EFFECT OF PHOTOPERIOD AND NUTRITIONAL-SHOCK ON INCREASING THE NUMBER OF MINITUBERS FROM APICAL ROOTED CUTTINGS GROWN IN COCO-PEAT

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 nuitritional-shock on growth performance and yield of first generation potato tuber production of potato plants. Potato variety ‘Atlantic’ was treated under 5 photoperiod – P1 (8h day^{-1}), P2 (9h day^{-1}), P3 (10h day^{-1}), P4 (11h day^{-1}) and P5 (natural light) under nutritional-shock process with reducing N concentration in nutrient solution (N1-0, N2-25%, N3-50%, N4-75%, and N5-100%), 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 (P2, P3) and nutritional-shock (N4, N5) on the formation and development of minitubers. The study revealed that photoperiod at 10h day^{-1} showed the best result with 7.4 tubers plant^{-1} and 569.7 tubers m^{-2}. 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 N5 treatment (100% reduction in N concentration) with 9.3 tubers plant^{-1}, higher than the control treatment 300%. The nutrient shock treatment at 30 DAT in 48 hours 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^{-1}, 308 tubers m^{-2}).

Keywords: Atlantic, minitubers potato, nutritianal-shock, photoperiod, tuberization

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 (Halterman et al., 2016). 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 (FAOSTAT, 2019). 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 (Islam et al., 2020). 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 (Beata et al., 2020). Over the years, the use of basic potato seed minitubers in seed production has revolutionized the potato production industry by shortening the seed production cycle in the field and creating a large number of desease clean seed potatoes supplying for production. Basic potato seed minitubers are obtained from seed production on in vitro-derived seedling by different growing methods (Gildemacher et al., 2009).

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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 (Navarrete Ortega, 2004; Wiresema et al., 1987), using thin film technology (NFT - Nutrient Film Technoque) or growing in hydroponics systems (Muro et al., 1997). 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 many outstanding advantages (Beata, 2020). 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 (Mohanty, 2019). 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 (Wang, 2008). Physiological responses, especially to temperature and to light (Rahman et al., 2021) have been studied. Research results of Ewing and Struik (1992), Struik and Ewing (1995) 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 (Sang, 1999). According to Krauss and Marschner (1976), Sattlemacher and Marschner (1979), 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 30^0C) did not form tubers (Krauss and Marschner, 1982).

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.

MATERIALS AND METHODS

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 true leaves, roots reached the bottom of the pot (about 15 days after cutting), were disease-free

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with bacterial wilt and virus using the Elisa kit produced by the International Potato Research Center before conducting experiments.

Experiment conditions

The experiments were conducted in controlled greenhouse on concrete raising beds with dimensions of 10m x 1m x 0.3m. 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^{-2} (distance 20 x 15cm). 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} (Novella et al., 2008) (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 Tables 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.

Table 1. Nutrient solution

Chemical name	**A Tank (100 L)**	**B Tank (100 L)**
$Ca(NO_3)_2.4H_2O$	8,400 g	
Fe(EDTA)	67 g	
KNO_3	2,900 g	
K_2SO_4		2,300 g
KH_2PO_4		1,500 g
MAP		450 g
$MgSO_4.7H_2O$		2,300 g
$MnSO_4.H_2O$		3.3 g
$CuSO_4.5H_2O$		0.4 g
$Na_2MoO_4.2H_2O$		2.5 g
$ZnSO_4.7H_2O$		3.5 g
H_3BO_3		1.48 g

Table 2. Nutritional irrigation regime for potato minnitubers production 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}

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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 design (Completely Randomized Design), meanwhile experiment of the combianation of photoperiod and nutritional-shock was designed by Randomized Complete Block Design to optimize the effect of photoperiod and nuitritional-shock on growth performance and yield of first generation potato minitubers production of potato plants. A plot area was $10m^2$ and the space between 2 plots was 0.2m. Potato variety 'Atlantic' was treated under 5 photoperiods – P1 (8h day^{-1}), P2 (9h day^{-1}), P3 (10h day^{-1}), P4 (11h day^{-1}) and P5 (natural light), under nutritional-shock process with reducing N concentration in nutrient solution (N1-0, N2-25%, N3-50%, N4-75%, and N5-100%), 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 (P2, P3) and nutritional-shock (N4, N5) on the formation and development of minitubers. In this research, the best rerults of initial experiments was used as inputs for experiment later.

Measurement of plant growth characteristics and seed tuber yield

Height growth (cm $week^{-1}$), leave growth (leave $week^{-1}$), leave area growth (cm^2 $week^{-1}$), number of tuber stolons $plant^{-1}$ (at 30 DAT), average number of tubers $plant^{-1}$, average weight of tubers (g), tuber yield (tubers m^{-2}) and standard tuber (Percentage of tubers over 3g).

Data analysis

Data was analysed 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.

RESULTS

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^{-1} had a height growth rate of 7.11 to 7.52 cm $week^{-1}$, higher than the control (5.13 cm $week^{-1}$), 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 $week^{-1}$ and was highest in the control treatment. The leaf area growth of the P3, P4 and P5 (control) had no difference and averaged from 8.12 to 8.24 cm^2 $week^{-1}$ (Table 3).

The number of tubers $plant^{-1}$ is the most interested parameter. The results showed that the average number of tubers $plant^{-1}$ was different and statistically significant, the P3 (short-day photoperiod of 10 hours day^{-1}) gave the highest number of tubers (7.4 tubers $plant^{-1}$), the

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lowest was P5 (control) with only 3.7 tubers plant^{-1}. 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 yield was different and higher than that of the control treatment, with an average of 394.2 to 569 tubers m^{-2}, the highest was P3 treatment, and the control treatment reached 299.4. tubers m^{-2}. There was no difference in the percentage of standard tubers among the experimental treatments, averaging from 81.8% to 86.7% (Table 3).

Table 3. Effect of light regime on minitubers production of cultivar Atlantic

Treatment	Height growth (cm week^{-1})	Leaves growth (leaves week^{-1})	leaves area growth (cm^2 week^{-1})	Average N_0 tubers Plant^{-1}	Average weight of tubers (g)	Tuber yield (tubers m^{-2})	Standard tuber (%)
P 1	7.52a	2.32	5.18c	5.3c	14.27	402.6c	82.4
P 2	7.41a	2.48	6.43b	6.1b	15.34	445.8b	81.8
P 3	7.24a	2.53	8.12a	7.4a	16.14	569.7a	83.2
P 4	7.11a	2.76	8.16a	5.0c	16.21	394.2e	82.6
P 5	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).*

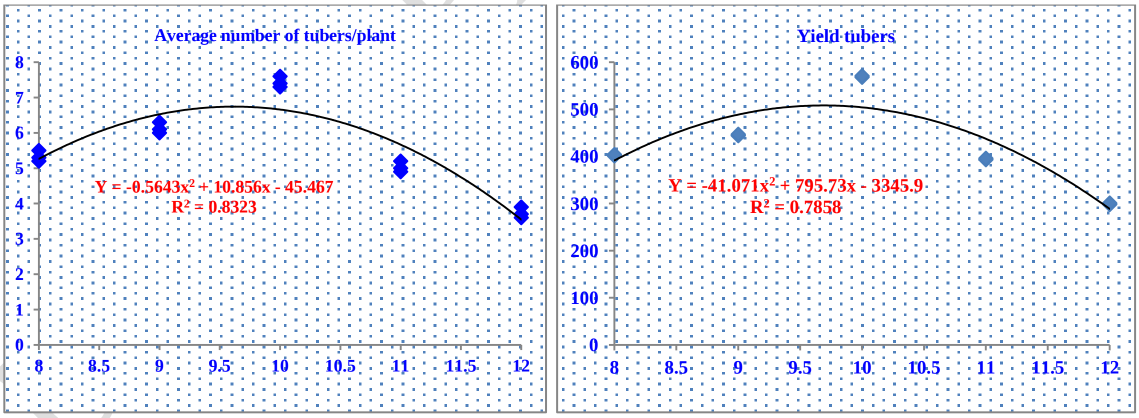

Figure 1. Correlation between number of tubers per plant, yield of Atlantic potato tubers and light regime in the production of minitubers of potato seedlings on substrates

According to Thach et al., (2008), production of minitubers of potato seed by aeroponics method for Atlantic variety, nutrient solution temperature of 20^{0}C combined with photoperiod of 9 hours day^{-1} for average weight of tubers was 4.14g, yield of 484 tubers m^{-2}, photoperiod of 10 hours day^{-1} for average weight of tubers of 6.98g, yield of 532 tubers m^{-2} and photoperiod of 11 hours day^{-1} for average tuber weight of 9.23g, yield of 686 tubers m^{-2}. The results of this study showed that the average number of tubers per plant was different and

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statistically significant, in which P3 treatment (short-day photoperiod of 10 hours day^{-1}) gave the highest number of tubers, the lowest was the control treatment (only 3.7 tubers plant^{-1}). 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 tubers m^{-2}, in which the highest was in P3 (10h day^{-1}). 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 (1978). Short day photoperiod had a positive effect on potato minitubers yield in greenhouses. The number of potato seed minitubers plant^{-1} varied significantly under the conditions of a day shorter than the standard 16h daylight during the *in vitro* period (Milinkovic et al., 2012).

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^{-1} was a ranged of from 0.26 to 3.0 tubers plant^{-1} (Ahlowalia, 1994), from 1.85 to 2.54 tubers plant^{-1} (Grigoriadou and Leventakis, 1999). However, Corrêa et al. (2008) suggested that the average number of tubers would be from 7.0 to 8.31 tubers plant^{-1} if there were appropriate technical measures to affect the tuber formation stage of potato plants. Cao and Tibbitts (1998), 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^{-1} 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^{-1} reached from 2.3 to 7.6 (tubers), the average number of tubers (3.0 - 9.3 tubers plant^{-1}), in which the highest was the N5 treatment (100% reduction in N concentration) with 9.3 tubers plant^{-1}, higher than the control treatment 300% (Table 4).

The variation of tuber fresh weight plant^{-1} 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^{-1} 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^{-1} and tuber yield m^{-2} was correlated with the reduction rate of N concentration in the nutrient solution at 30 DAT, represented by the following equations: Y = -0.5x2-0.029x + 17,266; R^2 = 0.91 and Y = 0.0069x2 + 0.9916x + 96,571; R^2 = 0.88, respectively (Figure 2).

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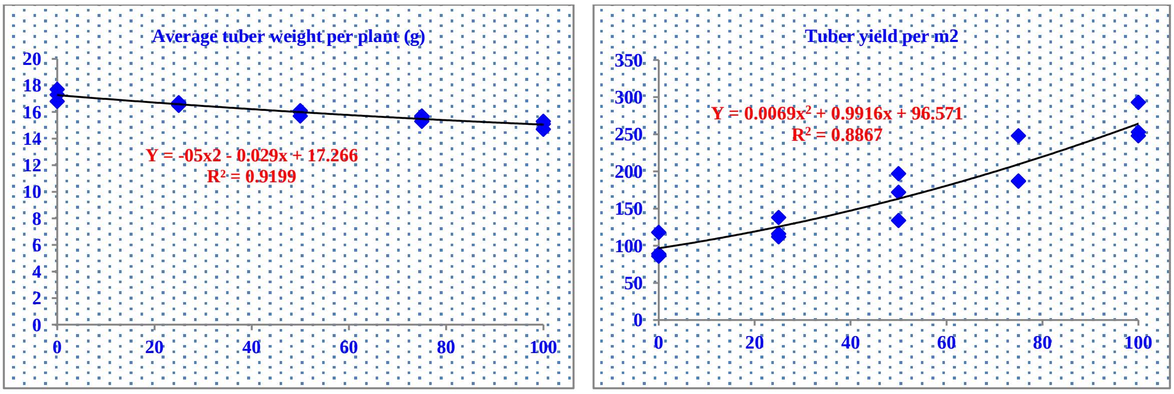

Figure 2. Correlation between average tuber weight, yield of Atlantic potato tubers and nutritional shock in the production of minitubers of potato seedlings on substrates

Table 4. Effect of nutritional shock on minitubers production of cultivar Atlantic

Treatment	N_0. stolons plant^{-1}	Average N_0. tubers plant^{-1}	Average tuber weight (g)	Tuber yield (tubers m^{-2})
N1	2.3 e	3.0e	17.2a	97.6c
N2	3.6d	4.3d	16.6b	122.0c
N3	5.0c	6.0c	15.9c	167.6b
N4	6.0b	7.3b	15.5d	207.3b
N5	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).*

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. (1994) 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 stolens.

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^{-1}, 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.

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Table 5. Effect of nutrient shock treatment time on the ability to produce minitubers of cultivar Alantic

Treatment (DAT)	N_0. stolons plant^{-1}	Average N_0. tubers plant^{-1}	Average tuber weight (g)	Tuber yield (tubers m^{-2})	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

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).

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^{-1}, the average tuber weight was 15.1g and the yield was 270.3 tubers m^{-2} (181.5%) compared to 12h treatment.

Table 6. Effect of nutrient shock duration on the ability to produce minitubers of cutivar Atlantic

Treatment	N_0. stolons plant^{-1}	Average N_0. tubers plant^{-1}	Average tuber weight (g)	Tuber yield (tubers m^{-2})
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).

The results of the regression analysis showed that the average number of tubers plant^{-1} and tuber yield m^{-2} was correlated with the duration of nutritional shock using root-cutting from in vitro cultured seedlings on the substrate, represented by the following equations: $Y = -0{,}0067x^2 + 0{,}05403x - 2{,}9556$; $R^2 = 0.59$ and $Y = -0{,}1921x^2 + 15{,}589x - 91{,}533$; $R^2 = 0.58$, respectively (Figure 3).

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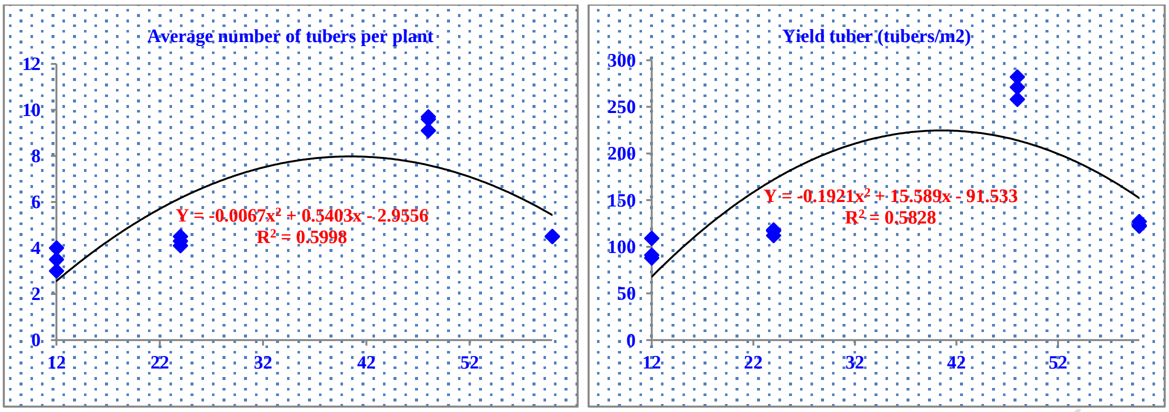

Figure 3. Correlation between average number of tubers, yield of Atlantic potato tubers and duration of nutritional shock in the production of minitubers of potato seedlings on substrates

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 signifcantly affected the yield characteristics. The interactions between the photoperiod and nutritional were recorded as being statically signifcant.

Table 7. Effect of photoperiod and nutrient shock on the ability to produce minitubers of cutivar Atlantic

Parameters	**Photoperiod**	**Reducing N concentration (%)**		Average P
		N4	N5	
Number of stolons plant^{-1}	P2	4.0c	5.6b	4.8B
	P3	5.6b	7.6a	6.3A
	Average N	4.5B	6.6A	
	CV% = 5.9; F(N)*; F(P)**; F(NxP)**			
Average number of tubers plant^{-1}	P2	5.6c	7.6b	6.6B
	P3	6.6bc	10.6a	8.6A
	Average N	6.1B	9.1A	
	CV% = 7.5; F(N)**; F(P)**; F(NxP)**			
Average tuber weight plant^{-1}	P2	16.2a	15.2b	15.7a
	P3	15.6ab	15.0b	15.3b
	Average N	15.9A	15.1B	
	CV% = 1.7; F(N)*; F(P)*; F(NxP)*			
Tuber Yield (tubers m^{-2})	P2	139.0d	230.6b	184.8B
	P3	164.3c	308.0a	236.1A
	Average N	151.6B	269.3A	
	CV% = 3.0; F(N)*; F(P)*; F(NxP)*			

*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).*

Averaging across photoperiod the number of tubers plant^{-1}, the average number of tubers plant^{-1} 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^{-1}, average tuber weight plant^{-1} and tuber yield m^{-2} (6.6; 9.1; 269.3), respectively. Similarly, averaging across reducing N concentration,

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photoperiod at 10h day^{-1} produced the highest yield characteristics (6.3; 8.6; 236.1), respectively. Overall, tuber yield was greater in the N5P3 (308.0 tubers m^{-2}) than in the other treatments.

DISCUSSION

Using aeroponics, potato plants can get 250–300 minitubers plant^{-1} (Terent'yeva and Tkachenko, 2018), produces an average of 8.5 times more minitubers than conventional soil production (Kakuhenzire et al., 2017). 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 (Banadysev, 2012; Mbiyu et al., 2012; and Mateus-Rodriguez et al., 2013). It is known that the potato tuberization phase is sensitive to the density of the solid substrate. Ritter et al. (2001) 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 (Lommen, 2008). In our study, production of first seed potato minitubers using apical rooted cutting from in vitro culture on coco-peat subtrates in controlled condition by greenhouse beds method gave a range of average number of tubers plant^{-1} (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 (Mohanty and Baruah, 2019). 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 developping contries like Vietnam.

According to Be'langer et al. (2000), 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. (2003) 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 (Silva et al., 2013).

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) (Novella, 2008; Vos and Putten, 1998). The authors (Errebhi, 1988; Meyer and Marcum, 1998) suggested that N affects stem and leaf development and the subsoil including the weight and number of tubers plant^{-1}. A excess of N can prolong tuber formation and development and reduce yield (Barry, 1996; Silva et al., 2013). Research results of Chang et al. (2006) showed that using aeroponics to produce minitubers of potato seed, the appropriate nitrogen fertilizer level was from 200-220 mg/L.

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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^{-2}). The data (Table 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 (Krauss, 1985). Scondly, N promotes shoot and root growth instead of stolons by reducing the ability to accumulate dry matter for stolon formation (Jackson, 1999). Finally, the rate of N absorption will affect the absorption of other elements, especially P, Ca 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 (Sattelmacher and Marschner, 1979).

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 (Sang, 1999). 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 (Dat, 2005). In this research, photoperiod affected on growth and yield characteristics of Alantic variety. In which, the treatment (short-day photoperiod of 10h day^{-1}) 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 of potato seed minitubers (Vander Zaag and Demagante, 1988; Vreugdenhil and Sergeeva, 1999; Oraby et al., 2015; and Wang et al., 2018). 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 yield, especially significant in the number of stolons $plant^{-1}$ and the average number of tubers $plant^{-1}$ ($P<0.01$). In which, the nutritional shock treatment (reduced N concentration by 100%) at 30 DAT for 48h combined with photoperid of 10h day^{-1} gave the highest yield of potato minitubers (308 tubers m^{-2}) (Table 7).

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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 coco-peat 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.

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