



Research Article

Production of G0 'Median' Potato Seeds by Stolons Induction Through Pruning, Nitrogen and Cytokinin Application

¹Dewi Hernawati, ²Jajang Sauman Hamdani, ²Sumadi and ²Syariful Mubarak

¹Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Sumedang 45363, Indonesia

²Doctoral Programs of Agricultural Science, Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Sumedang 43563, Indonesia

Abstract

Background and Objective: The use of pruning, nitrogen and cytokinin application to regulate plant growth and yield. The induction of stolon in potato plants is aimed to increase the number of tubers. The present study aimed to evaluate the effect of pruning, cytokinin and nitrogen application on stolon induction, growth and yield of G0 potato tuber (*Solanum tuberosum* L.) cv. Median in the medium-altitude land. **Materials and Methods:** This study was carried out at the screenhouse of Rancabango village, Tarogong Kaler Subdistrict, Garut District, West Java Province, Indonesia (700 m above sea levels). This study is arranged in split-plot design, with 2 factors, 12 combination treatments and 3 replications. The 1st factor was the pruning (P) consisted of 3 levels, i.e., no pruning (P0), pruned at 20 DAP (P1), pruned at 30 DAP (P2). The 2nd factor was the cytokinin and nitrogen application (H) that consisted of 4 levels, i.e., control (H0), 50 ppm cytokinin (H1), 100% nitrogen ZA (H2), 50 ppm cytokinin+100% nitrogen ZA (H3). **Results:** The results showed that there was an interaction effect between pruning, nitrogen and cytokinin on growth variables, such as leaf area, stomatal conductance, photosynthetic rate and production variables, such as number of stolons, percentage of stolons, number of tubers per plant, tuber weight per plant and plant dry weight. **Conclusion:** Pruning at 30 DAP combined with cytokinin and nitrogen application produced the best result, for about 9.33 tubers per plant.

Key words: Leaf area, photosynthetic rate, stomatal conductance, tuber, ZA

Citation: Hernawati, D., J.S. Hamdani, Sumadi and S. Mubarak, 2022. Production of G0 'median' potato seeds by stolons induction through pruning, nitrogen and cytokinin application. Asian J. Plant Sci., 21: XX-XX.

Corresponding Author: Jajang Sauman Hamdani, Doctoral Programs of Agricultural Science, Department of Agronomy, Faculty of Agriculture, Universitas Padjadjaran, Sumedang 43563, Indonesia

Copyright: © 2022 Dewi Hernawati *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is a top priority horticulture commodity due to its high potential to be developed as a source of carbohydrates. This plant is the 4th major food crop in the world, after rice, wheat and corn¹. This plant is mainly consumed as a carbohydrates-source tuber. The growth of population, food diversification and economic improvement causes the increasing demand for potato year by year.

Potato productivity in Indonesia is still relatively low compared to several European countries such as Belgium (44.3 t ha⁻¹) and the Netherlands (42.5 t ha⁻¹)². In tropical countries such as Indonesia, Malaysia and the Philippines, the main obstacle to increasing potato production is the availability and distribution of high-quality potato seed, especially for potato cultivation in the highlands³. Additionally, potato plantations in the highland cause some environmental damage, thus there is a need to expand the potato plantations to the lower altitude. Indonesia possess a lot of agricultural land at moderate elevation. Therefore, it is necessary to develop potato seed propagation in moderate plains with an altitude of 300-700 m above sea level that are widely available in Indonesia⁴⁻⁶. There is some modification of cultural practices that have been reported to help the seed production in the medium plains, i.e., the use of cytokinin and ethylene as PGR^{7,8} to induce stolons formation.

Cytokinin is the adenine-derived compound that contributes to the regulation of cell division and morphogenesis and is also used to stimulate bud formation, influence cell metabolism and stimulate the promotion of cell division. Benzyl amino purine (BAP) is one of the widely used cytokinins in the agricultural sector⁹. Cytokinin is used to accelerate plant growth, especially in the early vegetative stage of leaf development¹⁰.

However, the use of cytokinin solely is still less effective, thus there is a need to combine with nitrogen. Nitrogen is the macro element needed by a plant to increase their vegetative growth for supporting further generative growth, such as tuber formation and nutritional quality of tubers¹¹.

In addition to cytokinin and nitrogen application, certain culture practice such as pruning is reported to induce the formation of stolon. The basic concept of pruning is the elimination of apical dominance that commonly occurred due to the excessive level of endogenous auxin that made the inactive growth of lateral shoot. The more lateral bud growth, the larger canopy size, the more assimilates produced, the higher support for tuber formation. To achieve the best yield, there is still a challenge to determine the best time to prune.

Different pruning times may produce a different results. The potato plant generally consists of five growth phases, i.e., germination at 0-15 days, leaf development at 16-30 days, tuber initiation at 31-45 days, tuber filling at 45-90 days and tuber ripening at 91-120 days¹².

There are still limited studies on potatoes dealing with the combination of pruning, cytokinin and nitrogen application to support stolon and tuber production, especially for G0 potato. Therefore, this study aimed to evaluate the success of pruning combined with the application of cytokinin and nitrogen for inducing stolon and potato seed production.

MATERIALS AND METHODS

Study site: This study was carried out at the screen house of Rancabango Village, Tarogong Kaler Subdistrict, Garut District, West Java Province, Indonesia. The site was located at an altitude of ± 700 m above sea level. The study was carried out from July to October, 2021.

Experimental procedure: This study was arranged in a split-plot design. The main plot was pruning that consisted of 3 levels, i.e., no pruning (P0), pruning at 20 DAP (P1) and pruning at 30 DAP (P2). The second factor was the application of nitrogen and cytokinin that consisted of 4 levels, i.e., control (H0), 50 ppm cytokinin (H1), 100% nitrogen ZA (H2), 50 ppm cytokinin+100% nitrogen ZA (H3). 12 combination treatments replicated 3 times. In total there were 36 research units and each research unit consisted of 10 polybags. There was 1 plant per polybag so 360 plants were provided for present experiments.

The growing media used was a mixture of inceptisols soil, husk charcoal, compost and cocopeat with a ratio of 3:1:1:1. Furthermore, the planting media was mixed evenly and put into polybags with a size of 30×20 cm. Each polybag was labelled and arranged in a greenhouse. Potato cuttings from the selected runner were planted in polybags and then watered. Picking flowers at 60 DAP and harvesting at 100 DAP were marked by yellowing leaves and stems starting to dry out.

Measured variables: This study measured the microclimate of the greenhouse, e.g., daily relative humidity (Rh) and daily temperature by using a thermo-hygrometer at 08.00 am (morning), 01.00 pm (afternoon) and 05.00 pm (night). There result was then expressed in percentage (%) for relative humidity and Celsius for temperature observations. Both variables were measured by using the following formulas:

$$\text{Daily temperature (}^{\circ}\text{C)} = [(2 \times \text{morning temperature}) + \text{afternoon temperature} + \text{night temperature}] : 4 \quad (1)$$

$$\text{Daily relative humidity (\%)} = [(2 \times \text{Rh morning}) + \text{Rh afternoon} + \text{Rh night}] : 4 \quad (2)$$

This study also measured the leaf nutritional status, particularly the content of cytokinin and the nitrogen uptake at 8 weeks after planting (WAP) and expressed in percent (%). Plant growth was measured at 8 WAP in terms of leaf area, plant height (cm), chlorophyll content index (CCI), stomatal conductance ($\text{mmol m}^{-2} \text{ sec}$) and photosynthetic rate ($\text{CO}_2 \text{ m}^{-2} \text{ sec}$). Leaf area was measured by using the gravimetric method. Plant height was measured by a rolling meter, while CCI was determined by a chlorophyll concentration meter (Apogee). Stomatal conductance was determined by leaf porometer and the photosynthetic rate was determined by plant photosynthetic meter. At 8 WAP, the dry weight of plants and roots were measured by using the oven drying method for 2 days, at a temperature of $\pm 80^{\circ}\text{C}$. The number of tubers per plant, tuber-forming stolon (%), the number of stolons per plant were determined at harvesting day. Tuber-forming stolons (%) was calculated by dividing the number of tubers per plant by the number of stolons per plant and multiplied by 100%.

Data analysis: The data analysis in this study was carried out by using the SPSS statistical program. All obtained data were analyzed by using analysis of variance (ANOVA). If there was a significant result found, the analysis would be continued by using Duncan Multiple Range Test (DMRT) test at the α 5% level.

RESULTS AND DISCUSSION

The daily average temperature during the study was 23.8°C , the maximum temperature was 28°C and the daily minimum temperature was 18.5°C in Table 1. This condition has exceeded the optimum temperature suitable for the growth and development of potato plants, which is in the

range of $15\text{-}20^{\circ}\text{C}$ ¹³. Potato plants grown in places where the temperature exceeds the optimum can experience stress or high-temperature stress, while the temperature is an important factor for the growth and development of potato plants in addition to other environmental factors, such as relative humidity, light intensity and nutrient availability^{14,15}.

The average daily humidity during the experiment was 78.25% with the lowest daily humidity in September and the highest humidity in November (Table 1). Moisture conditions suitable for potato plants were 80-90%¹³. Relative humidity affected the rate of plant evapotranspiration, i.e., the low relative humidity, the high reduction of evapotranspiration¹⁶. In contrast, the dense humidity (too high Rh) could cause a high incidence of pest attack.

Table 2 showed there was no significant difference in cytokinin absorption level among tested treatments. The variation of cytokinin absorption in the observed potato plant was 42.927-60.086 ppm. Cytokinin levels in potato plants reached 60,086 ppm. These results explain that cytokinin was the adenine-derived compound that contributed to the regulation of cell division and morphogenesis. One of the popularly used cytokinins in the agricultural sector was benzyl amino purine (BAP)¹⁶⁻¹⁸.

Table 3 showed that there was no significant difference in nitrogen uptake level among tested treatments. The highest level of nitrogen uptake was observed in the combination treatment of no pruning and 100% Za nitrogen application for about 4.82%, while the lowest nitrogen uptake for about 3.20% was found in pruning at 30 DAP and combined with 100% Za nitrogen application.

Nitrogen is an essential macronutrient for plant growth and the nitrogen requirement for potato plants is relatively high. Nitrogen could stimulate plant vegetative growth and a lack of nitrogen elements could slow the growth. Nitrogen was needed as an energy source in the photosynthesis process. The increase in chlorophyll content was caused by the diversion of the reaction of the precursor compound geranylgeranyl pyrophosphate¹⁹.

Table 1: Microclimate data in the present study from August to November, 2021

Months	Average temperature ($^{\circ}\text{C}$)	Relative humidity (%)	Maximum temperature ($^{\circ}\text{C}$)	Minimum temperature ($^{\circ}\text{C}$)
August	23.8	75.00	28.0	18.0
September	24.5	73.00	30.0	19.0
October	24.0	80.00	28.0	18.0
November	23.0	85.00	28.0	19.0
Mean	23.8	78.25	28.5	18.5

Table 2: Cytokinin absorption levels of potato plant treated by different pruning, nitrogen and cytokinin application

Combination treatments	Cytokinin absorption (ppm)
P0H1	46.709
P0H3	45.141
P1H1	60.086
P1H3	48.001
P2H1	56.949
P2H3	42.927

P0H1: No pruning and 50 ppm cytokinin, P0H3: No pruning and combination of 50 ppm cytokinin and 100% ZA nitrogen, P1H1: Pruning at 20 DAP and 50 ppm cytokinin, P1H3: Pruning at 20 DAP and combination of 50 ppm cytokinin and 100% ZA nitrogen, P2H1: Pruning at 30 DAP and 50 ppm cytokinin, P2H3: Pruning at 30 DAP and combination of 50 ppm cytokinin and 100% ZA nitrogen

Table 3: Nitrogen total absorption of potato plant treated by different pruning, nitrogen and cytokinin application

Combination treatments	N-total (%)
P0H2	4.82
P0H3	4.62
P1H2	3.57
P1H3	4.09
P2H2	3.20
P2H3	4.11

P0H2: No pruning and 100% ZA nitrogen, P0H3: No pruning and combination of 50 ppm cytokinin and 100% ZA nitrogen, P1H2: Pruning at 20 DAP and 100% ZA nitrogen, P1H3: Pruning at 20 DAP and combination of 50 ppm cytokinin and 100% ZA nitrogen, P2H2: Pruning at 30 DAP and 100% ZA nitrogen, P2H3: Pruning at 30 DAP and combination of 50 ppm cytokinin and 100% ZA nitrogen

Table 4: Plant height (cm) and chlorophyll content index of potato plant treated by different pruning, nitrogen and cytokinin application

Treatments	Plant height (cm)	Chlorophyll Content Index (CCI)
P0	13.99 ^b	20.85 ^a
P1	11.83 ^a	22.52 ^a
P2	11.35 ^a	22.61 ^a
H0	11.40 ^a	17.89 ^a
H1	13.66 ^b	21.37 ^a
H2	13.90 ^b	24.55 ^b
H3	12.43 ^{ab}	24.16 ^b

P0: No pruning, P1: Pruning at 20 DAP, P2: Pruning at 30 DAP, H0: No treatment, H1: 50 ppm cytokinin, H2: 100% ZA nitrogen, H3: A combination of 50 ppm cytokinin and 100% ZA nitrogen. The mean is followed by different superscripts in the same column is significantly different based on the 5% DMRT test

Plant height observation carried out at 8 WAP showed that there was no interaction effect between pruning, cytokinin and nitrogen application Table 4. The absence of pruning in the treatment of 'no pruning' showed a higher yield of 13.99 cm. There is a significant reduction of plant height as the effect of pruning. However, the addition of 100% Za nitrogen was proved to successfully result from a high plant height, which was not significantly different to the 'no pruning' treatment. Nitrogen increased vegetative growth resulting in higher assimilation to supply tuber formation and increases protein and starch content in tubers²⁰. Nitrogen is a major macronutrient that is very essential because of its role

as a key element in the structure of proteins and nucleic acids, so its availability is often a limiting factor in the growth of plant communities²¹. An adequate supply of nitrogen could promote overall plant growth.

Observation of chlorophyll content index at 8 WAP resulted that there was no significant interaction effect between pruning, nitrogen and cytokine in application. The lowest est CCI was observed in the control (no treatment) for about 17.89, while the highest CCI was measured in the treatment of 100% ZA protein addition for about 24.55.

Chlorophyll was a molecule with a fibrinogen chain with a Mg atom as the central nucleus, which was surrounded by 4 nitrogen elements as branches in a chain. chlorophyll formation requires nitrogen. The amount of available nitrogen allowed the formation of more chlorophyll during the growth period. Chlorophyll content was an important factor indicating a plant in photosynthesis²². The content of chlorophyll in the leaves could affect the photosynthetic reaction, i.e., a small amount of chlorophyll certainly could reduce the rate of photosynthetic²³.

Cytokinins play a role in shoot stimulation, with the main role as cell division promoter compound²⁴. However, to achieve the desired results, the use of cytokinins solely was not sufficient. Early application of cytokinin resulted in higher chlorophyll content due to increased cytokinin which enhances chloroplast differentiation and chlorophyll biosynthesis, while also preventing chlorophyll degradation. Therefore, the initial application of cytokinin could increase the chlorophyll content.

Cytokinins maintain the integrity of the tonoplast membrane, thereby blocking the activity of an enzyme that degrades chlorophyll (chlorophyllase), which ensures that the cytoplasm could alter several aspects of cellular metabolism. This includes the continuous absorption and translocation of solutes to areas where they were needed to maintain tissue freshness. This phenomenon might be because gibberellins, cytokinins, abscisic acid and chlorophyll shared a common precursor, namely geranyl pyrophosphate, which was part of the gibberellin pathway²⁵.

Table 5 showed that there is a significant interaction effect of pruning, nitrogen and cytokinin application on leaf area, stomatal conductance and photosynthetic rate of potato plants. In the case of leaf area, the smallest leaf for about 27.7 cm² was observed in control (no pruning, no treatment of nitrogen and cytokinin). In contrast, the widest leaf was observed in the no pruning treatment combined with 50 ppm cytokinin and 100% ZA nitrogen, i.e., for about 42.63 In highland, the most suitable place for potato, the leaf well expanded. The moving of potato from highland to moderate

Table 5: Leaf area, stomatal conductance and photosynthetic rate of potato plant treated by different pruning, nitrogen and cytokinin application

Pruning treatments	Nitrogen and cytokinin treatment	Leaf area (cm ²)	Stomatal conductance (mmol m ⁻² sec)	Rate of photosynthetic (CO ₂ m ⁻² sec)
P0	H0	27.70 ^{Ab}	83.18 ^{Ab}	34.11 ^{Aa}
	H1	33.69 ^{Bb}	102.70 ^{Ba}	38.16 ^{Aa}
	H2	35.47 ^{Ba}	113.00 ^{Ba}	48.52 ^{Ba}
	H3	42.63 ^{Cb}	122.50 ^{Ca}	42.08 ^{Ba}
P1	H0	22.05 ^{Aa}	77.29 ^{Aa}	35.19 ^{Aa}
	H1	27.69 ^{Aa}	105.75 ^{Ba}	43.95 ^{Bb}
	H2	41.47 ^{Bb}	96.86 ^{Aa}	45.47 ^{Ba}
	H3	28.11 ^{Aa}	187.05 ^{Cb}	48.80 ^{Bb}
P2	H0	28.41 ^{Bb}	70.30 ^{Aa}	34.12 ^{Aa}
	H1	30.07 ^{Ba}	121.85 ^{Bb}	45.06 ^{Bb}
	H2	38.28 ^{Ca}	164.35 ^{Cb}	45.21 ^{Ba}
	H3	26.53 ^{Aa}	144.30 ^{Bb}	45.20 ^{Ba}

P0: No pruning, P1: Pruning at 20 DAP, P2: Pruning at 30 DAP, H0: No treatment, H1: 50 ppm cytokinin, H2: 100% ZA nitrogen, H3: Combination of 50 ppm cytokinin and 100% ZA nitrogen. The mean is followed by different lowercase superscripts in the same column is significantly different in terms of pruning treatment, based on the 5% DMRT test. The mean is followed by different uppercase superscripts in the same column is significantly different in terms of nitrogen and cytokinin application treatment, based on the 5% DMRT test

Table 6: Plant dry weight, root dry weight and root loss ratio of potato plant treated by different pruning, nitrogen and cytokinin application

Pruning treatments	Nitrogen and cytokinin application	Plant dry weight (g)	Root dry weight (g)	Root loss ratio (g)
P0	H0	2.62 ^{Aa}	1.08 ^{Aa}	3.10 ^{Ab}
	H1	3.77 ^{Ba}	1.42 ^{Bb}	2.72 ^{Aa}
	H2	3.33 ^{Ba}	0.71 ^{Ab}	4.58 ^{Bb}
	H3	3.86 ^{Bb}	0.89 ^{Ab}	4.57 ^{Bb}
P1	H0	2.23 ^{Aa}	1.43 ^{Bb}	1.66 ^{Aa}
	H1	5.62 ^{Bb}	1.28 ^{Bb}	4.79 ^{Bb}
	H2	2.93 ^{Aa}	1.44 ^{Bb}	7.48 ^{Cb}
	H3	1.54 ^{Aa}	0.66 ^{Aa}	2.68 ^{Aa}
P2	H0	2.93 ^{Aa}	1.16 ^{Ba}	2.93 ^{Ab}
	H1	3.78 ^{Ba}	0.83 ^{Aa}	4.80 ^{Bb}
	H2	4.94 ^{Bb}	0.13 ^{Aa}	4.53 ^{Ba}
	H3	2.58 ^{Aa}	0.80 ^{Ab}	3.49 ^{Ab}

P0: No pruning, P1: Pruning at 20 DAP, P2: Pruning at 30 DAP, H0: No treatment, H1: 50 ppm cytokinin, H2: 100% ZA nitrogen, H3: Combination of 50 ppm cytokinin and 100% ZA nitrogen. The mean is followed by different lowercase superscripts in the same column is significantly different in terms of pruning treatment, based on the 5% DMRT test. The mean is followed by different uppercase superscripts in the same column is significantly different in terms of nitrogen and cytokinin application treatment, based on the 5% DMRT test

land could cause the reduction of leaf size with a more number of leaf^{26,27}, to reduce the rate of leaf transpiration. Leaf area has also become one of plant growth variables that resulted from cell division and elongation activity that was influenced by the availability of nutrients including nitrogen²⁸, nitrogen is linearly related to leaf area in the period of vegetative growth²⁹.

In the case of stomatal conductance, there was a significant interaction effect between pruning, cytokinins and nitrogen application, as shown in Table 5. The pruning at 20 DAP combined with cytokinins and nitrogen resulted in the highest stomatal conductance value, i.e., 187.05 mmol m⁻² sec. Stomatal conductance was an important variable related to photosynthetic rate³⁰. The more stomata get closure, the lower the net assimilation rate of the plant itself. It was proved that the pruning at 20 DAP combined with cytokinins and nitrogen has also become the treatment with the highest photosynthetic rate.

Observation of plant dry weight was carried out at 8 WAP and the results was shown in Table 6. There was an interaction

between pruning, cytokinin and nitrogen application on plant dry weight, root dry weight and root loss ratio. Cytokinins had an important role in growth regulation. Cytokinins could affect various physiological, metabolic, biochemical and plant developmental processes such as cell division and enlargement, shoot and root morphology, chloroplast maturation, increase the rate of protein synthesis, delay leaf senescence and nutrient mobilization, regulate Ca in the cytosol and inhibit the effect of Auxins and GAs on stem elongation²⁵, that could be attributed to the higher dry weight of the plant.

Table 6 showed the highest plant dry weight was observed in pruning at 20 DAP combined with 50 ppm cytokinin, i.e., 5.62 g. The dry weight of the plant was not only reflect the rate of photosynthesis but also reflect the nutrient status of plants that involved in the metabolic process of dry matter products. The availability of nutrients could determine the dry matter production of plants. Dry matter production was the accumulation assimilate resulting through photosynthesis³¹.

Table 7: Number of bulbs and percentage of stolons of potato plant treated by different pruning, nitrogen and cytokinin application

Pruning treatment	Nitrogen and cytokinin application	Number of tubers per plant	Percentage of stolons (%)
P0	H0	5.00 ^{Aa}	88.18 ^{Aa}
	H1	8.00 ^{Ba}	111.58 ^{Aa}
	H2	8.33 ^{Bb}	208.33 ^{Cb}
	H3	8.33 ^{Bb}	95.24 ^{Aa}
P1	H0	5.67 ^{Aa}	107.32 ^{Aa}
	H1	8.67 ^{Bb}	185.58 ^{Bb}
	H2	6.33 ^{Ba}	106.80 ^{Aa}
	H3	7.67 ^{Ba}	153.33 ^{Bb}
P2	H0	5.30 ^{Aa}	96.97 ^{Aa}
	H1	8.33 ^{Bb}	199.84 ^{Bb}
	H2	7.30 ^{Ba}	131.66 ^{Aa}
	H3	9.33 ^{Cb}	210.68 ^{Cb}

P0: No pruning, P1: Pruning at 20 DAP, P2: Pruning at 30 DAP, H0: No treatment, H1: 50 ppm cytokinin, H2: 100% ZA nitrogen, H3: Combination of 50 ppm cytokinin and 100% ZA nitrogen. The mean is followed by different lowercase superscripts in the same column is significantly different in terms of pruning treatment, based on the 5% DMRT test. The mean is followed by different uppercase superscripts in the same column is significantly different in terms of nitrogen and cytokinin application treatment, based on the 5% DMRT test

Table 8: Stolon number and bulb weight of potato plant treated by different pruning, nitrogen and cytokinin application

Pruning treatment	Nitrogen and cytokinin application	Number of Stolons	Tuber weight per plant (g)
P0	H0	5.67 ^{Aa}	27.55 ^{Aa}
	H1	7.17 ^{Bb}	103.81 ^{Cb}
	H2	4.00 ^{Aa}	70.54 ^{Bb}
	H3	4.43 ^{Aa}	60.34 ^{Ba}
P1	H0	5.28 ^{Aa}	40.43 ^{Aa}
	H1	4.67 ^{Aa}	89.20 ^{Ca}
	H2	5.93 ^{Bb}	54.68 ^{Ba}
	H3	5.00 ^{Aa}	67.97 ^{Ba}
P2	H0	5.50 ^{Aa}	62.38 ^{Ba}
	H1	4.17 ^{Aa}	104.26 ^{Bb}
	H2	5.57 ^{Ab}	67.26 ^{Ba}
	H3	8.75 ^{Bb}	112.80 ^{Cb}

P0: No pruning, P1: Pruning at 20 DAP, P2: Pruning at 30 DAP, H0: No treatment, H1: 50 ppm cytokinin, H2: 100% ZA nitrogen, H3: Combination of 50 ppm cytokinin and 100% ZA nitrogen. The mean is followed by different lowercase superscripts in the same column is significantly different in terms of pruning treatment, based on the 5% DMRT test. The mean is followed by different uppercase superscripts in the same column is significantly different in terms of nitrogen and cytokinin application treatment, based on the 5% DMRT test

In terms of root dry weight, pruning treatment at 20 DAP and application of 100% ZA nitrogen (H2) gave the highest yield of 1.44 g. It was likely that pruning could stimulate stolons' growth. That root development in the field was influenced by root competition, texture (aeration) and nutrient availability²⁷.

The pruning, nitrogen and also cytokinin application also showed a significant interaction on the ratio of root loss. It was presumably due to plant physiology conditions in the late vegetative phase and high temperature inhibiting factors occurred. Potato plants were very susceptible to high temperatures during their life cycle, high temperatures could suppress the growth and development of plants including roots, stolons and tubers. The temperature did not cause roots to spread deeper, causing roots to become longer and more branched, causing a lower root loss ratio. Treatment in the form of pruning at 20 DAP and 100% ZA nitrogen application gave the best result for about 7.48 g.

Observation of the number of tubers per plant was carried out after harvest. The results of the analysis showed that there was an interaction between pruning, application of nitrogen

and cytokinins. Pruning at 30 DAP combined with 100% nitrogen ZA and 50 ppm cytokinin got the highest yield, which was 9.33 tubers in Table 7. The number of potato tubers was determined not only by the number of stolons formed but also by the absorption of water and nutrients from the growing media for photosynthesis and this treatment was thought to stimulate the growth of stolons. The highest percentage of tuber-forming stolons was also observed in the treatment of pruning at 30 DAP combined with 50 ppm cytokinin and 100% ZA nitrogen applications, which was 210.68.

Different pruning, nitrogen and cytokinin application resulted from a significant variation of the number of stolon and tuber weight per plant in Table 8. The pruning treatment at 30 DAP (P2) and the combination of cytokinins and nitrogen produced a good interaction effect on the number of stolons for about 8.47. Cytokinin function was to stimulate cell division and stimulate cell enlargement, thus, the increase of cytokinin application to plants could increase the number of stolons production in potato plants. Aside from cytokinin, nitrogen was also needed for supporting potato plant growth, especially stolon formation.

The pruning at 30 DAP combined with the nitrogen and cytokinins application resulted from the highest weight of tuber per plant, i.e., 112.80 g. It was likely that treatment could be produced, translocated and stored the highest amount of assimilates for supporting the expansion of potato tuber. Therefore, the practice of pruning, cytokinin and nitrogen was strongly recommended for our farmers especially farmers with tuber-G0 seed orientation products.

CONCLUSION

There was an interaction effect between the application of pruning, cytokinin and nitrogen on growth, leaf area, stomatal conductance, photosynthetic rate and quality parameters such as number of stolons, percentage of stolons formed by tubers, number of tubers per plant, tuber weight and plant dry weight. Pruning (P2) 30 DAP with the application of cytokinins and nitrogen affected the number of tubers per plant with the highest yield of 9.33 tubers, the highest percentage of stolons forming tuber and the highest tuber weight per plant.

SIGNIFICANCE STATEMENT

This study discovered the pruning, cytokinins and nitrogen that can be beneficial for potato stolon induction. This study will help the researchers to uncover the critical areas of stolon induction and its relationship to G0 potato seed production that many researchers were not able to explore. Thus a new theory on the modification of culture practice by applying the pruning, cytokinins and nitrogen to improve G0 potato seed production may be arrived at.

ACKNOWLEDGMENT

This study was financially supported by the Ministry of Education, Culture, Research and Technology through the Doctoral Dissertation Research scheme with contract No. 1207/UN6.3.1/PT.00/2021.

REFERENCES

1. Saleh, M.I., D. Rukmana and N. Hamid, 2020. Strategy development of commodity potato granola (*Solanum tuberosum* L.) in Tombolo Pao district Gowa Regency. IOP Conf. Ser.: Earth Environ. Sci., 10.1088/1755-1315/492/1/012125.
2. Supit, I., C.A. van Diepen, A.J.W. de Wit, J. Wolf, P. Kabat, B. Baruth and F. Ludwig, 2012. Assessing climate change effects on European crop yields using the crop growth monitoring system and a weather generator. Agric. Forest Meteorol., 164: 96-111.
3. Hamdani, J.S., Kusumiyati and S. Mubarak, 2018. Effect of shading net and interval of watering increase plant growth and yield of potatoes 'Atlantic'. J. Appl. Sci., 18: 19-24.
4. Hamdani, J.S. and Y.R. Suradinata, 2015. Effects of row intercropping system of corn and potato and row spacing of corn on the growth and yields of Atlantic potato cultivar planted in medium altitude. Asian J. Agric. Res., 9: 104-112.
5. Hamdani, J.S., Sumadi, Y.R. Suriadinata and L. Martins, 2016. Effects of shading and plant growth regulator on growth and yield of potato atlantik cultivar planted in medium altitude. J. Agron. Indonesia, 44: 33-39.
6. Nuraini, A., J.S. Hamdani, E. Suminar and D. Ardiansyah, 2017. Chitosan application to increase seed yield of G0 potato (*Solanum tuberosum* L.) granola cultivar on various types of growing media. J. Kultivasi, 16: 466-473.
7. Rosniawaty, S., I.R.D. Anjarsari, R. Sudirja, S.P. Harjanti and S. Mubarak, 2020. Application of coconut water and benzyl amino purine on the plant growth at second centering of tea (*Camellia sinensis*) in lowlands area of Indonesia Res. Crops, 21: 817-822.
8. Abelenda, J.A. and S. Prat, 2013. Cytokinins: Determinants of sink storage ability. Curr. Biol., 23: R561-R563.
9. Nuraini, A., P.S. Nugroho, W. Sutari, S. Mubarak and J.S. Hamdani, 2021. Effects of cytokinin and paclobutrazol application time on growth and yield of G2 potato (*Solanum tuberosum* L.) medians cultivar at medium altitude in Indonesia. Agr. Nat. Resour., 55: 171-176.
10. Shani, E., H. Ben-Gera, S. Shleizer-Burko, Y. Burko, D. Weiss and N. Ori, 2010. Cytokinin regulates compound leaf development in Tomato. The Plant Cell, 22: 3206-3217.
11. DaMatta, F.M., 2008. Ecophysiology of tropical tree crops: An introduction. Braz. J. Plant Physiol. 19: 239-244.
12. Bijma, F., E. Cator, H. Don, P. Hafkenscheid, J. Nowotarski and B. Ranjbar-Sahraei, 2016. Predicting Early Bulking in Potatoes. Proceedings of the 114th European Study Group Mathematics with Industry, 25-29 January 2016, Amsterdam, The Netherlands, 13-24.
13. Rykaczewska, K., 2015. The effect of high temperature occurring in subsequent stages of plant development on potato yield and tuber physiological defects. Am. J. Potato Res., 92: 339-349.
14. Fernie, A.R. and L. Willmitzer, 2001. Molecular and biochemical triggers of potato tuber development. Plant Physiol., 127: 1459-1465.

15. Kotak, S., J. Larkindale, U. Lee, P. von Koskull-Doring, E. Vierling and K.D. Scharf, 2007. Complexity of the heat stress response in plants. *Curr. Opin. Plant Biol.*, 10: 310-316.
16. Anjarsari, I.R.D., J.S. Hamdani, C. Suherman, T. Nurmala and H. Syahrian, 2019. Effect of pruning and cytokinin applications on growth and yield of tea (*Camellia sinensis*). *J. Ind. Beverage Crops*, 6: 61-68.
17. Mubarak, S., Kusumiyati, A.A. Fauzi, A. Nuraini, F. Rufaidah and M.A.H. Qonit, 2020. Effect of benzyl amino purine and 1-methylcyclopropene in maintaining rooting quality of chrysanthemum (*Chrysanthemum morifolium* Ramat cv. 'White Fiji') cuttings. *Res. Crops*, 21: 141-150.
18. Ngomuo, M., E. Mnene and P. Ndakidemi, 2013. The effects of auxins and cytokinin on growth and development of (*Musa* sp.) var. "Yangambi" explants in tissue culture. *Am. J. Plant Sci.*, 4: 2174-2180.
19. Rosyida, R. and A.S. Nugroho, 2017. Effect of doses of compound NPK fertilizer and PGPR (plant growth promoting rhizobacteria) on wet weight and chlorophyll levels of pakcoy plant leaves (*Brassica rapa* L.). *Bioma J. Ilm. Biol.*, 6: 42-56.
20. Sonnewald, S., J. van Harselaar, K. Ott, J. Lorenz and U. Sonnewald, 2015. How potato plants take the heat? *Procedia Environ. Sci.*, 29: 97-97.
21. Connor, D.J., R.S. Loomis and K.G. Cassman, 2011. *Crop Ecology: Productivity and Management in Agricultural Systems*. 2nd Edn., Cambridge Press, New York, ISBN: 9780511974199, Pages: 546.
22. Li, Y., N. He, J. Hou, L. Xu and C. Liu *et al.*, 2018. Factors influencing leaf chlorophyll content in natural forests at the biome scale. *Front. Ecol. Evol.*, Vol. 6. 10.3389/fevo.2018.00064.
23. Yohanis, N., 2009. *Biochemistry: Structure and Function of Biomolecules*. Graha Ilmu Indonesia Pages: 116.
24. Kieber, J.J. and G.E. Schaller, 2014. *Cytokinins*. Arabidopsis Book, Vol. 12. 10.1199/tab.0168.
25. Taiz, L., E. Zeiger, I.M. Møller and A. Murphy, 2014. *Plant Physiology and Development*. 6th Edn., Sinauer Associates, Sunderland, United States, ISBN: 9781605353265, Pages: 700.
26. Tekalign, T. and P.S. Hammes, 2005. Growth and productivity of potato as influenced by cultivar and reproductive growth: II. Growth analysis, tuber yield and quality. *Sci. Hortic.*, 105: 29-44.
27. Fleisher, D.H., D.J. Timlin and V.R. Reddy, 2006. Temperature influence on potato leaf and branch distribution and on canopy photosynthetic rate. *Agron. J.*, 98: 1442-1452.
28. Farquhar, G., T. Buckley and J. Miller, 2002. Optimal stomatal control in relation to leaf area and nitrogen content. *Silva Fenn.* 36: 625-637.
29. Lemaire, G., E. van Oosterom, J. Sheehy, M.H. Jeuffroy, A. Massignam and L. Rossato, 2007. Is crop N demand more closely related to dry matter accumulation or leaf area expansion during vegetative growth? *Field Crops Res.*, 100: 91-106.
30. Campbell, N.A., J.B. Reece and L.G. Mitchell, 2002. *Biologi*. 1st Edn., Penerbit Erlangga, Indonesia, Pages: 570.
31. Jakarta: Rajawali Press. 2002. *Plant ecology is an approach to physiology*. https://scholar.google.com/citations?view_op=view_citation&hl=en&user=kQDyht0AAAAJ&citation_for_view=kQDyht0AAAAJ:vV6vV6tmYwMC