Effect of Paclobutrazol concentration on initial formation stolon in potato plants by in vitro at different temperature conditions

H Y B T Pane, D S Hanafiah* and R I Damanik

Faculty of Agriculture, Universitas Sumatera Utara, Medan, Indonesia.

E-mail: *diana.hanafiah@usu.ac.id

Abstract. The increase in potato production is constrained condition in Indonesia grow in the area with an altitude of 1500m above sea level. It is known that at high temperatures the gibberellin acid (GA) content in plants will increase, thus inhibiting tuber formation. The use of growth retardants such as paclobutrazol can inhibit GA biosynthesis so that it can stimulate tuber formation. In this study, the aim of this study was to determine the effect of paclobutrazol concentration on the initial formation of potato plant stolons by in vitro applied at different temperatures. The seeding stage was carried out on potato explants that had been kept for 4 weeks under 16 h of radiation. Paclobutrazol was given by pouring it into an explant bottle and keeping it in dark conditions at different temperatures for 8 weeks. From the results of the study, it is known that the application of paclobutrazol concentration and different temperatures affect the formation of micro tubers. where at a concentration of 3 ppm (P1) paclobutrazol was able to inhibit stolon elongation, stimulating subapical stolon and tuber swelling. The interactions between the application of paclobutrazol concentrations and different temperatures affected subapical stolon and tuber swelling.

1. Introduction
Potato is an annual crop that has the potential to be exported to other countries. After rice, wheat and maize, potato is the fourth main food crop in the world [1]. One of the inhibiting factors for potato productivity in Indonesia is the temperature at which the potato is a low temperature crop with a minimum temperature of 15°C [2] at an altitude of 1500 m above sea level (masl).

Light intensity, photoperiod, temperature and genotype are factors that influence potato tuber formation [3]. However, the main obstacle in the tuberisation process is its thermosensitivity in most potato cultivations, so it becomes an obstacle to the expansion of potato cultivation to tropical areas.

In lowland areas, growing potatoes will experience an increase in the synthesis of endogenous gibberellins which will inhibit tuber formation [4] and reduce the number of available carbohydrates [5]. There are two processes of potato tuber formation, namely the development of stolons and the process of forming the tubers themselves. Stolon growth is influenced by the vegetative growth of the plant, namely the number of stems produced where a large number of stems allows a large number of tubers. The size development of these stolons will later become tubers [6].

Endogenous gibberelin is a component of the signal inhibitor in potato tuberisation during the day, because at night the biosynthetic activity of gibberelin is reduced [7]. One of the efforts to increase
potato productivity in tropical climates that experience high temperatures is the use of growth regulators such as paclobutrazol [8]. Paclobutrazol is a triazole growth regulator that can inhibit GA biosynthesis and is effective in inhibiting excessive vegetative growth and increasing tuber yield and quality of potatoes grown in high temperature conditions [9].

2. Materials and methods

2.1 Time and place of research
The research was carried out in the tissue culture laboratory and screen house, University of Medan Area, North Sumatera, Indonesia.

2.2 Materials and tools
The materials used in this study were 4 weeks old potato explant (variety Olympus) and paclobutrazol. The tools used in this study were Laminar Air Flow Cabinet (LAFC), black cloth, a box with a size of 100 cm x 100 cm, a temperature thermometer.

2.3 Research implementation
The explants were sieved by subculture. The plant part was cut consisting of one book for each explant. Each culture bottle consists of 5 plant explants that are maintained for 4 weeks with 16 h of exposure under a temperature of 16-25°C. The Paclobutrazol application was carried out after 4 weeks of explants. Paclobutrazol is given by pouring it into a bottle containing explants with a treatment dose of 0 ppm (P₀), 3 ppm (P₁), 6 ppm (P₂), 9 ppm (P₃), 12 ppm (P₄), and maintained in conditions without light for 8 weeks at different temperatures, namely temperatures of 25-35°C (S₁), 25-50°C (S₂). The observed variables were stolon elongation, subapical stolon swelling and tuber formation.

2.4 Data analysis
The data was analyzed using the variance test assisted by using Microsoft Excel 2013 software. After that, if there are differences between the treatments tested, then proceed with the Duncan Multiple Range Test (DMRT) data test method with a significance 5%.

3. Results and discussion

3.1 Effect of Paclobutrazol concentration at different temperatures on shoot elongation

| Paclobutrazol | Incubation temperature | Shoot Elongation (cm) |
|---------------|------------------------|-----------------------|
| P₀ (0 ppm)    | S₁ (25-35°C)           | 16.90 a               |
| P₁ (3 ppm)    | S₁ (25-35°C)           | 3.00 bc               |
| P₂ (6 ppm)    | S₁ (25-35°C)           | 3.20 bc               |
| P₃ (9 ppm)    | S₁ (25-35°C)           | 4.40 b                |
| P₄ (12 ppm)   | S₁ (25-35°C)           | 5.50 ab               |

Note: the same letter in each column shows no significant difference at the 5% significance level using the DMRT test.
The concentration of paclobutrazol on the effect of stolon elongation (table 1). It can be seen that the application of paclobutrazol was able to inhibit shoot formation, where concentration of 3 ppm formed less when compared to other concentrations. The temperature does not affect the shoot elongation but more bud formation occurs in conditions of a temperature of 2-50 °C.

3.2 Effect of Paclobutrazol concentration at different temperatures on subapical stolon Swelling

The concentration of paclobutrazol at different temperatures affected the subapical swelling of the stolon. And there is an interaction effect between the concentration of paclobutrazol and different temperatures (table 2).

| Interaction of paclobutrazol concentration and different temperatures |     |
|---------------------------------------------------------------|-----|
| P₀S₁                                                          | 0.00d |
| P₀S₂                                                          | 0.00d |
| P₁S₁                                                          | 8.79bc |
| P₁S₂                                                          | 11.09ab |
| P₂S₁                                                          | 5.24cd |
| P₂S₂                                                          | 11.40ab |
| P₃S₁                                                          | 0.00d |
| P₃S₂                                                          | 14.66a |
| P₄S₁                                                          | 0.00d |
| P₄S₂                                                          | 10.06b |

Note: the same letter on each line shows no significant difference at the 5% significance level using the DMRT test

The interaction between giving paclobutrazol concentration at different temperatures influenced the sub apical swelling of the stolon. The most stolon swelling occurred in the paclobutrazol treatment with a concentration of 9 ppm at a temperature of 25-50°C (14.66).

3.3 Effect of Paclobutrazol concentration at different temperatures on tuber formation

The interaction between the concentration of paclobutrazol at different temperatures affected tuber formation, where tuber formation occurs most frequently in the paclobutrazol treatment with a concentration of 3 ppm at a temperature of 25-50°C (table 3).

The interaction of giving paclobutrazol concentration at different temperatures affected tuber formation. The formation of shoots is caused by an increase in GA, especially at high temperatures. The increased activity of gibberellin is due to the influence of the microclimate where the temperature and light intensity increase [4]. According to Suharjo [4], at high temperatures (29±2°C), there is an increase in plant height and inhibits the release of tubers. Shoot growth can be inhibited by using growth regulators which capable of suppressing GA biosynthesis to inhibit shoot growth. One of the growth regulators can be used to inhibit GA biosynthesis is paclobutrazol. This is because the initial application of paclobutrazol can capture the activity of gibberelic acid in plant cells which is responsible for cell elongation in plants, so the application of paclobutrazol can reduce the elongation of stems or stolons [10].
Table 3. The effect of paclobutrazol concentrations in different temperatures on tuber formation.

| Interaction of paclobutrazol concentration and different temperatures |  |
|---|---|
| P₀ S₁ | 0.00 cd |
| P₀ S₂ | 0.00 cd |
| P₁ S₁ | 12.69 b |
| P₁ S₂ | 16.31 a |
| P₂ S₁ | 6.39 bc |
| P₂ S₂ | 13.82 ab |
| P₃ S₁ | 0.00 cd |
| P₃ S₂ | 5.17 c |
| P₄ S₁ | 0.00 cd |
| P₄ S₂ | 4.89 cd |

Note: the same letter on each line shows no significant difference at the 5% significance level using the DMRT test.

In this study, the concentration of paclobutrazol, incubation temperature and the interaction between them had a significant effect on the stolon formation process. This is evidenced by the formation of stolon in treatment paclobutrazol concentration of 3 ppm at a temperature of 25-50 °C. This phenomenon is associated with reduced gibberellin synthesis in response to the application of paclobutrazol, leading to decreased cell proliferation, resulting in decreased leaf expansion [4].

![Figure 1. Elongation Stolon in treatment P₀ (0 ppm (left)) and P₁ (paclobutrazol 3 ppm (right)) on lengthening Stolon](image)

In P₀ plants (figure 1) the number of shoots was higher than in P₁, but in P₁ plants there was swelling of the stolons. the swelling of the stolons in these plants was probably due to the reduction in shoot elongation observed in the experiment as more assimilation was channeled to increase the yield of tuber growth [11]. The addition of retardants (paclobutrazol) can reduce gibberellin activity by stopping the vegetative phase (stolon elongation) so that it focuses the photosynthate flow as tuber formation and tuber enlargement [10].
In this study, the swelling of the stolons only occurred at the lower part that was directly exposed to paclobutrazol. According to Balamani and Poovaiah [12], the observation of tuber formation of underground stolon has been induced higher for all treatments.

Figure 2. (a) Swelling of the subapical stolon (b) Establishment of tuber.

The application of paclobutrazol with a concentration of 3 ppm was able to inhibit the formation of shoots resulting in swelling of the stolons which later could produce tubers (figure 2b). The treatment of plants with paclobutrazol causes an increase in the number of enzymes responsible for starch biosynthesis such as starch synthase, thereby increasing the starch content accumulation in tubers [4,13].

4. Conclusions
The application of paclobutrazol with a concentration of 3 ppm was able to inhibit stolon elongation, at a temperature of 25-50°C, where the most stolon elongation occurred. The interaction between paclobutrazol concentration and different temperatures also affected stolon swelling and tuber formation.

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