Utilisation of Apical Stem Cutting for Fast Propagation of White Potato Seed Tubers

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Utilisation of Apical Stem Cutting for Fast Propagation of White Potato Seed Tubers

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The limited availability of disease-free, high-quality seed tubers is a major constraint for the production of white potato worldwide. This research aimed to develop a non-destructive method for fast multiplication of high-quality seed potato tubers using the apical stem cuttings that can be repeatedly harvested from the first generation of disease-free plants (G_0) for further propagation. Using soilless cultivation systems in the screen house, a higher growth rate and yield was obtained from G_0 cuttings grown in the aeroponics system compared with the hydroponics system. Both PE foam and rockwool were suitable to be used as stem holder for the aeroponically grown cuttings. Cultivation of the G_0 cuttings in the field was successful, with each cutting produced an average of 5.4 to 6.3 tubers per plant under different spacings and rates of NPK fertiliser applied. The yield of field-grown G_0 cuttings was influenced by plant spacing and application rate of NPK fertiliser. A plant spacing of 20 cm × 20 cm produced the highest seed tubers yield and the optimum application rate of NPK fertilizer was 600 kg/ha. Utilisation of the apical stem cuttings of G_0 plants may reduce the quantity of seed tubers required per unit of planting area, which in turn would reduce the production cost while increasing the supply of seedling and the yield of individual G_0 seed tubers.

Keywords: aeroponics; hydroponics; soilless culture; multiplication; rockwool; coconut fibre

I. INTRODUCTION

White potato (*Solanum tuberosum* L.) is ranked as the fourth most important staple crop in the world after rice, wheat and corn (Wattimena, 2000). In Indonesia, the land area for potato cultivation increased with the annual production of 1.2 million tons per year (Indonesian Central Bureau of Statistics, 2018a). The demand for potato in Indonesia in 2000 was 8.9 million tons (Wattimena, 2000), and it is expected to continue to increase with the increase in population, as a result of the success of food diversification program promoted by the government, the changes in the consumption patterns of the younger generation, the increase in the purchasing power, the development of potato-based industries, and the dietary needs for lower-sugar carbohydrates. The consumption of potato in Indonesia increased at a rate of 13.9% per annum, from 1,476 kg per capita per year in 2014 to 2,282 kg per capita per year in 2017 (Indonesian Central Bureau of Statistics, 2018b). As such, there is a need to increase the potato production in Indonesia by increasing the potato productivity.

The average potato productivity of 17.67 ton/ha in Indonesia (Directorate General of Horticulture, 2014) was much lower than that recorded in the Europe at 25 ton/ha (International Potato Center, 2018). One of the reasons for the lower potato productivity in Indonesia was the utilisation of low-quality seed tubers as the planting stock due to the limited availability and high cost of high-quality (certified) potato seed tubers. Until the year of 2008, the production of certified potato seed tubers in Indonesia was only 11% of the total national demand (Baharuddin *et. al.*, 2012; Ranu, 2009). In addition, the price of commercial seed tubers was 4 to 5 times higher than that of the commercial tubers. These situations highlighted the need to increase the supply and reduce the price of high-quality (certified) potato seed tubers in Indonesia.

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The high-quality seed potato production system (also seed potato propagation system) in Indonesia follows the general seed potato production schemes adopted internationally. The propagation system follows a one generation flow, which begins with the vegetative production of parent seeds in the forms of plantlets, seed tubers, and cuttings from a disease-free plantlet known as the nucleus seed. In order to maintain the quality of potato seed tubers, the propagation was monitored and certified by the seed certification authority (Directorate General of Horticulture, 2014). Most of the worldwide formal high-quality potato seed tuber production system begins with the production of true-to-type, virus- and disease-free plantlets or microtubers by tissue culture (Indonesian Central Bureau of Statistics, 2018a; Naik & Karihaloo, 2007; Ritter *et. al.*, 2001; Mbiyu *et al.*, 2012). The plantlets are then multiplied through cuttings that are used to produce the disease-free seed tubers known as the breeder seeds (designated as G_0 or generation-0 seeds) in the insect-proof screen house by soilless cultivation systems (hydroponics or aeroponics cultures) (Directorate General of Horticulture, 2014; Naik & Karihaloo, 2007; Ritter *et. al.*, 2001; Mbiyu *et al.*, 2012). The G_0 seeds are then propagated four to five times, under controlled conditions, to produce the foundation seeds (G_1–G_3) and eventually the commercial certified potato seed tubers (G_4 tubers) for the growers (Hirpa *et. al.*, 2010; Mbiyu *et. al.*, 2012; Naik & Karihaloo, 2007; Ritter *et al.*, 2001). During the propagation, potato plantation and the subsequently produced tubers can be easily infected by a wide range of insects, viruses, fungi and bacteria, which would reduce the quality of potato seed tubers produced in subsequent cycles of field propagation (Tegg & Wilson, 2016; Thomas-Sharma *et al.*, 2016).

In anticipation of the problem of high infection risks in the field, the Indonesian government has implemented a new legislation to reduce the number of propagations in the formal potato seed tuber production scheme, from five to a maximum of two cycles. Under the new production scheme, the legal commercial seed tubers now recognised in Indonesia are the G_2 tubers instead of the previously G_4 tubers (Indonesian Central Bureau of Statistics, 2018a). One tuber may produce 10 to 20 new tubers during each production cycle, depending on the field maintenance and cultivation system. Shortening the production system to two cycles under the new production scheme means a decrease in the multiplication rate of G_0 tuber by at least 100 times. Although the new regulation can increase the quality of commercial potato seed tubers, it might increase the production cost of commercial (G_2) seeds, which would decrease the supply of commercial seeds for potato growers and increase the price of certified seed potatoes. Therefore, approaches that can increase the multiplication rates of G_0 and G_1 potato seed tubers to increase the supply of certified commercial potato seed tubers are needed, and a possible alternative is by increasing the number of plants obtained from each G_0 and G_1 seed tuber via apical stem cuttings.

Stem cuttings from disease- and virus-free potato plantlets have been successfully employed in the commercial production of G_0 potato seed tubers worldwide, including in Indonesia (Directorate General of Horticulture, 2014; Mbiyu *et. al.*, 2012; Otazu, 2010). The propagation of plantlets by single-node stem cuttings for five to six cycles under controlled conditions (Naik & Karihaloo, 2007; Ritter *et. al.*, 2001; Mbiyu *et al.*, 2012) can generate 100 to 1000 virus-free plants from each plantlet. Therefore, apical stem cuttings from G_0 and G_1 plants are a promising planting stock that can be used to increase the number of G_0 and G_1 plants, which in turn would increase the supply of the foundation (G_1) and commercial (G_2) potato seed tubers for the farmers.

This study aims to investigate the use of apical stem cuttings from G_0 plants to increase the multiplication rate of foundation seeds (G_1 seeds) in soilless culture system, and also for the production of commercial potato seed tubers (G_2 seeds) in the field. Three experiments were carried out to complete the study. In the first experiment, the growth of apical stem cuttings in different soilless cultivation systems, namely hydroponics and aeroponics, for the production of high-quality seed potatoes under controlled conditions was evaluated in view of the tendency of disease-free potato seed tubers to be infected in the field. Materials other than rockwool that is commonly used in the soilless cultivation system were also tested for their suitability to substitute rockwool as cutting holder in the aeroponics system. Under the formal potato seed tubers production scheme in Indonesia, commercial seed tubers can be produced in field plantation using G_1

(foundation seeds) or potato seed tubers of higher classes, i.e. plantlets, plantlet cuttings or G_0 tubers (Directorate General of Horticulture, 2014). On the condition when less G_1 seed tubers are available, cuttings from the G_0 plants might be used for the commercial seed production in the field. However, each cutting will produce only one main stem while a seed tuber will produce five to ten haulms. Cuttings may thus have different spacing and fertilisation requirements than tubers when planted in the field. As such, in the second and third experiments, apical stem cuttings were grown in the field to examine the effects of plant spacing and rate of fertiliser application on the production of commercial potato seed tubers.

II. MATERIALS AND METHODS

A. Preparation of Apical Stem Cuttings

The same preparation was applied to apical stem cuttings sourced for all experiments. For experiment 1, the apical stem cuttings were obtained from G_0 potato plants (*Solanum tuberosum* L. variety Granola grown in an insect-proof screen house equipped with four hydroponic or aggregate culture blocks and an automatic watering system in Sembalun Timba Gading Village located at an altitude of *ca.* 1200 m above sea level. For experiments 2 and 3, the apical stem cuttings were obtained from the field-grown G_0 plants for the production of commercial seeds. Apical portions of three-node long were excised from the partially lignified stem of three- to five-week-old G_0 potato mother plants. Typically, four cuttings were made per plant. The lateral leaves of the cutting were trimmed, and the basal part of the cutting was treated with a thin layer of paste made from a mixture of IAA solution (2 ppm) and fungicide (1:1 v/w). The cuttings were planted in seedling trays (70 cuttings per tray) containing a medium composed of rice husk charcoal and coconut peat at a ratio of 3:1, and the seedling trays were placed in the screen house or in the field (as appropriate) under a 50% shading net. The medium was kept moist by regular watering and maintained until the cuttings take root for 2 weeks. The rooted cuttings with two to three leaves were then used for the experiments.

B. Experiment 1: Effect of Different Soilless Cultivation Systems on Growth and Yield of Apical Stem Cuttings

In this experiment, the growth and yield of apical stem cuttings grown in different soilless cultivation systems, namely the hydroponics (aggregate culture block) and aeroponics systems, in the insect-proof screen house at Sembalun Timba Gading Village of Sembalun District, West Nusa Tenggara, were evaluated in four replicates. Each replicate of the hydroponics system comprised of a concrete bed measuring 80 cm × 1200 cm, with a depth of 30 cm containing the mixed medium of rice husk charcoal and coconut peat. Each concrete bed held 240 cuttings planted at a spacing of 20 cm × 20 cm. The aeroponics culture systems comprised of 12 aeroponics racks, and 4 racks were allocated for each treatment testing for the use of different materials as the cutting holder, including rockwool, coconut fibre or synthetic polyethylene (PE) foam. Each replicate of the aeroponics rack comprised of 96 cuttings secured to a Styrofoam platform. The basal part of each cutting was wrapped with different materials, and the cuttings were inserted into the holes made in the Styrofoam cover at a spacing of 20 cm × 20 cm. Nutrient solution (AB hydroponic mix) was automatically sprayed to the hydroponics medium or to the cuttings' roots in the aeroponics system from the same medium tank. The growth and yield of the plants derived from apical stem cuttings was measured for five samples randomly selected from each replicate.

C. Experiment 2: Effect of Spacing on Yield of Field-grown Apical Stem Cuttings

The experiment was carried out in the field at Sembalun Bumbung Village, Sembalun District, East Lombok. Two-week-old rooted apical stem cuttings were planted in 12 planting beds of the dimension 5 m × 1 m × 30 cm ($l \times w \times h$) at different spacings (20 cm × 20 cm, 30 cm × 20 cm, and 40 cm × 20 cm). Each treatment was carried out in four replicates (four planting beds per treatment). For each replicate, the number of cuttings planted at different spacings of 20 cm × 20 cm, 30 cm × 20 cm and 40 cm × 20 cm were 125, 80 and 60, respectively. Organic fertiliser (Petroganik, PT Petrosida Gresik, Indonesia) was applied to the soil at a dose of 3000 kg/ha before planting. The planting beds were covered by silver plastic mulch with planting holes made one day before transplantation. The cutting-derived plants were maintained according to the common potato plants maintenance practice adopted by the farmers in Sembalun Bumbung Village. The plants were also given NPK (16:16:16) fertiliser at a rate of 700 kg/ha. The NPK fertiliser was applied three times, ½ dosage before planting, and ¼ dosage 4 weeks and 8 weeks after planting. Weeding was done before NPK fertiliser application. The plants were watered, weeded, and sprayed with insecticide (Ludo 310 EC, PT Deltagro Mulia Sejati, Jakarta) and fungicide (Revus 250 SC, Syngenta, Kenya) every two weeks. Watering and insecticide application were stopped 10 days before harvesting.

D. Experiment 3: Effect of NPK Fertilizer Application rate on Yield of Field-Grown Apical Stem Cuttings

In experiment 3, the two-week-old rooted apical stem cuttings from G_0 plants were planted in the planting beds of the dimension 5 m × 1 m × 30 cm at a spacing of 20 cm × 20 cm, with 125 cuttings planted per bed. Organic fertiliser (Petroganik) was applied to the soil at a rate of 3000 kg/ha during the preparation of planting bed, and the planting bed was covered with silver plastic mulch. The plants were treated with different rates of NPK fertiliser (400 kg/ha, 600 kg/ha and 800 kg/ha) in four replicates. The NPK fertiliser used in this experiment contained 16% N (NO_3 and NH_4), 16% P_2O_5 and 16% K_2O, 5% CaO and 1% MgO. Application of the fertiliser was carried out 3 times: ½ dosage before planting, ¼ dosage at 4 and 8 weeks after planting. Maintenance of the cutting-derived plants were carried out as described above.

E. Data Collection and Analysis

Data collected in the experiments included growth parameters such as plant height, number of leaves, and number of primary branches, as well as the yield in terms of the number of tubers per plant, weight of individual tubers, total weight of tuber per plant and per unit area, and tuber size distribution. The potato tubers were graded into size categories by weight according to the guidelines from the Directorate General of Horticulture (2014). The foundation (G_1) tubers were sorted into four sizes: large (>20 g per tuber), medium (10–20 g per tuber), small (5–10 g per tuber) and extra small (<5 g per tuber). The criteria of tuber size for commercial tubers were large (>50 g per tuber), medium (30–50 g per tuber), small (20–30 g per tuber) and extra small (<20 g per tuber). Analysis of variance (ANOVA) was conducted to detect if there was a significant difference among treatments at 5% significance level. Tukey's Multiple Range Tests were performed when the results from ANOVA were significant. All statistical tests were conducted using the Minitab for Windows version 16 software.

III. RESULTS

A. Effect of Different Soilless Cultivation Systems on Growth and Yield of Apical Stem Cuttings

Apical stem cuttings from G_0 plants were able to grow and produce seed tubers in the soilless culture systems. The first experiment was conducted to compare the growth and yield of apical stem cuttings grown in hydroponics and aeroponics systems. Data for the aeroponics system were average of the data for treatments of different materials used as the cutting holder. The growth, yield and size of G_1 seed tubers produced differed between cuttings grown in the hydroponics system than the aeroponics. At the age of 2 to 6 weeks after planting, there was no significant difference between the height of plants regenerated from apical stem cuttings grown in the hydroponics and aeroponics systems. The hydroponically grown plants regenerated from cuttings were taller than those grown in the aeroponics system at the age of 8 and 10 weeks after planting. The average height of potato plants from cuttings grown in the hydroponics system was 52.9 cm while those grown in the aeroponics system was 48.2 cm at the age of 10 weeks after planting (Table 1).

Table 1. The effect of different soilless cultivation systems on the height of plants regenerated from apical cuttings of G_0 potato plants over 8 weeks

Treatment	Plant height (cm)				
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
Hydroponics	22.7 ± 1.3 a	32.1 ± 2.6 a	40.0 ± 0.9 a	50.9 ± 1.0 b	52.9 ± 0.6 b
Aeroponics	26.0 ± 1.6 a	33.5 ± 1.1 a	42.3 ± 1.0 a	42.7 ± 1.5 a	48.2 ± 0.5 a

Means at each column followed by the same letter were not significantly different based on the Tukey's test at 5% significance level. The values were average of four replicates ± standard error.

However, the number of leaves was not significantly different in the cutting-derived plants grown in both hydroponics and aeroponics systems over 8 weeks of planting, with the exception that a significantly higher number of leaves was observed in the cuttings at the age of 6 weeks after planting in the aeroponics system. The numbers of leaves of the cuttings grown in the hydroponics and aeroponics system at the age of 10 weeks after planting were 47.5 and 56.4 (Table 2).

Although cuttings planted in aeroponics system was shorter than the hydroponics, the aeroponics produced 7-fold higher number of tubers per plant and four times higher yield than the hydroponics. In a hydroponics system, each cutting produced an average of only 6.1 seed tubers of 174.8 g whilst cuttings grown in aeroponics system produced an average of 44.6 tubers of 621.2 g (Table 3). However, the total tubers produced in the aeroponics system was dominated by small-sized (41.5%) and small-sized (22%) tubers. The medium-sized tuber constituted a higher proportion of the total tubers produced by the cuttings grown in the hydroponics system, with no extra small tubers produced (Table 3).

Table 2. The effect of different soilless cultivation systems on the number of leaves of G_0 cuttings over 8 weeks

Treatment	Number of leaves				
	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
Hydroponics	3.8 ± 0.4 a	13.8 ± 0.2 a	26.9 ± 0.6 a	39.0 ± 2.2 a	47.5 ± 4.6 a
Aeroponics	4.5 ± 0.2 a	14.8 ± 0.6 a	32.5 ± 0.8 b	43.7 ± 1.3 a	56.4 ± 0.8 a

Means at each column followed by the same letter were not significantly different based on the Tukey's test at 5% significance level. The values were average of four replicates ± standard error.

Table 3. The effect of different soilless cultivation systems on the yield of tubers produced by apical stem cuttings of G_0 plants

Tuber yield parameters	Types of soilless cultivation system	
	Hydroponics system	Aeroponics system
Number of tubers per plant	6.1 ± 0.3 b	44.6 ± 1.8 a
Weight of individual tubers (g)	28.5 ± 1.2 a	14.0 ± 0.2 b
Weight of tubers per plant (g)	174.8 ± 14.3 b	621.2 ± 17.8 a
Weight of tubers per unit area (g/m^2)	4369.3 ± 356.3 b	15530.7 ± 444.6 a
Proportion of tubers by size categories		
Large-sized tubers (%)	16.5 ± 1.2 b	20.4 ± 1.2 a
Medium-sized tubers (%)	61.2 ± 2.2 a	16.2 ± 0.6 b
Small-sized tubers (%)	22.3 ± 1.3 a	41.5 ± 0.9 a
Extra small-sized tubers (%)	0.0 ± 0.0 b	22.0 ± 0.6 a

Means followed by different letters across each row were significantly different based on the Tukey's test at 5% significance level. The values were average of four replicates ± standard error.

The growth of apical stem cuttings in the aeroponics system was evaluated in terms of increase in the cutting-derived plant height, number of leaves and number of primary branches of the cutting. The growth rate was inferred from the slope of the linear regression equation that described the growth parameter as a function of cutting age. The types of materials used to secure the cuttings in the aeroponics system affected the growth rates of cuttings. The plant height increments rates in cuttings kept in place with rockwool, PE foam and coconut fibre were inferred from the following linear regression equations: $y = 2.6744x + 22.492$, $y = 2.7308x + 20.854$ and $y = 2.1597x + 18.848$ (Figure 1A). Cuttings wrapped with rockwool and PE foam had a significantly higher rate of plant height increase compared with those secured in place, with coconut fibre. Similarly, higher rates of increase in the number of leaves and number of primary branches were observed in the apical stem cuttings wrapped with rockwool and PE foam compared with those held in place with coconut fibre (Figures 1B & 1C). The rates of increase in the number of leaves for cuttings wrapped with rockwool, PE foam and coconut fibre were inferred from the following linear regression equations: $y = 6.647x - 9.495$, $y = 6.6383x - 10.427$, and $y = 4.9844x - 7.1113$ (Figure 1B); those of the number of primary branches were inferred from the following equations: $y = 0.854x - 1.033$; $y = 0.8505x - 0.9441$ and $y = 0.7117x - 1.1534$ (Figure 1C).

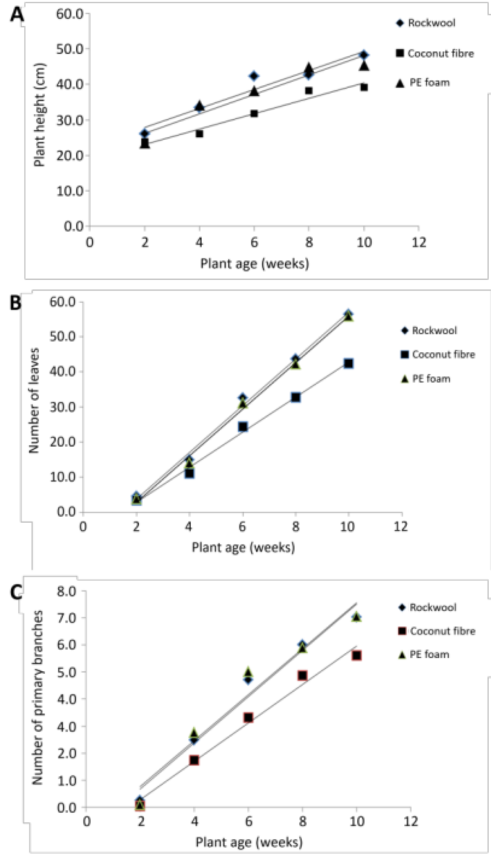

Figure 1. The effect of materials used as cutting holder in the aeroponics system on the (A) plant height, (B) number of leaves, and (C) number of primary branches of plants derived from apical stem cuttings.

The yield of G_1 tubers produced from apical stem cuttings of G_0 plants grown in the aeroponics system was influenced by the types of materials used as the stem holder. Rockwool and PE foam resulted in a two-fold higher number of tubers, and higher yield of tubers per plant and per unit area compared with plants held in place with coconut fibre. However, the average size of tubers produced by cuttings supported with different materials in the aeroponics system was not significantly different (Table 4). Similarly, there was no significant difference in the proportion of tubers of all sizes produced from cutting-derived plants held by different materials in the aeroponics system.

B. Effect of Spacing on Yield of Field-grown Apical Stem Cuttings

In the field, apical stem cuttings of G_0 mother plants were able to grow and produce an average of 5.4–5.6 tubers per plant. The number of tubers produced by the cuttings was not influenced by plant spacing. However, plant spacing had an effect on the weight of individual tubers, yield of tuber per plant and yield of tuber per unit area (Table 4). Narrower spacing of 20 cm × 20 cm resulted in the production of significantly smaller tubers (individual tubers with an average weight of 32.4 g) and lower yield per plant (175 g/plant) compared with wider spacings (30 cm × 20 cm and 40 cm × 20 cm). However, the yield of tubers per unit area decreased as the spacing increased.

Table 4. Effect of materials used as cutting holder in the aeroponics system on the yield of tubers produced by apical stem cuttings of G_0 plants

Tuber yield parameters	**Types of cutting holder**		
	Rockwool	**Coconut fibre**	**PE foam**
Number of tubers per plant	53.6 ± 4.8 b	28.1 ± 3.6 a	52.1 ± 1.6 b
Weight of individual tubers (g)	12.7 ± 2.5 a	14.6 ± 2.2 a	14.8 ± 0.9 a
Weight of tubers per plant (g)	680.3 ± 29.8 b	413.1 ± 112.0 a	770.3 ± 65.9 b
Weight of tubers per unit area (g/m²)	17006.3 ± 744.4 b	10327.4 ± 1475.2 a	19258.4 ± 1647.7 b
Proportion of tubers by size categories			
Large-sized tubers (%)	20.6 ± 1.6 a	21.5 ± 1.8 a	19.1 ± 2.2 a
Medium-sized tubers (%)	18.9 ± 1.5 b	13.4 ± 0.7 a	16.3 ± 0.9 ab
Small-sized tubers (%)	37.6 ± 2.0 a	42.2 ± 2.5 ab	44.6 ± 2.1 b
Extra small-sized tubers (%)	22.9 ± 2.2 a	23.0 ± 1.6 a	20.0 ± 1.4 a

Means followed by different letters across each row were significantly different based on the Tukey's test at 5% significance level. The values were average of four replicates ± standard error.

The yields of tubers from cuttings planted at the spacings of 20 cm × 20 cm, 30 cm × 20 cm, and 40 cm × 20 cm, were 4374.0 g/m², 3949.4 g/m² and 3164.0 g/m², respectively (Table 5).

The size of tubers produced from the cutting-derived plants was dependent on the plant spacing. The medium-sized tubers dominated the total tubers produced by cuttings grown at different spacings, with a higher proportion of medium-sized tubers produced from cuttings grown at the spacings of 20 cm × 20 cm and 30 cm × 20 cm compared with those grown at a spacing of 40 cm × 20 cm. On the other hand, the widest spacing of 40 cm × 20 cm produced a higher proportion of large-sized tubers than the narrower plant spacings (Table 5).

C. Effect of NPK Fertilizer Application Rate on Yield of Field-grown Apical Stem Cuttings

Rate The application rate of NPK fertiliser is an important factor that limits the plant growth and production. In this experiment, the application rate of NPK fertiliser did not significantly influence the number of tubers produced by each apical stem cutting from G_0 plants grown at a spacing of 20 cm × 20 cm. Each cutting produced an average of 5.6 to 6.3 tubers when treated with 400 kg/ha to 800 kg/ha NPK (16:16:16) fertiliser (Table 6). However, the application rate of NPK fertiliser did influence the weight of individual tubers, yield of tubers per plant and per unit

Table 5. Effects of plant spacing on the yield of tubers produced by apical stem cuttings of field-grown G_0 plants.

Tuber yield parameters	**Plant spacing**		
	20 cm × 20 cm	**30 cm × 20 cm**	**40 cm × 20 cm**
Number of tubers per plant	5.4 ± 0.2 a	5.6 ± 0.3 a	5.9 ± 0.2 a

Weight of individual tubers (g)	32.4 ± 0.8 a	40.3 ± 0.9 b	45.2 ± 1.4 b
Yield of tubers per plant (g)	175.0 ± 12.4 a	225.7 ± 27.0 b	253.1 ± 21.8 b
Yield of tubers per unit area (g/m^2)	4374.0 ± 61.1 c	3949.4 ± 115.6 b	3164.0 ± 23.1 a
Proportion of tubers by size categories			
Large-sized tubers (%)	19.6 ± 1.2 a	24.5 ± 1.6 ab	29.1 ± 2.9 b
Medium-sized tubers (%)	62.8 ± 3.0 b	63.3 ± 1.2 b	56.3 ± 0.8 a
Small-sized tubers (%)	17.6 ± 1.0 b	12.2 ± 1.5 a	14.6 ± 1.3 a

Means followed by different letters across each row were significantly different based on the Tukey's test at 5% significance level. The values were average of four replicates ± standard error.

Table 6. The effect of application rate of NPK fertiliser on the yield of tubers produced by apical stem cuttings of field-grown G_0 plants

Tuber yield parameters	**Application rate of NPK fertiliser (kg/ha)**		
	400	**600**	**800**
Number of tubers per plant	5.6 ± 0.9 a	6.2 ± 0.8 a	6.3 ± 0.9 a
Weight of individual tubers (g)	29.3 ± 0.9 a	38.2 ± 0.8 b	37.2 ± 0.5 b
Yield of tubers per plant (g)	164.1 ± 4.4 a	237.0 ±11.0 b	234.4 ± 5.1 b
Yield of tubers per unit area (g/m^2)	4102.0 ± 109.3 a	5925.7 ± 274.9 b	5859.0 ± 128.2 b
Proportion of tubers by size categories			
Large-sized tubers (%)	21.2 ± 0.7 a	19.2 ± 1.5 a	32.5 ± 2.6 b
Medium-sized tubers (%)	59.3 ± 1.9 a	69.6 ± 1.3 b	59.2 ± 0.7 a
Small-sized tubers (%)	19.5 ± 0.8 b	11.2 ± 1.7 a	8.3 ± 1.4 a

Means followed by different letters across each row were significantly different based on the Tukey's test at 5% significance level. The values were average of four replicates ± standard error.

area, and size of tubers produced from apical stem cuttings. Application of the lowest rate of NPK fertiliser (400 kg/ha) resulted in cutting-derived plants with smaller tubers, lower yield per plant and lower yield per unit area. A higher tuber yield per plant and per unit area was obtained from cuttings treated with 600 kg/ha and 800 kg/ha NPK, with no significant difference in the yield between treatments (Table 6).

IV. DISCUSSION

The limited availability of high-quality (certified) potato seed tubers in Indonesia is a major obstacle in the attempt to increase the potato plant productivity (Dianawati *et al.*, 2013). This was caused by many factors, including the high production cost and limitations in the availability of suitable areas for potato seed tuber production in Indonesia, number of potato seed producers, relevant production technology, as well as the nucleus and foundation seeds for mass propagation (Nikmatullah *et al.*, 2018). The limited availability and high price of nucleus and foundation seeds restricted the multiplication propagation and production of commercial potato seeds; thus, an alternative strategy needs to be developed in order to increase the supply of high-quality (certified) potato seed tubers for commercial potato production by the farmers.

Under the certified potato seed tuber production scheme, the production of G_1 tuber can begin with disease- and virus-free plantlets or G_1 tubers cultured in soilless media or sterilised soils under the controlled conditions similar to those required for the production of G_0 seeds (Directorate General of Horticulture, 2014). Apical stem cuttings from G_0 plants may be utilised in the seed potato production scheme in order to increase the multiplication rates of breeder and foundation potato seeds. Each G_0 plants commonly has 4 to 6 stems, and thus can produce an additional 4 to 6 plants by cuttings. The apical stem cuttings of G_0 plants were able to grow and produce potato seed tubers in the soilless cultivation systems (Figure 1; Tables 1–3). The results from this study suggested that cultivation of apical stem cuttings of G_0 plants by aeroponics system resulted in a higher number and yield of G_1 tubers than in hydroponics system. Cuttings grown in the hydroponics system were taller but had lower number of leaves, and produced less tubers than the cuttings grown in the aeroponics system. In line with this observation, several previous studies also reported that the aeroponics system produced higher number of stolons and tubers by 34% to 250% compared with the hydroponics system (Farran & Mingo-Castel, 2006; Ritter *et. al*., 2001; Tierno *et al*., 2014). In the aeroponics system, nutrient solution was directly applied to the roots by intermittent spraying to allow increased nutrient uptake. Chang *et al*. (2012) previously showed that plants grown in both aeroponics and hydroponics culture systems were not significantly different in shoot height and leaf areas, but the aeroponically grown plants had a higher root vitality, shoot fresh and dry weight than those grown in the hydroponics system. In addition, aeration at the root zone was higher as the root was not submerged in growing medium in the aeroponics system. The increase in root aeration has been reported to increase the plant growth and yield. In tomato plants grown under the salinity stress, for example, a higher aeration volume resulted in an increase in the total length, surface area and volume of roots, chlorophyll contents of the leaves as well as the photosynthetic rates (Li *et al*., 2019). In this study, apical stem cuttings grown by aeroponics showed a higher number of leaves at the age of 6 weeks after planting (Table 2). The higher number of leaves may increase the width of plant canopy. Plants with wider canopy and more leaves would intercept more sunlight. A higher leaf number and leaf area may result in a higher photosynthesis rate and production of carbohydrates which enhance the growth of stolon and thus tuber production (International Potato Center, 2018). In this study, each aeroponically grown cutting produced an average of 44.6 tubers compared with 6.1 tubers in the hydroponically grown cutting (Table 3). However, potato tubers produced in the aeroponics system were predominantly small and extra small, while the hydroponically grown cuttings produced mostly medium-sized tubers. The increased number of tubers produced by aeroponically grown apical stem cuttings increased the competition for nutrition and space among tubers, which resulted in smaller tubers. Further research is required to evaluate the growth and yield potential of the small and extra small tubers produced from the aeroponics system in the field. In addition, more studies are needed to increase the number of medium-sized tubers produced by cuttings in the aeroponics system.

A stem holder is required in the aeroponics system to keep the plants in place. Rockwool is commonly used as the stem holder in the soilless cultivation system because of its elasticity and water-holding capacity (Dannehl *et al*., 2015). Apart from having high total pore space for holding air and nutrient solution, rockwool is also chemically inert and sterile (Bussell & McKennie, 2004; Olympios, 1993). However, rockwool is less environmental friendly, for the material is neither degradable nor reusable (Choi *et. al*., 2014; Shinohara *et. al*., 1999; Van Os, 1994), and it is sometimes difficult to obtain in rural areas of Indonesia. The potential of using materials other than rockwool as stem holder in the aeroponics system was explored by evaluating the growth and yield of cuttings secured to the Styrofoam platform with different materials, including coconut fibre which are readily available in the villages and had been used in hydroponics culture (Shinohara *et al*., 1999). A previous study using rockwool and coconut fibre as the substrates for hydroponics culture of tomato plants showed that coconut fibre had similar water-holding capacity, electric conductivity, pH and elemental composition as rockwool, which resulted in a similar growth and yield of tomato (Shinohara *et al*., 1999). In contrast, when coconut fibre was used to hold the apical stem cuttings of potato plants in the aeroponics culture in this study, a lower growth and yield was observed compared with the cuttings kept in place with rockwool and PE foam (Figure 1 & Table 4). This may due to the different physical properties of the materials used as cutting holders. The fibres in rockwool and PE foam are less dense and softer, allowing more flexible adjustments following the growth and development of the cuttings. On the other hand, the denser, harder and less flexible coconut fibre might have presented more resistance to the penetration and elongation of roots and stolon to the base of the aeroponics system, thereby limiting nutrient uptake by the roots, which eventually led to lower growth and tuber production. Therefore, a higher number of G_1 tubers can be obtained by growing the apical stem cuttings in the aeroponics system, with PE foam used to substitute rockwool as the cutting holder during the production.

Although the aeroponics system produced higher yield than the hydroponics system, the former may not be readily adopted by the small-scale seed producers in Indonesia. The initial investment cost for setting up the an aeroponics system is considerably high, at about 24,000 dollars for a simple system to produce 80,000 minitubers a year (Mbiyu *et al.*, 2012). In view of the higher technology, infrastructure and operational costs incurred in setting up the aeroponics system (Mbiyu *et al.*, 2012), the hydroponics system is a more attractive option for the small-scale producers in Indonesia. Therefore, more studies are required to develop a simple and cost-effective aeroponics system for faster production of high-quality potato seed tubers.

Apical stem cuttings were used to produce mini cuttings from disease-free plantlets and to increase the multiplication rates of disease-free potato plantlets. This method has been used routinely in the formal seed potato production scheme including in Indonesia (Directorate General of Horticulture, 2014; Mbiyu *et. al.*, 2012; Naik & Karihaloo, 2007; Ritter *et al.*, 2001). The results presented here indicated that the apical stem cuttings of G_0 plants offer a reliable means to increase the production of G_0 tuber which is costly and of low availability by hydroponics and aeroponics systems. In addition, cultivation of the G_0 cuttings in the field was also possible with the yield of tubers dependent on the plant spacing and the application rate of NPK fertiliser.

Optimisation of the plant spacing and NPK fertiliser application rate for field-grown potato plants derived from apical stem cutting is necessary, as the plants derived from the G_0 plants cuttings grow to different sizes compared to those regenerated from plantlet cuttings or from potato seed tubers. The common spacing for production of potato in the field is 70–100 cm × 20–30 cm, depending on the size of the seed tubers used (Li *et. al.*, 2019; Masarirambi *et. al.*, 2012; Sutapradja, 2008). In the hydroponics system using apical plantlet cuttings to produce G_0 minitubers, the plant spacing ranged from 5 cm × 5 cm to 20 cm × 20 cm (Chindi *et. al.*, 2014; Naik & Karihaloo, 2007; Ritter *et. al.*, 2001; Tierno *et al.*, 2014). Adjustment of plant spacing is necessary in order to provide the optimal condition for plants and to increase the yield (Masarirambi *et. al.*, 2012; Sutapradja, 2008). A higher competition for water, nutrition and light resulted in a lower growth and yield in plants grown at narrow spacing. In this study, spacing also influenced the growth and yield of G_0 plants in the field, with wider spacings of 40 cm × 20 cm and 30 cm × 20 cm produced higher yield per plant and higher proportion of large-sized tubers. However, the narrowest spacing of 20 cm × 20 cm is more suitable for seed tubers production from G_0 cuttings in the field, as cuttings grown at this spacing produced the highest yield per unit area with the total tubers dominated by the medium-sized and small-sized tubers. Medium and small individual tubers weighing less than 50 mg are required by potato growers.

The application rate of fertiliser also influenced the growth and yield of field-grown G_0 potato cuttings. Potato plants are very responsive to fertiliser, and their productivity are greatly influenced by the rate of fertiliser applied. Potato plants commonly need about 100–150 kg N, 100–150 kg P_2O_5 and 150 kg K_2O per hectare (Kusandriani, 2014). The application rate of fertiliser is dependent on the soil fertility and potato variety. The recommended application rate of NPK fertilizer for growing potato from seed tubers at plantation in Sembalun was 700 kg/ha (Dawson & Marshall, 2012) while in West Java, the optimum application rate of ammonium nitrate-based NPK (15:15:15) fertiliser was 1000 ton/ha or 700 kg/ha for NPK (16:16:16) fertiliser (Sutrisna & Surdianto, 2014). In this study, with the exception of the number of tubers, other yield parameters such as the weight of tubers per plant and per unit area, as well as the size of tubers were significantly affected by the rate of NPK fertiliser applied (Table 6). The optimum application rate of NPK fertiliser for G_0 cuttings in the field was 600 kg/ha, which resulted in the production of 5925.7 g of tubers per unit area with medium-sized tubers constituting the highest proportion of the total tubers.

Collectively, the results of this study showed that it is feasible to use the apical stem cuttings of G_0 potato plants to increase the multiplication rate and availability of potato seed tubers. Further testing at a larger scale is required before the apical stem cuttings from G_0 plants are introduced for use under the seed potato production scheme.

V. CONCLUSION

Apical stem cuttings obtained from G_0 plants can be used to increase the multiplication rate of G_0 tubers. Of the soilless culture systems, a higher growth rate and yield was observed in the plants derived from G_0 cuttings grown in the aeroponics system compared with those grown hydroponically. Both PE foam and rockwool were found to be the better materials for use as the cutting holder in the aeroponics system. In addition, cultivation of the G_0 cuttings in the field was successful, with each cutting produced an average of 5.4 to 6.3 tubers, depending on the spacing and NPK fertiliser application rate. A higher yield of seed tubers was obtained from apical stem cuttings grown at a spacing of 20 cm × 20 cm, and the optimum rate of NPK fertilizer was 600 kg/ha.

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