Akta Agrosia

Promoting Tuber Formation *In Vitro* With Benzyl Amino Purine and Paclobutrazol at Different Concentrations

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ABSTRACT

This experiment was carried out at the Tissue Culture Laboratory, Department of Agronomy, College of Agriculture, Bengkulu University from November 2017 to June 2018. The experimental design used was completely randomized design (CRD) consisting of 18 treatments, a combination between BAP (0, 5, and 10 mgl^{-1}) and paclobutrazol (0.0, 2.5, 5.0, 7.5, and 10.0 mgl^{-1}). The results showed that a combination of 5 mgl-1 and 7.5 mgl-1 paclobutrazol demonstrated the fasting time for tuber emergence, the highest percentage of productive explants, the highest number of microtuber per bottle, the highest number of microtuber per plant, and the highest diameter of microtuber.

INTRODUCTION

Potato tuber (*Solanum tuberosum* L) has been known as one of the major source of staple food in the word. In Indonesia, potato crops are grown at high altitude (Idawati, 2012), because of the need for low temperature to produce tuber. However, the average yield of potato in Indonesia is considerable low (18.34 ton ha^{-1}) compared to those produced at temperate regions, like USA (37.40 $ton.ha^{-1}$) or the Netherland, up to 45.10 $ton.ha^{-1}$ (FAO, 2012).

In this respect, two main factors have been identified as the cause contributing to the low tuber yield, serious pathogen attack (Sari *et al.,* 2016) and the lack of high-yielding certified seeds (Sugihono and Hasbianto, 2014). Dewi (2017) reported that Indonesia only able to provide 15% of certified potato seeds needed by farmers. In addition, BPS (2017) reported that Indonesia has imported potato seeds from Canada (4172 ton), Germany (5329 ton), England (700 ton), and USA (240 ton). On the other hand, the need of potato seeds for 72 000 hectare land reaches up to 108 000 ton per year.

Providing a good potato seeds will solve nearly half of the problems faced by Indonesian potato farmers. Sugihono and Hasbianto (2014) explained that producing certified potato seeds may be started from

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producing micro-cutting or micro-tuber *in vitro,* which can be used to produce Go class seeds (Wattimena, 2000). Wattimena (1992) preciously described it very detail on how to produce certified seeds. It starts from Go class seeds produced by planting micro-cutting or micro-tuber at the screen house, followed by planting Go seeds still an the screenhouse to produce G1 class seeds. The G1 seeds will be planted in the controlled field to produce G2 seeds.

The key factor in producing potato tubers *in vitro* is the use of suitable type and proper concentration of plant growth regulator (Yusuf and Suminar, 2013). Ni'mah *et al.* (2012) reported that adding BAP into MS media promoted *in vitro* tuber formation and tuber growth. Sagala *et al.* (2012) found that 5 mgl^{-1} BAP was the best concentration compared to other concentrations tested (0, 2.5, 7.5 mgl^{-1}). At 5 mgl^{-1} BAP, Sagala *et al.* (2012) found the fastest tuber formation, the highest number of tuber formed, the biggest tuber diameter, and the highest tuber dry weight. In addition to cytokinin, tuber formation is also promoted by the application of retardant (Wattimena *et al*., 1983), such as coumarin, CCC, ancymidol, and paclobutrazol (Suharjo *et al*., 2008). These retardants promote tuber formation by inhibiting GA production and suppressing its activity (Wattimena, 1992). Recent study showed that tuber formation, tuber number, tuber size, and tuber weight were promoted as the concentration of paclobutrazol increased from 0.1 mgl^{-1} to 0.6 mgl^{-1} (Yusuf and Suminar, 2013). A combination of cytokinin and retardant has been reported by Ainanur (2004) to promote tuber formation *in vitro.* They used kinetin (0, 1, 2, 3 mgl^{-1}) and paclobutrazol (0, 2.5, 5.0, 7.5 mgl^{-1}) and found that the best concentration was 2.0 mgl^{-1} kinetin and 7.5 mgl^{-1} paclobutrazol. However, the effect of a combination between BAP and paclobutrazol at higher concentration in inducing potato tuber formation has not yet been evaluated. The objective of this research was to determine the best combination of BAP and paclobutrazol on tuber formation and growth.

MATERIALS AND METHODS

The experiment was carried out from November 2017 to June 2018 at the Tissue Culture Laboratory, Department of Agronomy, Faculty of Agriculture, Bengkulu University. The experiment used Completely Randomized Design (CRD) with single factor, resulting from a combination of BAP and Paclobutrazol, which consisted of 18 treatments. Each experimental unit was replicated 6 times. The treatments were presented in Table 1.

Table 1. Treatment combination of BAP and Paclobutrazol.

Treatment Combination	BAP (mgl^{-1})	Paclobutrazol (mgl^{-1})
A	0	0
B	0	2.5
C	0	5,0
I	0	7.5
E	0	10.0
F	0	12.5
G	5	0
H	5	2.5
I	5	5,0
J	5	7.5
K	5	10.0
L	5	12.5
M	10	0
N	10	2.5
O	10	5,0
P	10	7.5
Q	10	10.0
R	10	12.5

Microtuber Production. Microtuber production was done by following the method modified by Suharjo *et al.* (2008), known as two step methods, which were shoot induction and tuber initiation.

Shoot Induction. The media used were MS salt (Murashige and Skoog, 1962) supplemented with pyridoxine.HCl (0.5 mgl^{-1}), glycine (2 mgl^{-1}), thyamine (0.1 mgl^{-1}), nicotinic acid (0.5 mgl^{-1}), myoinositol (100 mgl^{-1}), Ca-P (2 mgl^{-1}), and sugar (30 gl^{-1}). The media pH was adjusted to 5.8 with NaOH (0.5 N) before adding agar (7 gl^{-1}), and heated it

until all agar was diluted. Once the agar diluted, the media was dispensed into bottle (20 ml each). The media were autoclaved for 20 minutes at 121 ^{o}C and 15 psi. Shoot induction was done by planting 5 cutting with 2 nodes, taken from 6 weeks old culture, in sterile media. The cuttings were incubated for 6 weeks in incubation room with 16 hours photoperiods and 18 $\pm$ 2 ^{o}C temperature.

Tuber Induction. When the cultures were 6 weeks old, liquid media containing BAP and Paclobutrazol were poured to the bottle. The media used for tuber induction were similar to those used for shoot induction, except that there was no agar, the sugar was increased to 80 g l^{-1} , and BAP and Paclobutrazol were added. The bottles containing tuber inducing media were wrapped tightly with black sheet and put in a room for 10 weeks at 21-22 ^{o}C. Every other days the bottle were check for tuber formation and culture maintenance.

Variable Measured. The variable measured in this experiment included: tuber emergence (days), percentage of productive plant (%), tuber number per productive plant, tuber number per bottle, tuber diameter, and tuber fresh weight (mg). Except for, tuber emergence observed every other day all variables were measured at harvest (10 weeks after tuber induction treatment).

Data Analysis. Data analysis was done by Analysis of variance followed by mean separation analysis with Duncan's Multiple Range Test at 5%.

RESULTS AND DISCUSIONS

Analysis of Variance. The results of analysis of variance showed that all treatment combinations of BAP and Paclobutrazol significantly affected all variable measured (Table 2).

Tuber Emergence. All treatment combinations produced tuber even without BAP or Paclobutrazol, which might be attributed to the absolute absence of light, as complete darkness has been known to promote potato microtuber formation (Suharjo *et al.*, 2008). It has also been reported that complete darkness promoted the synthesis of endogen cytokinin (Dewi, 2007). Furthermore, the fastest tuber emergence (12.8 days) was found when 5 mg l^{-1} BAP was combined with 7.5 mg l^{-1} while the longest tuber emergence (39.0 days) was found when no BAP and Paclobutrazol were added to the media (Table 2). Without BAP and Paclobutrazol, the tuber emerged after 39.0 days.

Table 2. Results of analysis of variance and coefficient of variance.

Variable observed	F-value	CV (%)
Time of tuber emergence	39.01*	11.97
% of productive plant	14.44 *	17.31
Tuber number per plant	3.44 *	19.85
Tuber number per bottle	26.61 *	18.37
Tuber diameter	6.63 *	19.05
Tuber fresh weight	45.89*	20.83

Increasing Paclobutrazol concentrations speeded up tuber formation about 7 days. However, when BAP was added to the media (5 mg l^{-1}), it speeded up tuberization up to 20 days (Table 2). This data indicated that Both BAP and Paclobutrazol promoted tuber formation. However, it seemed that BAP played more important role than Paclobutrazol in promoting tuberization. Zakaria (2010) reported that adding 4 mg l^{-1} Kinetin to the MS media promote tuberization of potato microtuber. Previous workers reported that the combination of BAP (5 mg l^{-1}) and Alar (10 mg l^{-1}) produced microtuber in the second week (Sagala *et al*., 2012). Futhermore, Gairah (2015) reported that adding BAP (5 mg l^{-1}) to MS media enriched with coconut water and 2,4 -D (10 mg l^{-1}) speed up tuber formation and increased tuber number.

The presence of retardants (Paclobutrazol, Ancymidol, CCC, or Coumarin) inhibited GA synthesis and activity, which in turned promote tuber formation (Masniawati, 2010; Suharjo et al., 2008). Lack of retardant has been reported to slow down tuber formation, as reported by Ni'mah (2015) where the tuber emerged after 10 weeks after being induced.

Productive Plan. Percentage of productive plant was measured by counting the number of plant producing tuber divided by all microcuttings planted for each experimental

unit and multiplied by 100%. The highest percentage of productive plant (93%) was found in treatment J (5.0 mg l^{-1} BAP + 7.5 mg l^{-1} Paclobutrazol while the least figure was found in media without any plant growth regulator (Table 3). These results confirmed previous research reporting that cytokinin and retardant, either applied individually or in combination, increased the number of plant producing tuber (Amalia *et al*., 2017; Setiadi *et al*., 2014). Amalia *et al*. (2017) added 1 mg l^{-1} Paclobutrazol and 90 mg l^{-1} sukrosa increased the percentage of plant producing tuber. Previous experiment reported that combining 5 mg l^{-1} and 400 ppm CCC increased the number of plant producing tuber and the number of tuber produced per plant (Setiadi *et al*., 2014). Not only in potatoes, BAP has also been reported to promote tuber formation in saitomo (Maretta *et al*., 2018)

Tuber Number per Plant. The highest number of tuber produced per productive plants (1.9 tubers per plant) was found when 5 mg l^{-1} BAP was combined with 7.5 mg l^{-1} Paclobutrazol (treatmen J) while the least number (1.0 tuber per plant) was found in control treatment (Table 3). Similar results have been reported by Ibrahim *et al*. (2015) in *in vitro* experiment. They found that 50 ppm cytokinin and 100 ppm paclobutrazol in nutrient film technique (NFT) increased the percentage of stolon become tuber per plant. Aryakia and Hamidogli (2010) reported that using cytokinin alone did not enough to promote microtuber formation, because cytokinin did not strong enough to inhibit GA activity or GA synthesis (Sakya *et al*., 2003). Therefore, to induce tuber formation, one needs to add retardant (Wattimena, 2000), such paclobutrazol, CCC, ancymidol, and coumarine (Salisbury and Ross, 1995; Suharjo *et al*., 2008). The number of tuber per plant is significantly related to the number of tuber produced per plant. It therefore the more the

Table 3. Effect of BAP and Paclobutrazol on tuber emergence, productive plants, and tuber number per plant

Treatments	BAP (mg l^{-1})	Paclobutrazol (mg l^{-1})	Time of tuber emergence (days)	Productive Plant (%)	Number of tuber per productive plant
A	0	0	39.0 a	23.2 f	1.0 d
B	0	2.5	32.1 b	30.0 f	1.4 b-d
C	0	5,0	31.8 b	46.0 e	1.3 b-d
D	0	7.5	31.0 b	56.0 de	1.1 cd
E	0	10.0	31.8 b	56.0 de	1.2 cd
F	0	12.5	31.6 b	63.2 cd	1.1 cd
G	5	0	18.1 ef	63.2 cd	1.6 b
H	5	2.5	16.6 f	72.0 bc	1.3 bc
I	5	5,0	19.8 d-f	70.0 b-d	1.4 bc
J	5	7.5	12.8 g	93.0 a	1.9 a
K	5	10.0	17.0 f	80.0 ab	1.4 bc
L	5	12.5	16.5 f	72.0 bc	1.6 b
M	10	0	19.3 d-f	70.0 b-d	1.4 bc
N	10	2.5	21.1 de	70.0 b-d	1.3 bc
O	10	5,0	20.8 d-f	70.0 b-d	1.3 bc
P	10	7.5	22.8 cd	83.2 ab	1.2 bc
Q	10	10.0	22.0 cd	72.0 bc	1.4 bc
R	10	12.5	25.3 c	72.0 bc	1.4 bc

Note: the same letter at the same column means no significantly different at 5%.

plants produced tuber, the more productive the plants were.

Tuber Number per Bottle. The combination of 5 mg l^{-1} BAP and and 7.5 mg l^{-1} Paclobutrazol (J) produced the highest number of tuber per bottle (9.3 tuber) while the control treatment (A) only produced 1.1 tuber (Table 4). These results were much higher than that reported by Gairah (2015). Using MS media supplemented with 2,4-D, coconut milk, and 5 mg l^{-1} BAP, Gairah (2015) produced 6.69 tubers per bottle. Dewi *et al*. (2016) reported that the highest number of tuber was produced when 5 ppm paclobutrazol and 150 g l^{-1} sucrose were added to the media. These results confirmed the work of Safli (2009) and Samanhudi *et al*. (2009) using retardant to increase the number of potato tuber produced *in vitro*.

Tuber Diameter. The highest tuber diameter (6.6 mm) was found in J treatment (5 mg l^{-1} BAP and and 7.5 mg l^{-1} Paclobutrazol) while the smallest (2.4 mm) was resulted by the control treatment (0 mg l^{-1} BAP and and 0 mg l^{-1} Paclobutrazol). Tuber diameter reflected the accumulation of starch in the tuber, which was very much affected by the amount of sugar absorb by the roots (Salisbury and Ross, 1992). Similar size of microtuber has also been reported by Sagala *et al*. (2012) and Misniawati (2016). The diameter of potato was 4.33 mm when Sagala *et al.* (2012) growing potato on MS media supplemented with BAP (5 mg l^{-1}) and alar (10 mg l^{-1}). Misniawati (2016) produced microtuber with 3.48 mm in diameter when the media was supplemented with 150 g l^{-1} sucrose and 5 ppm paclobutrazol. Furthermore, higher concentration of BAP seemed to inhibit tuber size, in which 10 mg l^{-1} BAP resulted in smaller tubers (Table 3). In this respect, Wattimena (1983) explained that application of cytokinin, like Kinetin, 2-ip, and BAP at 10 mg l^{-1} promoted the formation of axillary shoot, but inhibited the formation of potato tuber. In general, cytokinin promoted tuber formation only when it was applied with retardants. Yet, cytokinin has been known to promote plant growth by increase cell number (Zakaria, 2010).

Table 4. Effect of BAP and Paclobutrazol on tuber emergence, productive plants, and tuber number per plant

Treatments	BAP (mg l^{-1})	Paclobutrazol (mg l^{-1})	Number of tuber per bottle	Tuber fresh weight (mg)	Tuber diameter (mm)
A	0	0	1.1 d	50.1 i	2.4 f
B	0	2.5	2.0 d	100.2 hi	3.2 ef
C	0	5,0	3.0 c	200.1 gh	3.4 de
I	0	7.5	3.3 c	305.3 fg	3.9 bc
E	0	10.0	3.3 c	403.3 ef	3.7 cd
F	0	12.5	3.3 c	402.9 ef	4.3 bc
G	5	0	5.1 b	821.1 b	4.9 b
H	5	2.5	5.0 b	819.5 b	4.4 bc
I	5	5,0	5.0 b	817.5 b	4.6 bc
J	5	7.5	9.3 a	1614.8 a	6.6 a
K	5	10.0	5.8 b	712.1 bc	4.4 bc
L	5	12.5	5.8 b	813.4 b	4.3 bc
M	10	0	5.0 b	612.4 cd	4.7 bc
N	10	2.5	4.8 b	503,1 de	4.7 bc
O	10	5,0	4.8 b	613.2 cd	4.6 bc
P	10	7.5	5.1 b	609.1 cd	4.8 bc
Q	10	10.0	5.1 b	618.2 cd	4.5 bc
R	10	12.5	4.8 b	504.4 de	5.1 bc

Note: numbers followed by the same letter at the same column means no significantly different at α=5%.

Figure 1. Microtuber produced by a various combination of BAP and Paclobutrazol

Tuber Fresh Weight. This experiment again demonstrated that the combination of 5 mg l^{-1} BAP and 7.5 mg l^{-1} presented the best yield of potato tuber (1 614 mg) among the combinations of treatment tested (Table 3). The fresh wright of tuber was very much affected by the number and the size of tuber (Warnita, 2008). This media combination (J treatment) showed the highest number of tuber and the largest tuber size (Table 3). Cell growth takes place due to the increase in cell number, controlled by the presence of cytokinin, or due to the increase in the cell size, controlled by the activity of gibberrelic acid (Salisbury and Ross, 1995; Sakya *et al.*, 2013). In this respect, it was unlikely that increasing tuber growth was due to the increase in cell size, as the activity of gibberelic acid was inhibited by the presence of retardant. It was therefore the presence of cytokinin (BAP) and retardant (paclobutrazol) in the media was expected to promote tube growth. Previous report showed that the number and the weight of tuber increased significantly by the application of 5 mg l^{-1} cytokinin and 15 mg l^{-1} (Nuraini et al. (2016). Reducing the concentration of cytokinin and retardant to 1 mg l^{-1} (kinetin) and 7.5 mg l^{-1} (paclobutrazol) was reported to reduce tuber number and its size (Ainanur, 2004).

CONCLUSION

The best growing media to induce tuber formation, improve tuber size, and increase tuber weight *in vitro* was 5 g l^{-1} BAP combined with 7.5 mg l^{-1} paclobutrazol.

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