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### Effect of Photoperiod and Nutritional-Shock on Increasing the Number of Minitubers from Apical Rooted Cuttings Grown in Coco-Peat

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#### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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#### ABSTRACT

Currently, a variety of techniques for producing potato minituber have been investigated, including soilless aeroponic, hydroponic and deep-water culture systems. However, minitubers potato production in the greenhouse from apical rooted cutting grown coco-peat under a photoperiod and nutritional - shock system is a new and creative idea. Therefore, the objective of the study was to optimize the effect of photoperiod and nutritional-shock on growth performance and yield of first generation potato tuber production of potato plants. Potato variety 'Atlantic' was treated under 5 photoperiod - (8 h/day, 9 h/day, 10 h/day, 11 h/day and natural light) under nutritional-shock process with reducing N concentration in nutrient solution (0;25%; 50%;75% and 100%), the growth stage of plant to conduct nutritional-shock (30, 35, 40, 45 and 50 DAT), the implementation time of nutritional-shock (12, 24, 48 and 60 hours) and the combination of photoperiod (9 h/day, 10 h/day) and nutritional-shock (75%, 100%) on the formation and development of minitubers. The study revealed that photoperiod at 10h/day showed the best result with 7.4 tubers/ plant and 569.7 tubers m<sup>-2</sup> The number of tuber stolons, average number of tubers and tuber yield was directly proportional to the decreasing in N concentration in the nutrient solution, in which the highest was the 100% reduction in N concentration with 9.3 tubers/plant, higher than the control treatment 300%. The nutrient shock treatment at 30 DAT in 48 ours gave the best tuber production efficiency. There was a strong interaction between photoperiod and nutritional-shock on the formation and yield of potatoes (10.6 tubers/plant, 308 tubers m<sup>-2</sup>).

Keywords: Atlantic; minitubers potato; nutritional-shock; photoperiod; tuberization.

#### **1. INTRODUCTION**

Potato (*Solanum tuberosum* L.) is one of the most valuable crops in the world, ranked as the 4th most important food crops after rice, wheat and maize [1]. The potato area in the world reached 19.30 million hectares, with an average yield of 20.11 tons/ha, with an output of 388.2 million tons [2]. It consits of a high source of carbohydrates, proteins, vitamins, and minerals, so potato is currently consider a solution for feeding more than a billion people [3]. Therefore, enhancing the productivity of this root crop may be a key tool in fulfilling the nutritional requirement and the demand of minitubers for production practices.

Potato seed quality is an important factor determining yield. The long-term use of seed tubers produced in the field contributes to the accumulation of pathogens, especially viruses, leading to seed degradation and reduced yield and quality of potatoes [4]. Over the years, the use of basic potato seed minitubers in seed production has revolutionized the potato production cycle in the field and creating a large number of disease clean seed potatoes supplying for production. Basic potato seed minitubers are obtained from seed production on in vitro-derived seedling by different growing methods [5].

According to Ranalii (1997), the production of basic potato seed minitubers by in vitro culture has high propagation coefficient and reduced genotype variations when grown in production. Basic potato seed minitubers can also be produced by growing plants in greenhouses with high density [6,7], using thin film technology (NFT - Nutrient Film Technique) or growing in hydroponics systems [8]. Besides the production of basic potato seed minitubers without soil as above, aeroponics technology has also been researched and perfected and used to produce basic potato seed minitubers with manv advantages outstanding [4]. However. aeroponics technology still has limitations in the production of potato seed with the high investment cost, strict cultivation process, difficult to spread the model, especially the quality of seed tubers in terms of biology must be compared with other cultivation methods.

In Vietnam, Potato Vegetable & Flower Research Center has researched the production of basic potato seed minitubers on soil, coco-peat substrate and isolated in a greenhouse to overcome the limitations of the hydroponic and aeroponic methods [9]. However, these are still preliminary studies, not yet determined the best technical measures to control the process of potato tubers formation, with the best quantity and quality of tubers.

Potato tuberization is a distinctive developmental process controlled by many factors; genotypes determine tuber size, number and yield potential, while yield performance is influenced by the physiological status of the seed tuber [10]. Physiological responses. especially to temperature and to light [11] have been studied. Research results of Ewing and Struik [12], Struik and Ewing [13] have confirmed that in potato production, low temperature and short day light will promote the formation and development of potato tubers, dry matter accumulation in tubers.

Nutrition is a very important factor, which is the basis for determining the survival, growth, development, yield and quality of plants [14]. According to Krauss and Marschner [15], Sattlemacher and Marschner [16], N at high concentrations inhibits potato tuber formation, while at low concentrations promotes tuber formation. Cycles of repeated high/low N will lead to tuber chain formation. However, reducing N under conditions of long day (18h) and high temperature (stable at 30<sup>o</sup>C) did not form tubers [17].

The study aimed to determine the influence of photoperiod and nutritional-shock on the production of disease-free potato seed minitubers grown on coco-peat substrates, using hydroponic solutions and isolated under greenhouse conditions at Dalat, Vietnam.

#### 2. MATERIALS AND METHODS

# 2.1 Plantlet Production and Growth Conditions

Root-cutting seedlings of cultivars Atlantic were obtained from tissue culture plants. Seedlings were grown in pots with an average height of 6-7cm. Seedlings had 5-6 trueleaves, roots reached the bottom of the pot (about 15 days after cutting), were disease-free with bacterial wilt and virus using the Elisa kit produced by the International Potato Research Center before conducting experiments.

#### 2.2 Experiment Conditions

The experiments were conducted in controlled greenhouse on concrete raising beds with dimensions of 10 m x 1 m x 0.3 m. The growing substrate was clean coco-coir and watered with 80% moisture before planting 2 days. Potato seedlings with full roots (the roots reached the bottom of the pot - about 15 days after dipping), 6-7cm in height, 5-6 true leaves were planted in pots. Plant density was 30 plants m<sup>-2</sup> (distance 20 x 15 cm). The basis fertigation was (ppm) 182 N. 46.5 P. 253.5 K. 160 Ca. 36 Mg. 48 S. 4.0 Fe. 0.06 Cu, 0.22 Zn, 0.5 Mn and 0.26 B, pH = 6.0,  $EC = 1dS m^{-1}$  [18] (Table 1). Nutrition will be mixed according to the defined formula. Mix into 2 tanks, tank A and tank B. The composition and amount of phase are as in Table 1. When watering the nutrient solution, use 1 liter of solution A + 1 liter of solution B and 98 liters of water. Nutrients and water are provided through a drip irrigation system, using a drip line of the Netafim Company, Israel. The irrigation regime depends on the growth stage of the plant (Table 2). Measures to care for, prevent pests and diseases were carried out according to the procedure of Vegetable and Flower Research Center, Datlat, Vietnam.

#### 2.3 Experiment Design

A study was conducted from 2018 October to 2020 December at the Vegetable and Flower Research Center, Dalat, Vietnam. A series of experiment was set up as one - way factorial (Completely Randomized Design). desian meanwhile experiment of the combianation of photoperiod and nutritional-shock was designed by Randomized Complete Block Design to optimize the effect of photoperiod and nutritionalshock on growth performance and yield of first generation potato minitubers production of potato plants. A plot area was 10 m<sup>2</sup> and the space between 2 plots was 0.2 m. Potato variety 'Atlantic' was treated under 5 photoperiods – (8 h/day: 9 h/day: 10 h/day: 11 h/day and natural light), under nutritional-shock process with reducina Ν concentration in nutrient solution (0%; 25%; 50%; 75% and 100%), the growth stage of plant to conduct nutritional-shock (30, 35, 40, 45 and 50 DAT-day after transplanting), the implementation time of nutritional-shock (12, 24, 48 and 60 hours) and the combination of photoperiod (9 h/day, 10 h/day) and nutritional-shock (75%, 100%) on the formation and development of minitubers. In this research, the best results of initial experiments was used as inputs for experiment later.

Table 1. Nutrient solution composition for potato minnitubers production of cultivar Atlantic
using root-cutting seedlings from tissue culture plants on coco-coir substrate

Chemical name	A Tank (100 L)	B Tank (100 L)
Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	8,400 g	
Fe(EDTA)	67 g	
KNO <sub>3</sub>	2,900 g	
$K_2SO_4$		2,300 g
KH <sub>2</sub> PO <sub>4</sub>		1,500 g
MAP		450 g
MgSO <sub>4</sub> .7H <sub>2</sub> O		2,300 g
MnSO <sub>4</sub> .H <sub>2</sub> O		3.3 g
CuSO <sub>4</sub> .5H <sub>2</sub> O		0.4 g
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O		2.5 g
ZnSO <sub>4</sub> .7H <sub>2</sub> O		3.5 g
H <sub>3</sub> BO <sub>3</sub>		1.48 g

# Table 2. Nutritional irrigation regime for potato minitubers production of cultivar Atlantic using root-cutting seedlings from tissue culture plants on coco-coir substrate

Growing duration	Irrigating times per day	Irrigation amount
1-40 days after transplanting	5	400L ha⁻¹
41-70 days after transplanting	3	500L ha⁻¹

#### 2.4 Measurement of Plant Growth Characteristics and Seed Tuber Yield

Height growth (cm/week), leave growth (leave/week), leave area growth (cm<sup>2</sup>/ week), number of tuber stolons/plant (at 30 DAT), average number of tubers/plant, average weight of tubers (g), tuber yield (tubers  $m^{-2}$ ) and standard tuber (Percentage of tubers over 3 g).

#### 2.5 Data Analysis

Data were submitted by analysis of variance (ANOVA), followed by Ducan test using SAS software 9.1. Treatment differences were regarded as significant at P<0.05 or P<0.01. Regression was analysed by using Minitab statistical software.

#### 3. RESULTS

#### 3.1 The Effect of Light Regime on the Ability to Produce Minitubers

Potato plants form and develop tuber stolons under photoperiod conditions of short day and low temperature. Experimental results showed that the short-day photoperiodic treatments from 8 to 11h/day had a height growth rate of 7.11 to 7.52 cm/week, higher than the control (5.13 cm/week), however, all of these treatments had lighter green leaf color than the control. Regarding the growth of leaves, the difference between treatments was not statistically significant, the average was from 2.32 to 3.12 leaves/week and was highest in the control treatment. The leaf area growth of the 10 h/day, 11 h/day and P5 (control) had no difference and averaged from 8.12 to 8.24 cm<sup>2</sup>/week (Table 3).

The number of tubers/plant is the most interested parameter. The results showed that the average number of tubers/plant was different and statistically significant, the P3 (short-day photoperiod of 10 hours/day<sup>1</sup>) gave the highest number of tubers (7.4 tubers/plant), the lowest was P5 (control) with only 3.7 tubers/plant. The average weight of tuber between treatments did not differ, averaging from 14.27 to 16.27g and was higher in treatments with longer daylight hours. The tuber vield was different and higher than that of the control treatment, with an average of 394.2 to 569 tubers m<sup>-2</sup>, the highest was 10h/day treatment, and the control treatment reached 299.4. tubers m<sup>-2</sup>. There was no difference in the percentage of standard tubers among the experimental treatments, averaging from 81.8% to 86.7% (Table 3).

According to Thach et al., [19], production of minitubers of potato seed by aeroponics method for Atlantic variety, nutrient solution temperature of 20°C combined with photoperiod of 9 hours/day for average weight of tubers was 4.14 g, yield of 484 tubers m<sup>-2</sup>, photoperiod of 10 hours/day for average weight of tubers of 6.98 g, vield of 532 tubers m<sup>-2</sup> and photoperiod of 11 hours/day for average tuber weight of 9.23 g, yield of 686 tubers m<sup>-2</sup>. The results of this study showed that the average number of tubers per plant was different and statistically significant, in which P3 treatment (short-day photoperiod of 10 hours/day) gave the highest number of tubers, the lowest was the control treatment (only 3.7 tubers/plant). The average weight of tuber between the experimental treatments did not differ, averaging from 14.27 to 16.27g and higher in treatments with longer day. The actual tuber yield was different and higher than that of the control treatment, averaging from 394.2 to 569.7

 
 Table 3. Effect of light regime for potato minitubers production of cultivar Atlantic using rootcutting seedlings from tissue culture plants on coco-coir substrate

Treatment	Height growth (cm/week)	Leaves growth (leaves/ week)	Leaves area growth (cm <sup>2 /</sup> week)	Average N <sub>0</sub> tubers/ Plant	Average weight of tubers (g)	Tuber yield (tubers m <sup>-2</sup> )	Standard tuber (%)
8 h/day	7.52a	2.32	5.18c	5.3c	14.27	402.6c	82.4
9 h/day	7.41a	2.48	6.43b	6.1b	15.34	445.8b	81.8
10 h/day	7.24a	2.53	8.12a	7.4a	16.14	569.7a	83.2
11 h/day	7.11a	2.76	8.16a	5.0c	16.21	394.2e	82.6
natural light	5.13b	3.12	8.24a	3.7d	16.27	299.4f	86.7
CV %	4.62	4.14	5.02	5.11	4.87	5.13	-
F-test	*	ns	**	**	ns	**	-

In the same average group, the values with the same accompanying characters do not have statistical significance P <0.05; ns: none significant; significant difference (p<0.05); significant difference (p<0.01)

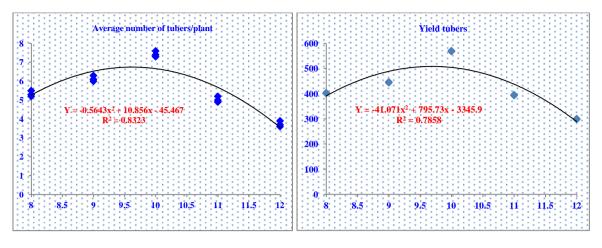


Fig. 1. Number of tubers per plant (A) and yield of tubers (B) inlight regime for potato minitubers production of cultivar Atlantic using root-cutting seedlings from tissue culture plants on coco-coir substrate

tubers  $m^{-2}$ , in which the highest was in 10h/day. The percentage of standard tubers among the experimental treatments did not differ, averaging from 81.8% to 86.7% (Table 3).

This research result is also consistent with the study of McDole et al. [20]. Short day photoperiod had a positive effect on potato minitubers yield in greenhouses. The number of potato seed minitubers/plant varied significantly under the conditions of a day shorter than the standard 16h daylight during the *in vitro* period [21].

#### 3.2 Effects of Nutritional Shock on Tuber Yield

At the time of 30 days after planting, the Atlantic variety grew and developed well, without the appearance of pests and diseases. This is also the time when these potato varieties begin to form tubers. The experiment performed reducing the concentration of N in the nutrient solution from 25% to 100%, within 48 hours.

According to authors, the average number of tubers/plant was a ranged of from 0.26 to 3.0 tubers/plant [22], from 1.85 to 2.54 tubers/plant [23]. However, Corrêa et al. [24] suggested that the average number of tubers would be from 7.0 to 8.31 tubers/plant if there were appropriate technical measures to affect the tuber formation stage of potato plants. Cao and Tibbitts [25], when studying the response of potato plants to different nitrogen concentrations found that nitrogen changes at the stage of tuber stolon

formation plays an important role in determining the number of tubers per plant. Reducing N to 0% in 2-3 days stimulated the number of tuber stolon formation to increase 3-4 times.

The results showed that the number of tuber stolons and the average number of tubers/plant was directly proportional to the decreasing in N concentration in the nutrient solution within 48 hours at 30 DAT, *showing significant differences* (P<0.01). The number of tubers/plant reached from 2.3 to 7.6 (tubers), the average number of tubers (3.0 - 9.3 tubers/plant), in which the highest was the N5 treatment (100% reduction in N concentration) with 9.3 tubers/plant, higher than the control treatment 300% (Table 4).

The variation of tuber fresh weight/plant and tuber yield  $m^{-2}$  was observed under different treatments (Table 4). There was a statistically significant difference between the treatments. The tuber weight per plant decreased along with the reducing in N concentration. The highest tuber weight/plant was recorded from the treatment N1, followed by N2 and N3. The tuber yield (number of tubers  $m^{-2}$ ) of the treatments with reduced N concentration from 25% to 100% was higher than that of the control treatment from 26% to 171% (from 122.0 to 264.6 tubers  $m^{-2}$ ) (Table 4).

The results of the regression analysis showed that the average tuber weight/plant and tuber yield  $m^{-2}$  was correlated with the reduction rate of N concentration in the nutrient solution at 30 DAT, represented by the Fig. 2.

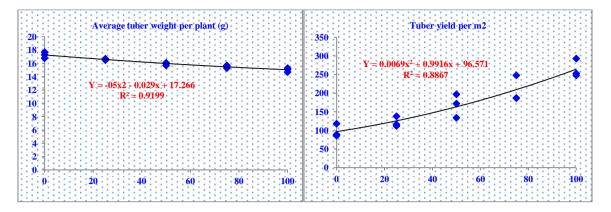


Fig. 2. Tuber weight per plant (A) and tuber yield (B) in nutritional shock for potato minitubers production of cultivar Atlantic using root-cutting seedlings from tissue culture plants on cococoir substrate

 
 Table 4. Effect of nutritional shock for potato minitubers production of cultivar atlantic using root-cutting seedlings from tissue culture plants on coco-coir substrate

Treatment %	N <sub>0</sub> . Stolons/plant	Average N₀. Tubers/plant	Average tuber weight (g)	Tuber yield (tubers m <sup>-2</sup> )
0	2.3 e	3.0e	17.2a	97.6c
25	3.6d	4.3d	16.6b	122.0c
50	5.0c	6.0c	15.9c	167.6b
75	6.0b	7.3b	15.5d	207.3b
100	7.6a	9.3a	15.0e	264.6a
F-test	**	**	*	**
CV (%)	7.4	9.1	1.0	13.4

In the same average group, the values with the same accompanying characters do not have statistical significance P <0.05; ns: none significant; significant difference (p<0.05); significant difference (p<0.01)

#### 3.3 Effect of Nutrient Shock Time on the Ability to Produce Minitubers

Studying the role of N in the nutrient solution of the potato production system in Korea, Kim et al. [26] confirmed that at the beginning of tuber formation and development (about 30-35 DAT), it was necessary to reduce the amount of nitrogen in the nutrient solution, combined with low temperature conditions to stimulate the formation and development of tuber stolons.

The experiment of nutrient shock treatment (100% reduction in N concentration in the nutrient solution) at the time of the strongest growth and development of potato plants (30, 35, 40 and 45 DAT). The results in Table 5 showed that there was a statistically significant difference in the research parameters. However, nutrient shock treatment at 30 and 35 DAT had no statistically significant difference, the yield and yield components were all higher than those at 40, 45 DAT. The average number of tubers was

from 9.0 to 9.2 tubers/plant, the tuber yield was 262.3 to 270.7 tubers  $m^{-2}$ , the percentage of standard tubers was from 81.1 to 87%, of which the highest in treatment 30 DAT.

### 3.4 Effect of Nutrient Shock Duration on the Ability to Produce Minitubers

The study results showed that the nutrient shock treatment in 48 hours gave the best tuber production efficiency of 9.5 tubers/plant, the average tuber weight was 15.1g and the yield was 270.3 tubers  $m^{-2}$  (181.5%) compared to 12h treatment.

The results of the regression analysis showed that the average number of tubers/plant and tuber yield m<sup>-2</sup> was correlated with the duration of nutritional shock using root-cutting from in vitro cultured seedlings on the substrate, represented by the Fig. 3. A máxima produção por m2 (224 tubers/m2) foi obtida duration of nutritional shock de 40,57.

Treatment (DAT)	N₀. Stolons/plant	Average N₀. Tubers/plant	Average tuber weight (g)	Tuber yield (tubers m <sup>-2</sup> )	Standard tuber (%)
30	7.4a	9.2a	15.2b	270.7a	87.0a
35	7.2a	9.0a	15.4b	262.3a	81.1b
40	6.6b	7.9b	15.7a	234.0b	79.5c
45	6.0c	7.5b	15.9a	216.3c	76.8d
F – test	*	**	*	**	**
CV (%)	2.6	2.8	0.8	1.9	0.9

Table 5. Effect of nutrient shock treatment time for potato minitubers production of cultivar Atlantic using root-cutting seedlings from tissue culture plants on coco-coir substrate

In the same average group, the values with the same accompanying characters do not have statistical significance P <0.05; ns: none significant; significant difference (p<0.05); significant difference (p<0.01)

Table 6. Effect of nutrient shock duration for potato minitubers production of cultivar Atlantic using root-cutting seedlings from tissue culture plants on coco-coir substrate

Treatment	N₀. Stolons/plant	Average N₀. Tubers/plant	Average tuber weight (g)	Tuber yield (tubers m <sup>-2</sup> )
12h	3.1c	3.5c	16.1a	96.0c
24h	3.8b	4.3b	16.1a	115.7b
48h	7.3a	9.5a	15.1b	270.3a
60h	4.1b	4.5b	15.6a	124.0b
F-test	**	**	*	**
CV (%)	7.4	5.7	1.7	5.6

In the same average group, the values with the same accompanying characters do not have statistical significance P < 0.05; ns: none significant; significant difference (p < 0.05); significant difference (p < 0.01)

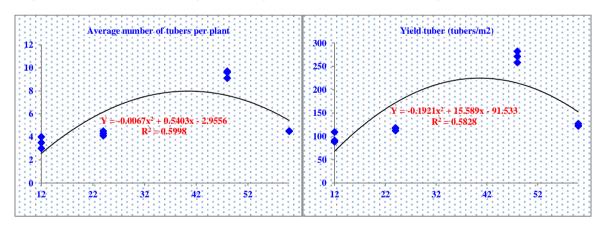


Fig. 3. Number of tubers per plant and yield tubers in duration of nutritional shock for potato minitubers production of cultivar Atlantic using root-cutting seedlings from tissue culture plants on coco-coir substrate

#### 3.5 Effects of Photoperiod and Nutritional Shock on the Ability to Produce Minitubers

The photoperiod and nutritional shock effects on yield characteristics of the experiments are summarized in Table 7. The individual treatments of photoperiod or nutritional shock significantly affected the yield characteristics. The interactions between the photoperiod and nutritional were recorded as being statically significant.

Averaging across photoperiod the number of tubers/plant, the average number of tubers/plant and the tuber yield increased proportionally to the decrease in N concentration in the solution. Reducing the concentration of N in the solution to 100% at 30 DAT within 48h gave the highest number of stolons/plant, average tuber

Parameters	Photoperiod	Reducing N concentration (%)		Average P
		75	100	
Number of	9h/day	4.0c	5.6b	4.8B
stolons/plant	10h/day	5.6b	7.6a	6.3A
	Average N	4.5B	6.6A	
	CV% = 5.9; F(N)*;	F(P)**; F(NxP)**		
Average number	9h/day	5.6c	7.6b	6.6B
of tubers/plant	10h/day	6.6bc	10.6a	8.6A
	Average N	6.1B	9.1A	
	CV% = 7.5; F(N)**;	F(P)**; F(NxP)**		
Average tuber	9h/day	16.2a	15.2b	15.7a
weight/plant	10h/day	15.6ab	15.0b	15.3b
	Average N	15.9A	15.1B	
	CV% = 1.7; F(N)*;	F(P)*; F(NxP)*		
Tuber Yield	9h/day	139.0d	230.6b	184.8B
(tubers m <sup>-2</sup> )	10h/day	164.3c	308.0a	236.1A
	Average N	151.6B	269.3A	
	CV% = 3.0; F(N)*;	F(P)*; F(NxP)*		

Table 7. Effect of photoperiod	and nutrient shock for potato minitubers production of cultiva	r
Atlantic using root-cutting	seedlings from tissue culture plants on coco-coir substrate	

In the same average group, the values with the same accompanying characters do not have statistical significance P < 0.05; ns: none significant; significant difference (p < 0.05); significant difference (p < 0.01)

weight/plant and tuber vield  $m^{-2}$  (6.6: 9.1: 269.3). respectively. Similarly. averaging across reducina N concentration, photoperiod at produced 10h/day the highest vield characteristics (6.3; 8.6; 236.1), respectively. Overall, tuber yield was greater in the N5P3  $(308.0 \text{ tubers m}^{-2})$  than in the other treatments.

#### 4. DISCUSSION

Using aeroponics, potato plants can get 250-300 minitubers/plant [27], produces an average of 8.5 times more minitubers than conventional soil production [28]. However, the authors pointed out the unjustified high costs of infrastructure and equipment as one of the reasons why soil-less seed potato production was not widespread [29,30,31]. It is known that the potato tuberization phase is sensitive to the density of the solid substrate. Ritter et al. [32] confirmed the delay in tuberization in the aeroponic production of minitubers which could be due to the lack of mechanical stress acting on stolons. In addition, because of multiple harvests so light can go into and stimulates the production of glycoalkaloids. These compounds, at a high level of concentration and consumption, can be toxic to humans [33]. In our study, production of first seed potato minitubers using apical rooted cutting from in vitro culture on coco-peat substrates in controlled condition by greenhouse beds method gave a range of average number of tubers/plant (5.6 10.6), tuber yield \_

(approximately 300 minitubers  $m^{-2}$ ) and tuber standard (81.8 – 86.7%). This method gives a lower tuber yield  $m^{-2}$  than hydroponic production. However, in this system, the tissue culture plantlets are used as mother plants in coco pits for producing cuttings. In six weeks, one mother plant can be multiplied to produce 8 plants and the number goes to more than 15 in 12 weeks [9]. For all, this greenhouse beds method still produces the high tuber yield and standard compared to aeroponics method, with low cost and suitable for the developing countries like Vietnam.

According to Be'langer et al. [34], nitrogen (N) affects the process of tuber formation, stimulates the growth and development of leaves, and increases the photosynthetic capacity of plants. N fertilization also increases the yield of tubers as well as the percentage of commercial tubers. Darwish et al. [35] suggested that applying too little or too much N, inappropriate fertilization time, and incorrect fertilization method will reduce potato yield. In any growing system, adequate N nutrition is fundamental for the growth and production of crops, especially potatoes. Both N deficiency and excess can impair tuber production [36].

In studies on the effect of N on the production of potato seed minitubers, many authors found that the concentration of N in the solution affects the development of potato stem (plant height, number of leaves, leaf area) [18,37]. The authors [38,39] suggested that N affects stem and leaf development and the subsoil including the weight and number of tubers/plant. A excess of N can prolong tuber formation and development and reduce yield [40,36]. Research results of Chang et al. [41] showed that using aeroponics to produce minitubers of potato seed. the appropriate nitrogen fertilizer level was from 200-220 mgL<sup>-1</sup>. However, it should be combined with some technical measures to affect the tuber production stage, usually from 30-45 DAT depending on the potato variety. We saw a similar trend early in our experiments.

In the study, reducing N concentration influenced significantly on the number of stolons, average number of tubers and tuber yield (P<0.01). Reducing N concentration (100%) gave the highest yield characteristics (7.6 stolons; 9.3 tubers; 264.6 tubers m<sup>-2</sup>). The data (Tables 5, 6) showed that reducing N concentration at the stage of 30 DAT within 48h produced the highest yield characteristics (except average tuber weight). The following three hypotheses have been proposed to explain the effect of N on tuber formation: Firstly, N suppresses the influence of plant hormones, leading to a decrease in GA concentration and an increase in ABA concentration [42]. Secondly, N promotes shoot and root growth instead of stolons by reducing the ability to accumulate dry matter for stolon formation [43]. Finally, the rate of N absorption will affect the absorption of other elements, Ρ, Ca especially and Mg, and high concentrations of N can limit the beneficial effects of other nutrients on tuber formation. It was observed that high foliar spray of N did not inhibit tuber formation, which is seen as additional evidence for N-induced tuber formation [16].

Light is essential for plants to photosynthesize to accumulate dry matter. Although varieties initiate tubers at any day-length, development is very much accelerated under short day condition. Stem elongation terminates earlier, tubers are initiated earlier and the plants die earlier than under long day condition [14]. In short day conditions, in the tropics and subtropics, yields can be high in the highlands or during the cold season. However, in each stage of growth and development they require different light. From the time of sprouting from the ground until the plant has flower buds, potatoes require long day light to benefit the development of leaves and promote photosynthesis. To the stage of potato tuber formation requires a short lighting time. When tubers thrive, potatoes require dark conditions [44]. In this research, photoperiod affected on growth and yield characteristics of Atlantic variety. In which, the treatment (shortday photoperiod of 10 h/day) gave the highest height growth, leaves area growth, average number of tubers and tuber yield (7.24; 8.12; 7.4; 579.7 respectively) (Table 3).

There are many studies on environmental factors (light, temperature, nutrition, pH and hormones) affecting the growth, development and formation potato seed minitubers [45,46,47,48]. of However, up to date, there have been no studies on the effects of photoperiod and nutritional shock on the ability to form potato minitubers. The results of this study showed that there was an interaction between photoperiod and nutritional shock on the components of potato vield, especially significant in the number of stolons/plant and the average number of tubers plant<sup>1</sup> (P<0.01). In which, the nutritional shock treatment (reduced N concentration by 100%) at 30 DAT for 48h combined with photoperiod of 10h/day gave the highest yield of potato minitubers (308 tubers  $m^{-2}$ ) (Table 7).

#### 5. CONCLUSION

Overall, the current study focused on the photoperiod and nutritional shock (reducing N concentration) so as to promote the ability of formation of potato seed minitubers using apical shoot cutting from in vitro plantlets grow on cocopeat substrates in controlled greenhouse. The effect of nutritional shock was obvious and the results were remarkable. Photoperiod and nutritional shock successfully increased the yield by improving the vegetative growth of plants.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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