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Growth and yield of apical stem cuttings of white potato (*Solanum tuberosum* L.) derived from disease-free G_0 plants

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Abstract

Growth and production of apical stem cuttings under various soil-less culture conditions for mass production of potato G_1 seed tubers was investigated. Different cutting lengths, Indole Acetic Acid (IAA) concentration, and age of mother plants from which the cuttings were taken, were evaluated for survival and growth of the cuttings. In separate experiments, successful cuttings were planted at different spacings and fertilizer rates. The highest survival rate was obtained from apical cuttings with three nodes treated with 1 ppm IAA. The best cutting growth was obtained from cuttings derived from 2 to 3 week-old mother plants. Plant height and individual leaf areas were higher at low spacing (10 cm x 10 cm), and a higher dose of NPK fertilizer (40 g/plot). The number of tubers and tuber weight per plant were higher at a spacing of 20 x 20 cm and fertilizer dose of 40 g/plot. Although higher plant density resulted in smaller tuber size, the combination of close spacing and higher doses of fertilizer resulted in the highest yield per unit area. Importantly, stock plants showed no significant decrease in plant yield after cutting. The results indicate that apical cuttings can be used for the mass-propagation of G_0 seed plants to speed up and increase the efficiency of production of G_1 seed tubers.

Key words: Apical stem cutting, auxin concentration, planting space, seed-potato tuber, soilless culture

Introduction

White potato (*Solanum tuberosum* L.) is an important cash crop in Indonesia. High quality seed-potato tubers, free from disease, are required in order to increase potato production and reduce production costs. Most of the seed potato production system is started from production of true-to-type and disease-free basic/ breeder seeds (called Generation 0 or G_0) which are regenerated from virus- and disease-free tissue culture plantlets or microtubers in a soil-less culture system (Naik and Karihaloo, 2007; Ritter *et al.*, 2001, Mbiyu *et al*., 2012). The breeder seeds are then propagated by authorized seed producers for four to five cycles under a controlled multiplication system to produce commercial certified potato seeds (Ritter *et al*., 2001; Naik and Karihaloo, 2007; Hirpa *et al*, 2010; Mbiyu *et al*., 2012). Although the plantation has been started from virus- and disease-free seed tuber**,** potato crops (and hence potato tubers used for seed) become rapidly infected by a large range of viruses, bacteria, fungi and nematodes, and these cause considerable constraints worldwide to potato-seed tuber multiplication and certification schemes (Tegg and Wilson, 2016; Thomas-Sharma *et al*., 2016). In the tropics, the possibility of pest and disease infection increases due to an increase in pest and disease diversity, and more favorable conditions such as temperature and humidity for microbial growth (Arora *et al*., 2014; Ahmed *et al*., 2015; Ata-ul-Haq, 2016), thus reducing the quality of regenerated potato seed-tubers.

For this reason, since November 2015 the Indonesian government has implemented a new legislation on certified seed potato production in order to increase the quality of seed potato in Indonesia (Direktorat Perbenihan Hortikultura, 2014). In the new legislation, the virus- and disease-free breeder/basic G_0 can only be multiplied for two cycles: 1) multiplication of G_0 seeds to produce foundation G_1 seeds under protective conditions, and then 2) multiplication of G_1 seeds to produce commercial G_2 seeds in the field (Direktorat Perbenihan Hortikultura, 2014). Under the new legislation, the approved method for production of foundation G_1 seeds is cultivation of G_0 seeds in sterilized media (soil or soil-less media) inside a screen house. Using this method, each G_0 seed may produce 10 to 20 foundation (G_1) seeds, depending on crop maintenance conditions**.** On the other hand, under previous legislation, G_0 seed could be propagated three times (*i.e.*, production of G_1 seed in protective conditions followed by production of G_2 seed in the field and then production of G_3/previously-recognized foundation seeds in the field), and each G_0 seed may result in 1,000–2,000 foundation (G_3) seeds, 100 times higher than under the current legal system in Indonesia. Therefore, there is a less availability of foundation seed for production of commercial seeds by seed producers, and this will hence decrease the availability of commercial seeds for potato growers. Therefore, there is a need to increase the multiplication rates of G_0 seed tubers to increase availability of foundation (G_1) seeds, and a visible alternative for this is *via* apical stem cuttings of G_0 plants to produce more than one G_0 plant per seed tuber.

Apical stem cuttings can be successfully employed for the mass propagation of disease-free plantlets, which enhances production of G_0 potato seeds. This system has been used for fast-multiplication of G_0 seeds in many developing countries, including Indonesia (Dawson, 2005; Naik and Karihaloo, 2007; Otazu, 2010; Mbiyu *et al*., 2012; Sumarni *et al*., 2014). Therefore, apical stem cuttings from disease-free G_0 plants may also provide an efficient mass-propagation method for production of *true-to-type,* disease-free G_1 seed tubers. The G_0 mini-tubers produce multiple-budded shoots that can be used advantageously to further

multiply the G_0 stocks under less robust conditions, if handled appropriately. In theory, this system may offer a reliable method to increase the production of disease-free G_1 tubers in a short period of time, a procedure that could be used for larger scale commercial production of G_1 potato seed tubers.

This study aims to investigate growth of apical stem cuttings from G_0 potato plants as well as growth and yield of potato plants derived from apical stem cutting under different cutting length, Indole Acetic Acid (IAA) concentration, age of mother plants, spacing and fertilizer rates. The study was undertaken with a view to establishing an efficient method for the mass propagation of G_0 seed tubers of white potato var. Granola L. to produce certified G_1 tubers using a soil-less aggregate culture system.

Material and methods

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/aggregate culture blocks and an automatic watering system. Experiment 1 comprised of two stages. The first stage was designed to investigate the effect of size of stem cuttings containing two, three or five nodes treated with different IAA concentrations (0, 0.5, 1. 2 and 4 ppm, w/v) on subsequent cutting growth and survival. The second stage involved evaluating the growth of the host G_0 plants after removing the cuttings compared with control plants from which no cuttings were removed.

The first stage was conducted as a completely randomized factorial design (two factors) while the second stage was a completely randomized block design. Both stages were in four replicates. The G_0 plants were grown in 16 experimental plots on four concrete blocks. Each block was divided into four experimental plots of 200 cm x 200 cm with 40 cm distance between each plot. The G_0 mother plants were established from certified G_0 potato-seed tubers with sprouts of *ca* 2 cm. They were grown in an aggregate culture system composed of a mixture of paddy-rice husk charcoal and coconut peat (3:1) enriched with 25 g/plot (equal to 62.5 kg/ha) NPK (containing 15 % KNO_3, 15 % P_20_5, 15 % K_2O and 10 % sulfur), 25 g/plot (equal to 62.5 kg/ha) SP36 (containing 46 % phosphorus acid), and 0.64 g/plot (equal to 1.6 kg/ha) 'Growmore' sulfur micro mix (containing 14 % S, 1 % B, 3, 2 % Cu, 7.5 % Fe, 8 % Mn, 0.4 % Mo, and 4.5 % Zn). The tubers were planted at 20 cm x 20 cm spacing, resulting in 100 plants per plot.

Apical cuttings (with 0, two, three, or five nodes) were made from 3-week old plants, and taken from all haulms on each plant. Cuttings were made using sterile surgical blades, the leaves trimmed, leaving only the apical leaf and one subsequent leaf. The cuttings (100) were treated with 0 ppm, 0.5 ppm, 1 ppm, 2 ppm and 4 ppm IAA (w/v) in four replicates. The cuttings were placed in plastic trays (15 x 25 x 4 cm, 100 cuttings/tray) containing the required concentration of IAA solution (at *ca* 1 cm depth) for 5 min. The cuttings were then planted in a seedling tray containing paddy rice/coconut husk media as described previously (50 cuttings/seedling try), and placed inside a screen house under shading net (50 %) with daily watering for 2 weeks for growth evaluation.

Survival of the cuttings is expressed as a percentage of the total cuttings that survived after 2 weeks. The height of the surviving cuttings and the number of roots and length of the roots were measured in samples selected randomly (10 samples/replicate) after the media was removed by washing carefully. The G_0 mother plants were maintained to evaluate the growth and yield after removing the cuttings. At 4 weeks after severance, NPK fertilizer (60 g/plot or equal to 150 kg/ha) and ammonium sulphate fertilizer/ZA containing 21 % of N and 24 % sulfur were applied at a dosage of 60 g/plot (equal to 150 kg/ha). The plants were harvested 100 days after planting, and 10 plants/plot were sampled for yield analysis.

In Experiment 2, all procedures were as described for the first, except where described otherwise. G_0 plants were established in 16 plots (100 plants/plot) at one-week intervals. Cuttings (with 3 nodes) were taken from the series of plants with different age (*i.e.*, plants at 2, 3, 4 and 5 weeks old, four plots for each age group). A total of 400 cuttings per plant age group were treated with 1 ppm IAA for 5 min (100 cuttings/replicate), and placed in seedling trays, 50 cuttings/ per tray. They were maintained for 14 days, and then evaluated for cutting survival and growth, as described for the first experiment.

Experiment 3 (a completely randomized block design, factorial, with four replicates) was undertaken to examine growth and yield of apical stem cuttings grown at different spacing (10 cm x 10 cm; 15 cm x 15 cm, and 20 cm x 20 cm) and dose of NPK fertilizer (20 g/plot, 30 g/plot, and 40 g/plot, equal to 125 kg/ha, 188 kg/ha and 250 kg/ha, respectively). Stock plants were established as previously described and apical stem cuttings possessing three nodes were made with sterile surgical blades, the leaves trimmed, the base of the cuttings dipped in 1 ppm IAA solution for 5 min and rooted in seedling trays for 14 days. The cuttings were transplanted onto plots (80 cm x 200 cm) containing media mixture as described for the first experiment at the planting as above, resulting in plant densities of 160, 65, and 40 plants/plot respectively. Growth and yield measurements were undertaken in plant samples (5 plants/plot) selected randomly. NPK fertilizer was given twice at half dosage (before transplanting and at 4 weeks after transplanting). In addition, the plants were given 'Growmore' sulfur micro mix (0.25 g/plot or equal to *ca.* 1.6 kg/ha), applied twice: before transplanting and at 4 weeks after transplanting), SP-36 (20 g/plot or equal to 125 kg/ha) before transplanting, and ZA (20 g/plot or equal to125 kg/ha) at 4 weeks after transplanting. The plants were watered every day to maintain the media humidity, sprayed regularly (at 2 week intervals) with insecticide (Ludo 310EC) and fungicide (Revus 250 SC), and media was added to cover the root zone at 4 and 8 weeks after transplanting. The watering and insecticide applications were stopped 10 days before harvesting.

Data collected in the third experiment included plant height, individual leaf area, time of tuber initiation, tuber weight/plant, number of tubers/plant, weight/tuber, and yield (total tuber weight/plot). The leaf area was the average of three leaves per plant (first fully expanded leaf, one leaf in the middle, and one leaf at the base of the plant). The leaf area was obtained by (1) measuring the actual leaf area of 15 different sized potato leaves using a leaf area meter and was (2) calculated by multiplying the width and length of the respective leaves. Both data sets were plotted and analyzed by linear regression to obtain an equation

relating actual leaf area and calculated leaf area. In the field, only the length and width of each leaf were measured, and the actual leaf area was determined by the linier regression equation. Data were analyzed for variance at 5 % confidential level followed by Honestly Significant Difference (HSD) test to separate actual mean values that were significantly different.

Results

Survival rate and growth of apical cuttings: Survival rate and growth of apical cuttings were dependent on cutting size, the IAA concentration applied, and the age of the mother plants used for the cuttings (Tables 1 to Tables 4). The size of cutting and IAA concentration interacted to influence the survival rate, number of roots, the length of the roots and the height of regenerated cuttings. The survival rate of plants without IAA treatment increased as the size of cutting increased. However, survival rate at the same cutting size increased as IAA concentration increased. The highest survival rate was obtained with cuttings with three nodes treated with 1 to 4 ppm IAA. In addition, the plant height increased as the IAA concentration increased, regardless the cutting size (Table 1).

There was an interaction between size of the cutting and IAA concentration in influencing the number of roots and root length of the cuttings. In cuttings treated without IAA and IAA up to 1 ppm, the number and length of roots decreased as length of cutting increased. For plants derived from cuttings of the same size, the number and length of the roots mostly increased as the concentration of IAA applied increased.

The results (Table 1 and Table 2) indicate that cuttings of good quality with high survival rates can be obtained from apical cutting bearing three nodes treated with IAA at a concentration of 1.0 ppm.

Experiment 2 was undertaken to determine whether the age of the mother plant is an important factor in producing cuttings of good quality. The results (Table 3) show that cutting survival and growth were influenced by the age of the mother plant; cuttings taken from young plants had a higher survival rate and better growth.

Table 3. Survival rate, shoot elongation, number of roots and length of roots of cuttings 2 weeks after removing them from mother plants of different age (Experiment 2)

Age of mother plant	Survival rate (%)	Shoot elongation (cm)	Number of root	Length of root (cm)
2 weeks	100 c*	9, 2 b*	15.2 b*	8.3 b*
3 weeks	100 c	8.4 ab	15.6 b	8.6 b
4 weeks	73 b	6.8 a	6.1 a	4.2 a
5 weeks	52 a	6.3 a	4.3 a	4.8 a
$HSD_{0.05}$	10.2	2.1	3.3	2.4

* Means at the same column followed by the same letters are not significantly different according to HSD test (at 5 % significant level), n =4

Table 4. Yield of G_0 potato plants following apical cutting with different number of nodes

Treatment	Number of tuber/ plant	Weight of tuber/ plant (g)	Weight per tuber (g)
Control	7.4	172.4 bc*	20.5 ab*
2 nodes cutting	8.2	182.3 c	22.2 b
3 nodes cutting	7.8	162.4 b	20.8 ab
5 nodes cutting	6.6	120.3 a	18.6 a
$HSD_{0.05}$	-**/	14.1	3.4

* Means at the same column followed by the same letter are not significantly different according to HSD test (at 5 % significant level), n=4

**/ Means without HSD values were no significant different according to analysis of variance (at 5 % significant difference)

In this study, apical stem cuttings were made for propagation of the G_0 stock plant, and therefore it is important to evaluate the seed potato production of the stock plants in order to obtain cuttings with less destructive effect to the stock plants. For this purpose, comparison was made between G_1 plants without apical stem cutting and G_1 plants with stem cuttings (three cuttings per plant) at different cutting size. The seed potato production of G_1 stock plants from which the cuttings were taken in Experiment 2 was examined in order to determine whether removal of the

Table 1. Interaction between cutting size and IAA concentration on survival rate (%) of apical cuttings and plant height after 2 weeks (Experiment 1)

IAA Concentration (ppm)	Survival Rate (%)			Plant height (cm)		
	Two nodes	Three nodes	Five nodes	Two nodes	Three nodes	Five nodes
0	7.5 a*	54.5 bc	44.8 b	5.6 a*)	8.2 b	10.4 bc
0.5	80.3 de	82.8 e	65.5 cd	6.2 ab	9.4 bc	11.3 c
1	83.8 e	100 f	75.3 de	7.6 ab	10.2 bc	13.9 d
2	85.5 ef	100 f	90.8 ef	8.5 b	11.3 c	14.6 dc
4	75.3 de	100 f	100 f	8.4 b	12.6 cd	16.4 c
$HSD_{0.05}$	16.4			2.46		

* Means of the same parameter followed by the same letter are not significantly different according to HSD test (at 5 % significant level), n=4

Table 2. Effect of cutting size and IAA concentration on the number of roots and root length after 2 weeks (Experiment 1)

IAA Concentration (ppm)	Number of roots			Length of roots (cm)		
	Two nodes	Three nodes	Five nodes	Two nodes	Three nodes	Five nodes
0	10.2 bc*	4.5 a	5.2 a	8.3 ab*)	6.4 a	6.3 a
0.5	14.4 cd	14.4 cd	8.3 b	10.1 b	8.9 ab	7.4 ab
1	16.6 d	17.1 d	12.2 c	9.4 abc	10.6 bc	8.3 ab
2	12.2 c	13.4 c	13.8 c	9.8 bc	10.2 bc	11.3 c
4	10 bc	12.1 c	12, 2 c	10.2 bc	9.4 abc	15.4 d
$HSD_{0.05}$	3.34			3.20		

* Means of the same parameter followed by the same letter are not significantly different according to HSD test (at 5 % significant level), n = 4

cuttings had a deleterious effect on the stock plants. The data (Table 4) show that removing cuttings with two or three nodes had little effect on seed potato production as indicated by the number of tubers/plant and weight of tubers/plant, but removal of cuttings with five nodes had a deleterious effect on these parameters.

Growth and yield of apical stem cuttings under different

Table 5. The effect of fertilizer treatment and plant spacing on the onset of tuber formation (Experiment 3)

Treatment	Time to onset of tuber formation (days after planting)
NPK (0.02 kg/plot)	20.4
NPK (0.03 kg/plot)	22.3
NPK (0.04 kg/plot)	27.6
$HSD_{0.05}$ */	-
Spacing distance 10 x 10 cm	23.7
Spacing distance 15 x 15 cm	21.3
Spacing distance 20 x 20 cm	22.8
$HSD_{0.05}$	-

*Means without HSD values were no significant different according to analysis of variance (at 5 % significant difference)

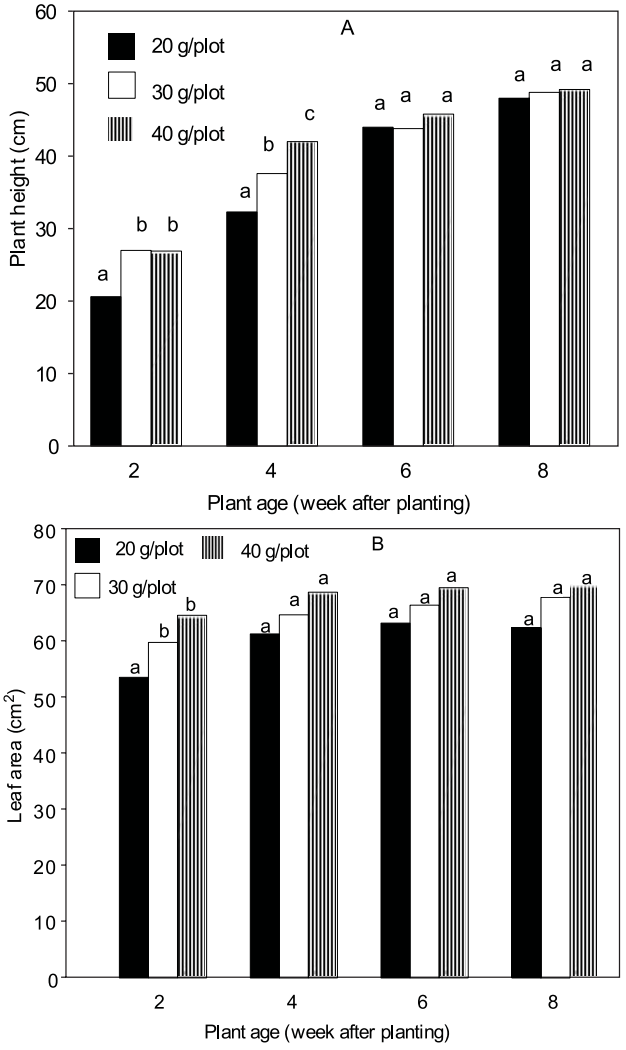

Fig. 1. Plant height (A) and leaf area (B) derived from apical stem cuttings at 2 to 8 weeks after planting treated with different rates of NPK fertilizer (20, 30, and 40 g/plot). Each value is the mean of four replicates. Mean values with the same letter are not significantly different at P=0.05 by Tukey's multiple range test

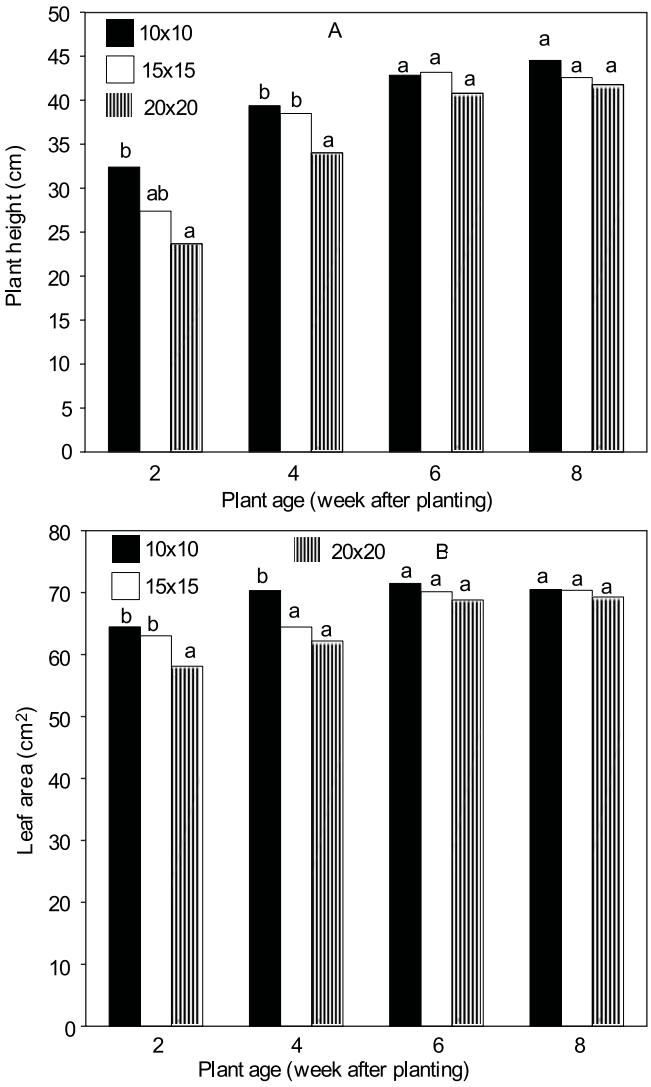

Fig. 2. Plant height (A) and leaf area (B) derived from apical stem cuttings at 2 to 8 weeks after planting treated with different spacing (10x10 cm, 15 x 15 cm, 20 x 20 cm). Each value is the mean of four replicates. Mean values with the same letter are not significantly different at P=0.05 by Tukey's multiple range test

spacing and fertilizer rates: Planting space and rates of fertilizer significantly altered plant height and the leaf area of cuttings from G_0 plants at 2 and 4 weeks after transplanting. At 2 and 4 weeks, the plant height and leaf area increased as the dosage of fertilizer increased, but it decreased as the spacing space increased (Figs. 1 and 2). However, at 6 and 8 weeks after transplanting, the plant height and leaf area of all plants were not significantly different, regardless of the NPK fertilizer rates and plant spacing.

Although fertilizer rate and planting density significantly altered the growth of the plants derived from cuttings after 2 and 4 weeks, they did not significantly affect the onset of tuberization from apical stem cuttings of G_0 plants (Table 5). The plants obtained from apical stem cuttings produced tubers at 20 to 28 days after transplanting.

Seed tuber formation was influenced by an interaction between fertilizer rate and planting space (Fig. 3). The highest number of tubers was obtained with cuttings planted at 15 x 15 cm and 20 x 20 cm and treated with 30 g/plot or 40 g/plot NPK, The weight of tubers per plant and the size of individual tubers were also higher at planting spaces of 15 x 15 cm and 20 x 20 cm.

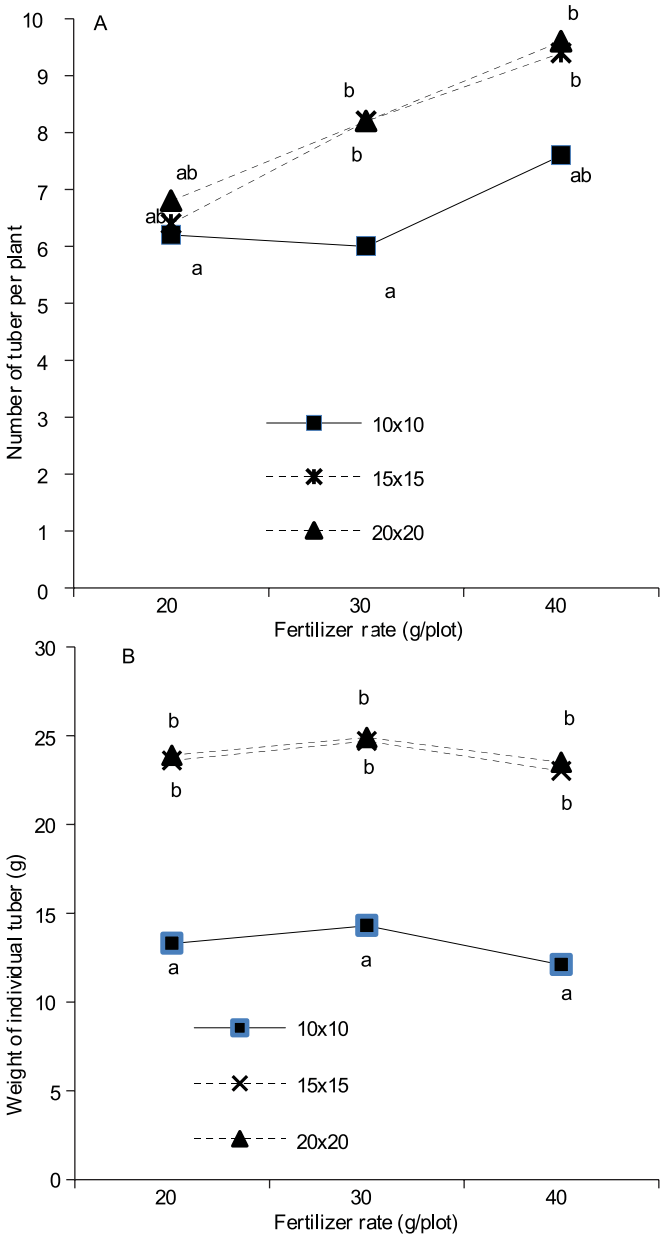

Fig. 3. Number of tuber per plant (A) and weight of individual tuber (B) derived from apical stem cuttings treated with different fertizer rates (20, 30 and 40 g/plot) and planting sapace (10 cm x 10 cm; 15 cm x 15 cm; 20 cm x 20 cm). Each value is the mean of four replicates. Mean values with the same letter are not significantly different at P=0.05 by Tukey's multiple range test

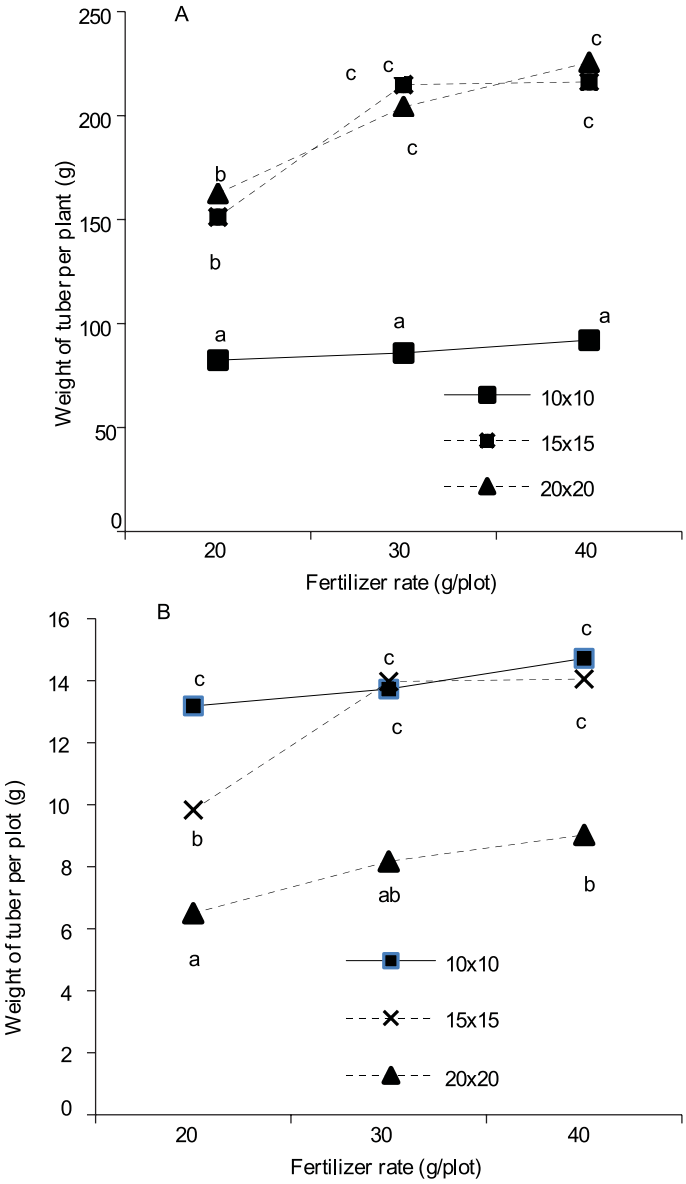

Fig. 4. Weight of tuber per plant (A) and weight of tuber per plot (B) derived from apical stem cuttings treated with different fertizer rates (20, 30 and 40 g/plot) and planting sapace (10 cm x 10 cm; 15 cm x 15 cm; 20 cm x 20 cm). Each value is the mean of four replicates. Mean values with the same letter are not significantly different at P=0.05 by Tukey's multiple range test

The weight of individual plant increased as the dosage of NPK fertilizer and spacing distance increased (Fig. 3). Although the tuber size obtained from wider plant spacing was higher, the total yield (weight of tubers/plot) obtained from widely spaced plants was significantly lower than those of narrower spacing (Fig. 4).

Discussion

The data described in this study evaluate the conditions required for the successful production of clone-derived cuttings of seed potato from G_0 seed tubers. The investigation was undertaken in order to develop an efficient method to increase multiplication rates of disease-free G_0 tubers. Using aggregate or sterile soil cultivation inside an insect-proof screen house, other studies show that each G_0 tuber may produce 5 to 25 G_1 seed tubers, depending on the cultivation system (Naik and Karihaloo, 2007; Sumarni *et al*., 2016; Azima *et al*., 2017) . The G_0 and G_1 seeds potatoes have lower risk of contamination by a pest or disease as they are the earlier stage of generation, and produced in controlled conditions inside an insect-proof house. Therefore, the price of G_0 and G_1 seed potato is predictably high.

Under previous potato seed multiplication and certification schemes in Indonesia, the G_0 and G_1 seeds were designated as pre-basic seeds and allowed to multiply for three to four stages in the field to produce foundation and commercial seeds. Therefore, foundation seeds were sufficiently available for production of commercial seeds. Under the current potato seed multiplication and certification scheme in Indonesia, G_1 seeds are now recognized as foundation seeds, and can only be propagated in one cycle to produce commercial seeds of G_2 tubers (Direktorat Perbenihan Hortikultura, 2014). The decrease in permitted multiplication cycles of the G_0 may result in less availability

and hence increased prices of G_1 seed tubers, and subsequent commercial G_2 seeds tuber. Therefore, there is a need to increase availability of affordable G_1 foundation seeds for production of commercial seeds for sustainable potato cultivation in Indonesia. Our suggested alternative is the multiplication of the G_0 plants *via* apical cutting to increase multiplication rates of G_0 plant.

The data show that the success rate to obtain cuttings is influenced by the number of nodes on the cutting (cutting length), IAA treatment, and the age of the mother plant when the cuttings are taken. The survival rate, number and length of roots were highest with cuttings with two and three nodes, and these were increased if the cuttings were treated with IAA. In theory, the length of the cutting is correlated with the endogenous reserves (*e.g.* carbohydrates) to support early growth of the cutting. In this case, under the same environmental conditions, larger cuttings would be expected to have higher reserves to support cell division and enlargement, and thus promote better root initiation and elongation. However, the data in Table 2 show that cuttings with five nodes have less ability to produce roots and have a lower survival rate, perhaps because they contained more ligneous material. Interestingly, the survival rate of larger cuttings increased as the concentration of IAA increased, but this did not occur in the smaller cuttings. IAA is widely used to promote root formation and elongation in many plant species. In plant including cuttings, IAA is produced in the plant apex, and transported basipetally (Parker & Briggs 1990; Tanimoto, 2007; Rovere *et al.*, 2013). It is possible that short cuttings accumulated a higher concentration of endogenous IAA at the base, so that when exogenous IAA was supplied, the concentration of IAA at the base may have exceeded the required concentration for the promotion of cell elongation, and thus decreased the rate of cell division and elongation.

Apical cuttings of potato plants derived from 2 and 3 week old mother plants had faster root and shoot growth and a higher survival rate. The age of the mother plants determines the maturity of the stem from which the cuttings are taken: maturity increases as the physiological age of the mother plant increases (Rasmussen *et al*., 2015). Juvenile cuttings have been shown to root better than mature cuttings possibly due increase of lignifications in older cutting and the production of a rooting inhibitor as the stem age is increased (Milborrow, 1994).

Cutting growth and yield are important aspects if the use of G_0 apical cutting as a multiplication system in seed potato and certification schemes is to be economically viable. Several cultivation treatments play an important role in the growth and yield of plants, including planting space (plant density) and rate of fertilizer (Oliveira *et al*., 2000; Ma *et al*., 2003; Zheng *et al*., 2016). In potatoes, many investigations demonstrated that tuber yield and size are influenced by planting density and planting space. Decrease in spacing resulted in an increase in plant density, and this may increase the tuber number/unit area, but decrease individual tuber weight (Negi *et al*., 1995; Gronowiz *et al*., 1990; Oliveira *et al*., 2000; Zheng *et al*., 2016). On the other hand, individual tuber weight may increase with the increasing distance but total yield may decrease (Negi *et al*., 1995; Gronowiz *et al*., 1990; Oliveira *et al*., 2000; Zheng *et al*., 2016). Therefore, obtaining suitable planting space is very important for seed potato production in order to produce a high yield plantation with high percentage of tuber with favourable seed-tuber weight (size). Common spacings for seed potato production from seed tuber in the field are 60-100 cm (row spacing) and 20-30 cm (intra row spacing) (Qasim *et al*., 2013; de Almaida *et al*., 2016; Lehar *et al*., 2017), depending on the size of seed tuber, fertilizer rates, cultivation areas and system. When cuttings are used as starting material for plantation, smaller plant size (canopy) is likely as cutting will produce only one main stem/plant compared to 5 to 10 haulms/seed tuber, and thus spacing requirement will be different. Similarly, fertilizer requirements will also different between plants derived from cutting and seed tuber.

In this study, planting of apical cuttings in narrower spacings (10 x 10 cm) and at a higher rate of NPK fertilizer (0.04 kg/ plot) resulted in taller plants with wider leaf areas at 2 and 4 weeks after transplanting. After 4 weeks, any spacing and rate of fertilizer treatment had no difference in growth parameter. In addition, planting space and fertilizer rate altered the yield of seed tubers obtained from each cutting. Tuber number, tuber size and individual tuber weight increased as the planting space increased from 10 x 10 cm to 20 x 20 cm. However, the narrower space resulted in higher total yields as the population doubled in the narrower space. Interestingly, planting space and fertilizer dosage did not alter the onset of tuber formation. Previously, using seed tuber as planting material, Oliveira *et al*. (2000) showed that narrower spacing resulted in an increase in above ground stem elongation and leaf area index before 70 days of planting, and this increase was suggested to be the plants' natural response to light competition. Higher light competition occurs at narrower spaces and the plant compensates to different photosynthetically-active radiation by changing above ground plant morphology, such as increasing plant height and specific leaf areas in the upper part of the plant (Oliveira *et al*., 2000). In addition, such changes were also influenced by the application rate of nitrogen fertilizer, whereby increased nitrogen application led to an increase in leaf area index and total yield of potato (Ma *et al*., 2003).

The results of our study using apical stem cuttings as starting material are similar to Gronowizc *et al*.'s (1990) study using seed tubers, in which it was found that there was an interaction between planting space and fertilizer dosage. In contrast to the data in Table 6 using stem cuttings, Oliveira *et al*. (2000) found that higher nitrogen applications delayed the onset of formation of first tuber when tubers were used as the starting material. These authors suggested that at a higher dosage of second application of fertilizer, the nitrogen enhanced new haulm formation and above ground growth from the seed tuber and thus delayed the onset of new tuber formation by 10 days.

This situation does not occur if apical stem cuttings are used as the source material, as apical cuttings have only one shoot. The onset of tuberization in apical cuttings reported in our study occurred earlier than in tuber-regenerated plants, and the plants were harvested at 80 to 90 days after transplanting whilst tuber regenerated plants of the Granola L variety had an average age of harvest of 100 days. Thus the formation of minitubers from apical cuttings can shorten the production time needed for the production of G_1 minitubers.

Overall, the results reported here suggest that the use of apical stem cuttings offers a fast and efficient method for mass propagation of G_0 plants, and can be utilized to increase yield of individual G_0 tubers. Cuttings of two or three nodes did not

have deleterious effect on mother plants, and thus by having an additional three to five plants/G_0 tuber will result in increased yields of resulting G_1 seed tuber. However, studies need to be carried out at a larger scale before this system can be utilized commercially as a strategy to speed up the production of quality G_1 seeds, particularly if the increased demand of basic seed (G_1 tubers) for production of commercial potato seeds is to be met under the new regulation in Indonesia.

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References

Ahmed, N., M.A. Khan, N.A. Khan and M.A. Ali, 2015. Prediction of potato late blight disease based upon environmental factors in Faisalabad, Pakistan. *J. Plant Pathol. Microbiol.,* S3:008. doi:10.4172/2157-7471.S3-008

Arora, R.K., S. Sharma and B.P. Singh, 2014. Late blight disease of potato and its management. *Potato J.,* 41(1): 16-40.

Ata-ul-Haq, Y. Iftikar, M.I. Ullah, Q. Shakeel, M. Mubeen, Q. Shaleel, F. Bakhtawar and I. Bilqees, 2016. Disease progression in potato germplasm from different reaction groups against potato virus Y in relation to environmental factors. *Trop. Plant Res.,* 3 (3): 600- 605.

Azima, N.S., A. Nuraini, J. Sumadi and S. Hamdani, 2017. Growth and yield respons of G0 potato seed to times and methods of paclobutrazol applicatıon in moderate altitude. *Jurnal Kultivasi,* 16 (2): 313-319

Dawson, P. 2005. *AGB/2005/167 Potato seed system development – alternative potato seed supply system for Indonesia.* Final Report, Appedix 7. Austral. Ctr. for Intl. Agric. Res., Australia.

de Almeida, F.M., J.A. Sánchez, A.T. de la Noval and J.A.C. Rodríguez, 2016. Effects of different plant spacings and seed tuber sizes on some morpho-productive characteristics of potato in Huambo, Angola. *Cul. Trop.*, 37 (2): 88-95. http://dx.doi.org/10.13140/RG.2.1.1452.1842

Direktorat Perbenihan Hortikultura, Direktorat Jenderal Hortikultura, 2014. *Teknis Perbanyakan dan Sertifikasi Benih Kentang.* Direktorat Perbenihan Hortikultura, Direktorat Jenderal Hortikultura, Jakarta, Indonesia.

Gronowizc, Z., A. Zielinska and K. Wencl-Owics, 1990. Effect of some agrotechnical factors on the yield of potatoes *cv.* Duet. *Acta Acad. Agric. ac Tech. Olstenesis,* 51: 43-51.

Hirpa, A., M.P.M. Meuwissen, A. Tesfaye, W.J.M. Lommen, A.O. Lansink, A. Tsegaye and P.C. Struik, 2010. Analysis of seed potato systems in Ethiopia. *Amer. J. Pot. Res.,* 87*:*537-552. DOI 10.1007/s12230-010-9164-1

Lehar, L., T. Wardiyati, M. Dawam and A. Suryanto, 2017. Influence of mulch and plant spacing on yield of *Solanum tuberosum* L .cv. Nadiya at medium altitude. *Inter. Food Res. J.,* 24(3): 1338-1344. *http://www.ifrj.upm.edu.my*

Ma, L., L.M. Dwyer and C. Costa, 2003. Row spacing and fertilizer nitrogen effects on plant growth and grain yield of maize. *Can. J. Plant Sci.,* 83: 241-247

Mbiyu, M.W., J. Muthoni, J. Kabira, G. Elmar, C. Muchira, P. Pwaipwai, J. Ngaruiya, S. Otieno and J. Onditi, 2012. Use of aeroponics technique for potato (*Solanum tuberosum*) minitubers production in Kenya. *J. Hort. For.,* 4(11): 172-177, DOI: 10.5897/JHF12.012

Milborrow, B.V. 1994. Inhibitors. In: *Advanced Plant Physiology*. M.B. Wilkins (ed.). Pitman Publishing, London,

Naik, P.S. and J.L. Karihaloo, 2007. *Micropropagation of production of quality potato seed in Asia Pacific.* Asia Pacific Consor. on Agric.Biotech. (APCoAB), Asia-Pacific Assoc. of Agric. Res. Ins. (APAARI), Bangkok, Thailand.

Negi, S.C., J. Shekhar and J.P. Saini, 1995. Effect of seed size and spacing on potato production*. Indian J. Agr. Sci.,* 65: 286-287

Oliveira, C.A.D.S., 2000. Potato crop growth as affected by nitrogen and plant density. *Pesquisa Agropecuaria Brasileira,* 35(5): 940-950. http://dx.doi.org/10.1590/S0100-204X2000000500011

Otazu, V. 2010. *Manual on quality seed potato production using aeroponics*. International Potato Center (CIP). Lima. Peru.

Parker, K.E. and W.R. Briggs, 1990. Transport of Indole-3-Acetic Acid during gravitropism in intact maize coleoptiles. *Plant Physiol*., 94: 1762-1769.

Qasim, M., S. Khalid, A. Naz, M.Z. Khan and S.A. Khan, 2013. Effects of different planting systems on yield of potato crop in Kaghan Valley:a mountainous region of Pakistan. *Agr. Sci.,* 4: 175-179. http://dx.doi.org/10.4236/as.2013.44025

Rasmussen, A., S.A. Hosseini, M.R. Hajirezaei, U. Druege and D. Geelen, 2015. Adventitious rooting declines with the vegetative to reproductive switch and involves a changed auxin homeostasis. *J. Exp. Bot.*, 66 (5):1437-1452. doi:10.1093/jxb/eru499

Ritter, E., B. Angulo, P. Riga, C. Herran, J. Relloso and M. Sanjose, 2001. Comparison of hydroponic and aeroponic cultivation systems for the production of potato minitubers. *Potato Res.,* 44: 129-135

Rovere, F.D., L. Fattorini, S.D. Angeli, A.Veloccia, G. Falasca and M.M. Altamura, 2013. Auxin and cytokinin control formation of the quiescent centre in the adventitious root apex of Arabidopsis. *Ann. of Bot.,* 112: 1395-407. doi:10.1093/aob/mct215

Sumarni E., Ardiansyah and N. Farid, 2014. Aplikasi nozel pada dua varietas kentang untuk produksi benih secara aeroponik. *J. Biofisika,* 10(1): 1-7

Tanimoto, 2007. Regulation of root growth by plant hormones - role for auxin and gibberellins*. Crit. Rev. in Plant Sci.,* 24(4): 249-265. http://dx.doi.org/10.1080/0735268050019610

Tegg, R.S. and C.R. Wilson, 2016. QPCR testing seed potato tubers for pathogens – what value for potato seed certification? *Acta Hort.*, 1118: 117-124.

Thomas-Sharma, S., A. Abdurrahman, S. Ali, J.L. Andrade-Piedra, S. Bao, A.O. Charkowski, D. Crook, M. Kadian, P. Kromaan, P.C. Struik, L. Torance, K.A. Garrett and G.A. Fobes, 2016. Seed`tuber degradation in potato: the need for a new research and development paradigm to mitigate the problem in developing countries. *Plant Patho.,* 65: 3-16. https://doi.org/10.111/ppa.12439.

Zheng, S.L., L.J. Wang, N.X. Wan, L. Zhong, S.M. Zhou and J.C. Yuan, 2016. Response of potato tuber number and spatial distribution to plant density in different growing seasons in Southwest China. *Front. Plant Sci*., 7: 365. doi: 10.3389/fpls.2016.0036

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