{-3}	57.77 a	10.67 a	0.822 a
$5x\ 10^{-3}$	61.33 a	8.00 b	0.390 b
10^{-2}	52.00 ab	8.67 ab	0.352 b

Remarks= Numbers followed by the same letters in one column show no significant difference at confident level of 0.05

On the other hands, dark photoperiod of 20 hours promoted microtuber emergence faster than it did at other treatments, i.e. only 41.75 days. Dark photoperiod of 20 hours promoted microtuber growth. It seems that certain dark photoperiod is required to perform microtuber formation. This correlates with normal growth of potato as SDP, which lives mostly with less than 12 hours day length in average.

Explants exposed to continuous dark photoperiod even form microtubers latest, i.e. 66.25 days (Table 2). Microtuber formation depends largely on the photosynthates produced by the plant itself. When sucrose is sufficiently available in the media, it can be used as raw material for microtuber formation. Nevertheless, it seemingly takes longer to form microtubers. Continuous dark photoperiod was reported to affect microtuber induction in some cultivars. In this study, continuous dark photoperiod resulted in relatively large microtubers. Coleman and Coleman (2000) said, however, that microtubers formed in continuous dark photoperiod tended to undergo longer dormancy in compare to those formed in light photoperiod. Consequently, microtubers are not readily available as potato seeds. Moreover, fewer shoots were found in the microtubers formed in continuous dark photoperiod in compare to those formed in light photoperiod. This means that continuous dark photoperiod tends to produce potato seeds with fewer shoots than those in light photoperiod. When such potato seeds were grown in the field, a relatively fewer number of shoots will be obtained and this will affect the number of plants grown from each tuber. Microtubers grown in continuous dark photoperiod have greenish-white colour and green shoots.

Table 2. The effect of dark period on the date of potato microtuber emergence

Dark Photoperiod (hours)	Date of Microtuber Emergence (days)
16	55.08 b
20	41.75 c
24	66.25 a

Remarks = Numbers followed by the same letters show no significant difference at confident level of 0.05

Number of Microtubers

Anova of alar application and dark photoperiod on the number of microtubers showed that either interaction betweeen alar and dark photoperiod or individual dark photoperiod had no sifnificant effect. Individually, however, alar application had significant effect on the number of microtubers. Further analysis (Table 1) showed that alar concentration of 10^{-3} mg/L resulted in most number of microtubers, i.e. 10.67. The most number of microtubers was found at alar concentration of less than 10^{-2} mg/L.

Alar concentration of 10^{-3} mg/L is sufficiently good to promote the number of microtubers. The number of microtubers in this study was higher than that reported by Garner and Blake (1989), i.e. 2 and was commonly found at the base of explants. If converted to this study, the number of microtubers formed in each bottle (four plants) was 8. From visual examination, microtubers were found not only at the bottom, but also at the upper layer of media, as observed at alar concentration of 0 mg/L and light period of 16 hours (Figure 2).

Remarks = (a) white microtuber
(b) green microtuber

Figure 2. Microtuber formed at upper layer of media

Highest number of microtubers was mainly found at alar concentration of 10^{-3} mg/L with continuous dark photoperiod. Five to seven microtubers were observed in one stolon at upper layer of media. Donnelly *et al.* (2003) said that the number of microtubers resulting from each explant was used to predict tubers produced in the field. More number of microtubers which resulted from *in vitro* culture is expected to give more tubers when the plants are grown in the field. More number of microtubers means more shoots because three weeks after harvested, microtubers can be stimulated to form shoots.

Fresh Weight of Microtubers

It is shown from the anova that dark photoperiod had not significant effect both individually and in interaction with alar concentration on fresh weight of microtubers. However, alar concentration individually had highly significant effect on fresh weight of microtubers.

Further analysis on alar treatment (Table 1) showed that concentration of 10^{-3} mg/L had highest fresh weight of microtubers. Fresh weight of potato microtubers at alar concentration of 10^{-3} mg/L could reach up to 0.822 g. So, in addition to highest number of microtubers, highest fresh weight of microtubers was also obtained at alar concentration of 10^{-3} mg/L.

In vitro production of tubers represents *in vivo* production of tubers, so that treatment resulting in highest number of microtubers with sufficiently high fresh weight of microtubers was necessarily explored. Selection for microtubers was carried out either on their weight, shape, or colour. Donnelly *et al.* (2003) said that small microtubers of 0.09 to 0.12 g would result in plants with low production of tubers. Optimum fresh weight of microtubers to be used as seeds was 0.50 g. Therefore, alar concentration met the requirement for good seed preparation is 10^{-3} mg/L. Meanwhile, Kusmana and Sofiari (2007) recommended shortened oval yellowish green tubers to be used as good potato seeds. In this study, microtubers produced by control plants had very low fresh weight (0.174 g), low number and was not suitable to be used as seeds.

CONCLUSIONS AND SUGGESTION

CONCLUSIONS

Alar and dark photoperiod had individual effect on the date of microtuber emergence, while the best alar concentration to increase microtuber production was 10^{-3} mg/L with 10.67 microtubers/cutting. Dark period did not have significant effect on the induction of potato microtuber.

SUGGESTION

Based on the results, it could be recommended that microtuber stimulation can be carried out from single node microtuber applied with alar of 10^{-3} mg/L. Further study on the stimulation of dormancy breakage of potato microtubers and attemps to stimulate microtuber resistance against diseases is needed.

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