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In vitro propagation and microtuberization of potato (*Solanum tuberosum* L.) Spunta variety in Lebanon

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: One of the factors that causes low productivity of potatoes in Lebanon is the limited availability of certified seeds. The aim of this study was to establish a rapid protocol for *in vitro* propagation and microtuberization of potato (*Solanum tuberosum* L.) of Spunta variety. Meristems culture associated to thermotherapy (one month/37°C) constituted the first step. The highest percentage of reactive meristem (92%) was observed on MS medium devoid of growth regulators while MS medium containing Kin 0.4 mg.l^{-1}, GA3 0.5 mg.l^{-1} and IBA 0.5 mg.l^{-1} yielded the highest average number of shootlets (7.8) in the seventh subculture. The lowest number of days obtained for microtuber formation was 10 and the highest average number of microtuber (1.49) was obtained with shootlets incubated under C2 culture conditions (16-h day/8-h night for initial 7 days at 25±2°C; for remaining period: continuous dark at 17±2°C). Contrary the highest microtubers average length (10.75 mm), average width (7.41 mm) and average weight (646.26 mg) were produced under C1 culture conditions (16-h day/8-h night at 25±2°C). Medium supplemented with 5 mg.l^{-1} BAP and 6% sucrose presented the highest average number of microtubers of 2.36 and 1.94 respectively. Type and concentration of cytokines and sucrose concentration did not have significant effect on the average length, width and weight of microtubers produced.

1. Introduction

Potato (*Solanum tuberosum* L.) is the most important non-cereal food crop in the world (Bamberg *et al.,* 2016; Nikitin *et al.,* 2018). It is cultivated in about 150 countries (Basera *et al.*, 2018). Potato cultivation is considered as strategic as it occupies the world fourth place in production

after Maize, Wheat, and Rice (FAO, 2019).

In potato production, the quality of seed potatoes planted is an important determinant of the final yield and quality (Roy, 2014). When farmers use their farm-saved seed potatoes for several cropping cycles without renewing the seed lot from a reliable source, seed-borne diseases accumulate and cause severe yield and quality losses (Fuglie, 2007). Potato propagation takes place primarily asexually, through tubers and microtubers (Zhang *et al*., 2006).

Potato microtubers are minute tubers produced through *in vitro* culture technique yielding disease-free and high-quality seeds that can be preserved for a long time (Badoni and Chauhan, 2009). Extensive physiological research has revealed that tuberization is controlled by several factors, such as hormonal combination, ratio of photoperiod**,** nutrient compositions among others (Naresh *et al.,* 2011). Many researchers used different growth regulators for *in vitro* induction of microtubers in potato (Hossain, 2005). Cytokinins, such as benzylaminopurine (BAP), and kinetin (Kin) are among these growth regulators (Al-Safadi *et al.,* 2000; Sarkar *et al.,* 2006; Aksenove *et al.,* 2009). In addition, sucrose, the cheap and safe disaccharide, is considered as a superior agent and a critical stimulus for inducing microtubers (Hussain *et al.,* 2006; Nistor *et al.,* 2010).

In Lebanon, potato production is important for food security as well as a source of revenue in rural areas. It is a strategic crop for Lebanese agriculture, covering about 19,000 ha in the Bekaa plain (MOA, 2012) and with production of approximately 300,000 tons per year constituting the greatest field crop tonnage in Lebanon. It is cultivated mainly in the Bekaa valley (central-eastern Lebanon, 900-1000 m above the sea level, 70% of total area) and in Akkar plain (northern Lebanon, 25-30% of total area) (Choueiri *et al.,* 2017).

Lebanon does not produce certified potatoes' seeds, they are imported mainly from the European Union (EU) Member States. In the early years of the 21st century, Lebanon imported between 15,000 and 20,000 tons of potato seeds each year and relied heavily on this import with prices ranging between $750 and $1,000 per ton (Abou-Jawdah *et al.,* 2001). The absence of a seed certification program, the introduction and exchange of potato seeds of unknown sanitary status and the lack of phytosanitary measures resulted in increased incidence and severity of potatoes' diseases (Abou-Jawdah *et al.,* 2001). To prevent the further spread of these diseases and to avoid the introduction of new pathogens, it is important to reinforce the seed certification scheme that mandates using and trading of only certified potato seeds. Such a scheme will help the Lebanese farmers to improve their production, get involved in a certified seed production program and reduce the incidence of diseases, and thus economical losses. The implementation of a potato seed production program in Lebanon is possible; especially that Lebanon is characterized by a wide range of microclimates favorable to produce them (Abou-Jawdah *et al.,* 2001). In a report published by the International Potato Center (IPC) in 1975, areas in Lebanon including the Laklouk, Daher el Beidar, and Northern Bekaa were listed among the qualified areas for potato seeds production.

Hence, the present study was initiated with the objective to determine optimum concentration of sucrose, the effect of two cytokinins (BAP and Kin) on the microtuberization capacity of the potato cultivar Spunta under different incubation conditions.

2. Materials and Methods

Plant material

The experiment was conducted at the Lebanese Agricultural Research Institute (LARI), Department of Plant Biotechnology. The mother plants used in this study were of Spunta variety. Thermotherapy combined with meristem culture has been successfully established for efficient eradication of the potatoes' viruses. About 10 tubers were subjected to a temperature of 37°C for 50 days, under a photoperiod of 16 hours/day. At the end of the thermal treatment, the sprouts from the treated tubers were separated in portions of 3 cm and surface sterilized using 70% ethanol for 1 min followed by 5% (v/v) sodium hypochlorite containing two drops of Tween-20 for 10 minutes. Finally, the explants sources were washed 4 times with sterile distilled water (10 min each wash) before being used for the collection of meristems.

In vitro propagation

Meristems have been cut with the first pair of leaf primordial and cultivated in 9 cm Petri dishes on 3 MS basal medium (Murashige and Skoog, 1962). P1 medium lacked any additional growth regulators. P2 medium contained Kinetin (Kin), Gibberellic acid (GA3) and indole-3 butyric acid (IBA) at concentra-

tions of 0.2 mg.l^{-1}, 0.5 mg.l^{-1} and 0.5 mg.l^{-1} respectively. P3 medium was supplemented with Kin at 0.4 mg.l^{-1}, GA3 at 0.5 mg.l^{-1} and IBA at 0.5 mgl^{-1}. In addition, to all media, a vitamin mixture consisting of nicotinic acid (5 mg.l^{-1}), ascorbic acid (20 mg.l^{-1}), pyridoxin (5 mg.l^{-1}), thiamin (10 mg.l^{-1}) and myo-inositol (100 mg.l^{-1}) was added. The pH of all media was adjusted to 5.7 prior to the addition of agar (0.7%). The media were autoclaved at 121°C for 20 min. Each medium was prepared in ten replicas with 5 meristems per replica for testing. The *in vitro* cultivated 150 meristems were then shifted to a culture growth room at 25±2°C with 16 h photoperiod under white light intensity of 3000 lux. Thirty days later, survival rate of meristems was recorded. The regenerated shootlets from the meristems were fragmented to obtain uninodal cuttings and transferred onto fresh medium. Subcultures of 30 days interval were re-conducted seven times using the same 3 initiation media i.e., P1, P2 and P3, under the same culture conditions described above with a set of 60 shootlets per medium (10 repetitions of 6 shootlets). The newly regenerated shootlets per explant were recorded by the end of each subculture to calculate the multiplication rate as follows:

Multiplication rate= Number of new shootlets/Number of initial shootlets

Sanitary control

To check the sanitary status of the shootlets that arose from the thermotherapy-treated meristems, the Tissue-Blot Immunoassay (TBIA), a reliable, routine and cost-efficient serological test that allow processing of large numbers of individual plant samples serological test and that is commonly used to detect plant viruses in field and vegetable crops (Makkouk and Kumari, 1996) was performed to detect six main potato viruses PVA, PVX, PVY, PVM, PVS and PLRV. In addition, all samples were tested by the double-antibody sandwich enzyme-linked immunosorbant assay (DAS - ELISA, Loewe, Germany) using specific antibodies (Clark and Adams, 1977). All shootlets resulting from the meristems culture were subjected to the sanitary control. Each shootlet was numbered and divided into 2 parts, the first part was sent to the plant protection laboratory at LARI to be analyzed, while the second part of each plant remained in the culture medium pending the sanitary control results.

Pre-tuberization

Shootlets from the fifth subculture were aseptically transferred into culture-tubes containing 10 ml of pre-tuberization medium (P1). Cultures were grown for 20 days in culture growth room at 25±2°C and 16 h photoperiod under white light intensity of 3000 lux.

Tuberization

Eight MS Liquid media with two sucrose concentrations 6% and 8% respectively, supplemented with 2 cytokinins, Kin (0, 2 and 4 mg.l^{-1}) or BAP (0 and 5 mg.l^{-1}) or their combinations were used as an inducing medium for microtuber production. Ten ml of each liquid medium were added to the tubes containing the pre-tuberization MS solid medium after 20 days of culture. For each culture medium, two culture conditions were studied. In the first culture condition, the incubation was in culture growth room for 16-h day, 8-h night at 25±2°C and 3000 lux for 60 days. In the second condition, an initial incubation at 16-h day, 8-h night at 25±2°C and 3000 lux, for 7 days followed by continuous dark at 19±2°C to 60 days. Conditions are summarized in Table 1. For each culture medium and condition, 32 replicas were prepared. Microtubers produced were harvested at day 60. Data were recorded on days to microtubers formation, average number, average length, average width and average weight of microtubers.

Statistical analysis

Means ± standard deviations were recorded for each step of the propagation protocol and analyzed by using standard analysis of variance (ANOVA). Duncan's multiple range test was used to show differences among the treatments' means. All statistical analyses were performed using SAS for Windows (SAS Institute Inc., 1995).

3. Results

In vitro propagation

The survival rate of meristems was recorded after 30 days. The highest percentage of reactive meristem was formed on P1 medium that is devoid of growth regulators with an average of 920±4.8%, whereas a significant decrease of surviving reactive explants of 74±5.8% and 58±5.0% ($p<0.05$) was obtained on P2 and P3 media respectively as shown in figure 1 and 2 (A-C). No significant changes in percent of reactive meristems was observed between P2 and P3 media.

The effect of growth regulators added in the culture medium is summarized in Table 2. Medium P3

Table 1 - Culture media and culture conditions tested for *in vitro* microtubers production of potato

Medium	Medium composition	Culture condition C1
M1	20 ml of MS solid medium with 6% sucrose + 0 Hormones	16-h day, 8-h night at 25±2°C
M2	20 ml of solid MS medium with 8% sucrose + 0 Hormones	16-h day, 8-h night at 25±2°C
M3	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M1 medium + 5 mg.l^{-1} BAP	16-h day, 8-h night at 25±2°C
M4	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M2 medium + 5 mg.l^{-1}BAP	16-h day, 8-h night at 25±2°C
M5	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M1 medium + 4 mg.l^{-1} Kin	16-h day, 8-h night at 25±2°C
M6	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M2 medium + 4 mg.l^{-1}Kin	16-h day, 8-h night at 25±2°C
M7	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M1 medium + 5 mg.l^{-1}BAP + 2 mg.l^{-1}Kin	16-h day, 8-h night at 25±2°C
M8	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M2 medium + 5 mg.l^{-1}BAP + 2 mg.l^{-1}Kin	16-h day, 8-h night at 25±2°C
Medium	*Medium composition*	*Culture Condition C2*
M1	20 ml of MS solid medium with 6% sucrose + 0 Hormones	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C
M2	20 ml of solid MS medium with 8% sucrose + 0 Hormones	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C
M3	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M1 medium + 5 mg.l^{-1} BAP	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C
M4	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M2 medium + 5 mg.l^{-1} BAP	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C
M5	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M1 medium + 4 mg.l^{-1} Kin	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C
M6	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M2 medium + 4 mg.l^{-1} Kin	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C
M7	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M1 medium + 5 mg.l^{-1}BAP + 2 mg.l^{-1}Kin	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C
M8	MS solid medium, after 20 days of culture, addition of 20 ml of liquid M2 medium + 5 mg.l^{-1}BAP + 2 mg.l^{-1}Kin	For initial 7 days, 16-h day, 8-h night at 25±2°C, for remaining period continuous dark at 19±2°C

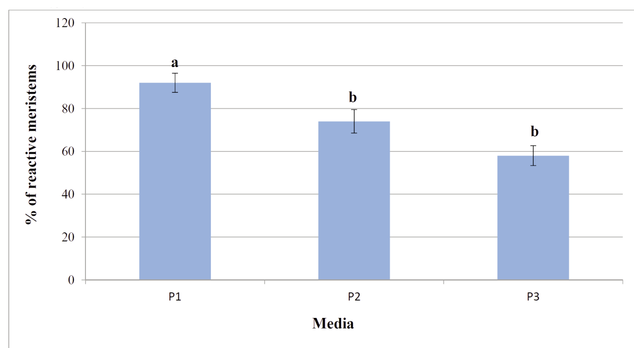

Fig. 1 - Effect of culture medium on the percentage of reactive meristem. Means with the same letter are not significantly different according to Duncan's.

presented the highest multiplication rate of 7.8±1.2 in the seventh subculture. Whereas in medium P2, the best multiplication rate obtained was 6.9±0.7 in the sixth subculture. Illustrations related to shootlets proliferation are presented in figure 2 (D and E).

It is important to note that all shootlets obtained in the meristems' cultures coupled with thermotherapy were free of the tested PVA, PVX, PVY, PVM, PVS and PLRV viruses.

In vitro microtuber formation

In vitro tuberization was obtained after propaga-

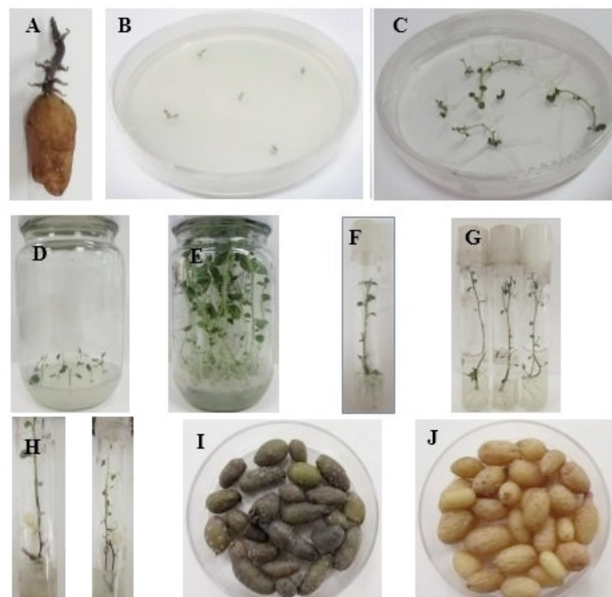

Fig. 2 - Represent the stages of the *in vitro* propagation and microtubers production of a representative under the three pre-tuberization conditions. A= Sprout of the treated tuber. B= Meristem initiation. C= Shootlets elongation. D= Uniodal cutting explants. E= Shootlet proliferation. F= Shootlet on pretuberization medium. G and H = Shootlets on tuberization medium. I= Microtubers produced under culture conditions C1. J= Microtubers produced under culture conditions C2.

Table 2 - Effect of culture media on the average number of shootlets per explant as recorded along the 7 subcultures

Media	Average number of shootlets per explant						
	Subculture 1	Subculture 2	Subculture 3	Subculture 4	Subculture 5	Subculture 6	Subculture 7
P1	0.70±0.3	1.17±0.2	3.87±0.4	4.07±0.5	5.08±0.5	5.4±0.2	4.00±0.6
P2	1.0±0.0	0.9±0.5	2.63±0.7	6.2±0.7	6.07±1.1	6.9±0.7	5.40±0.4
P3	1.2±0.6	1.5±1.0	5.57±0.7	7.33±0.9	6.00±0.2	7.4±0.9	7.80±1.2

P1= medium without any additional growth regulators
P2= medium contained Kinetin (0.2 $mg\cdot l^{-1}$), Gibberellic acid (0.5 $mg\cdot l^{-1}$) and indole-3 butyric acid (0.5 $mg\cdot l^{-1}$).
P3= medium was supplemented with Kinetin at 0.4 $mg\cdot l^{-1}$, Gibberellic acid at 0.5 $mg\cdot l^{-1}$ and indole-3 butyric acid at 0.5 $mg\cdot l^{-1}$

tion of the shoots (Fig. 2 E-H). The effect of BAP, Kin, or their combination in the presence of sucrose (6% or 8%) under both culture conditions were studied for microtubers' formation and development. Table 3 summarizes the results of microtubers' formation under all treatments.

Both the cytokinin type and its concentration significantly ($p<0.0001$) affected the average number (AN) of microtubers produced. Media containing BAP alone or in combination with Kin was superior to other media. The highest AN of microtubers per shootlet, i.e. 2.36 and 2.22, were produced when shootlets were propagated on M3 and M7 media with 5 $mg.l^{-1}$BAP or 5 $mg.l^{-1}$BAP + 2 $mg.l^{-1}$Kin respectively under C2 conditions. On the other hand, no statistically significant effect was observed when varying the type nor the concentration of the cytokines on the average length (AL) and average width (AW) or the average weight AWe of microtubers ($p=0.12$, $p=0.44$ and $p=0.39$ respectively) as shown in Table 3.

There is a linear relation between size and weight of microtuber implying that each factor that influenced the microtuber weight directly influenced microtuber size.

Effect of culture condition on microtuber formation

Results showed that the effect of culture condition was highly significant with p value <0.0001 for AN, AL,

Table 3 - Effect of cytokinin type and concentration on the average number (AN), average length (AL), average width (AW) and average weight (Awe) of microtubers produced

Culture media	Culture conditions	Average number of microtubers	Average length of microtubers (mm)	Average width of microtubers (mm)	Average weight (mg)
M1	C1	0.22±0.42 h	8.07 cd	5.05 e	375.3 d
M2	C1	0.78±0.70 fg	11.14 ab	7.28 abc	622.2 abc
M3	C1	1.53±0.62 cd	12.00 a	7.34 abc	673.1 ab
M4	C1	1.44±0.91 cde	10.07 abc	6.90 abcd	593.4 abcd
M5	C1	1.00±0.71 efg	11.8 a	7.96 a	734.5 a
M6	C1	0.94±0.82 fg	10.78 ab	7.23 abc	621.9 abc
M7	C1	1.58±0.95 cd	11.07 ab	7.43 ab	635.4 abc
M8	C1	1.25±0.85 def	11.1 ab	7.75 ab	643.3 abc
M1	C2	0.87±0.50 fg	9.45 bcd	6.8 abcd	535.4 abcd
M2	C2	0.55±0.80 gh	7.24 d	5.00 e	369.1d
M3	C2	2.36 a±1.05	8.89 bcd	5.64 de	473.5 bcd
M4	C2	1.56±0.98 cd	8.84 bcd	5.91 cde	561.5 abcd
M5	C2	1.00±0.65 efg	9.12 bcd	5.86 cde	487 bcd
M6	C2	1.58±1.05 cd	7.85 cd	5.34 e	406.3 cd
M7	C2	2.22±1.15 ab	7.85 cd	5.08 e	416.3 cd
M8	C2	1.80±1.16 bc	9.78 abc	6.26 bcde	600.7 abcd

Means followed by different letters in each column are significantly different for P≤0.05.

AW and AWe of microtubers (Table 4). AN was 1.49 for shootlets incubated under C2 condition and 1.09 for shooltlets cultivated under C1 condition. Values of AL, AW and AWe are 10.75 mm, 7.41 mm and 646.26 mg for microtubers produced under C1 condition and 8.63 mm, 5.65 mm and 481.23 mg for microtubers obtained under C2 condition respectively.

Under C1 culture condition, most of the cultures produced green microtuber, and this could be due to synthesis of the alkaloid solanine (Hoque, 2010). On the other hand, brown-colored tuber was observed in C2 culture condition (Fig. 2, I and J).

Effect of sucrose concentration on microtuber formation

The effect of sucrose on microtuber production is presented in Table 5. Results revealed a highly significant effect of sucrose concentration on the AN of microtubers produced. The media added with 5 $mg.l^{-1}$ BAP or 5 $mg.l^{-1}$ BAP + 2 $mg.l^{-1}$ Kin, both supplemented with 6% of sucrose, presented the highest AN of microtubers, 1.95 and 1.9 respectively. On the other hand, sucrose concentration had no effect on AL, AW and the AWe of microtubers (Table 5).

Time-dependent effect of growth regulators, sucrose concentration and culture condition on microtuber formation

Microtuber formations was followed in a time dependent manner (Fig. 3). Shootlets incubated under C1 conditions in media supplemented with sucrose (6% or 8%) devoid of any growth regulators, i.e. M1 and M2, needed 60 days for microtubers formation as compared to C2 conditions that needed 40 and 45 days respectively. When incubated in the presence of the diverse cytokinins (M3 till M8) under C1 conditions, microtubers' formation was evident at day 30. Shootlets incubated in M3 and M7 media, supplemented with 5 $mg.l^{-1}$ BAP and 5 $mg.l^{-1}$ BAP + 2 $mg.l^{-1}$ Kin respectively, showed the fastest microtubers' formation at day 10. Other cytokinin combinations (M4, M5 and M8) required between 15 to 20 days. This confirm that M3 and M7 combination were the best for microtubers' formation.

Table 4 - Effect of culture condition on the average number (AN), average length (AL), average width (AW) and average weight (Awe) of microtubers produced

Culture conditions	Average number of microtubers	Average length of microtubers (mm)	Average width of microtubers (mm)	Average weight (mg)	Colour of microtuber
C1	1.09 b	10.75 a	7.41 a	646.26 a	Green
C2	1.49 a	8.63 b	5.65 b	481.23 b	Brown

C1 culture conditions= 16-h day, 8-h night (25±2°C),
C2 culture conditions= For Initial 7 days, 16-h day, 8-h night (25±2°C), For remaining period: continuous dark (19±2°C)
Means followed by different letters in each column are significantly different for P≤0.05.

Table 5 - Effect of culture condition on the average number (AN), average length (AL), average width (AW) and average weight (Awe) of microtubers produced

Culture media	Sucrose concentration	Average number of microtubers	Average length of microtubers (mm)	Average width of microtubers (mm)	Average weight (mg)
M1	6%	0.55 d	8.76 a	5.93 a	455.35 a
M2	8%	0.67 d	9.19 a	6.14 a	495.65 a
M3	6%	1.95 a	10.45 a	6.49 a	573.3 a
M4	8%	1.5 b	9.46 a	6.41 a	577.45 a
M5	6%	1 c	10.46 a	6.91 a	610.75 a
M6	8%	1.26 bc	9.32 a	6.29 a	514.1 a
M7	6%	1.9 a	9.46 a	6.26 a	525.85 a
M8	8%	1.53 b	10.44 a	7.01 a	622 a

C1 culture conditions= 16-h day, 8-h night (25±2°C),
C2 culture conditions= For Initial 7 days, 16-h day, 8-h night (25±2°C), For remaining period, continuous dark (19±2°C)
Means followed by different letters in each column are significantly different for P≤0.05.

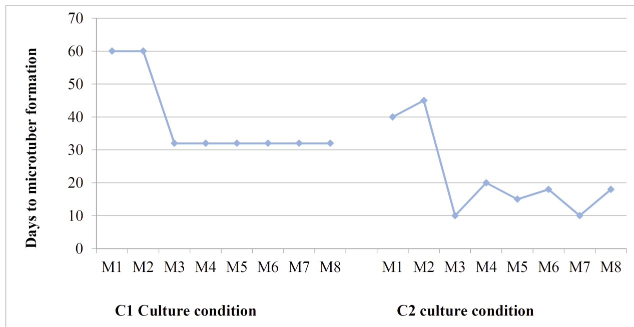

Fig. 3 - Days to microtuber formation under different conditions.

4. Discussion and Conclusions

Accumulation of seed-borne diseases is a major cause of low yield and quality losses in agriculture. Potato is a strategic crop for many populations. Producing disease-free potato microtubers is vital to improve its production. This study aimed to determine the best conditions and agents (sucrose and cytokinins) on the microtuberization capacity of the potato cultivar Spunta. As meristem tips are free from viruses, elimination and generation of virus free plants were shown by many researchers through meristem culture to obtain disease free potato plantlets (Jha and Ghosh, 2005; Bhuiyan, 2013).

Our findings provide evidence on the importance of having media devoid of growth regulators on the formation of reactive meristem in order to obtain the highest percentage. These results are in contradiction with others where Al-Taleb *et al.* (2011) indicated that the medium supplemented with 0.5 mg.l^{-1} of IBA was the best for shoots development and where Hajare *et al.* (2021) showed that best shoot initiation was obtained on MS medium supplemented with 1.5 mg.l^{-1} BAP + 3.0 mg.l^{-1} NAA for Gudiene variety, whereas 1.0 mg.l^{-1} BAP and 2.0 mg.l^{-1} NAA produced more shoots in Belete variety. According to a study done by Kanwal *et al*. (2006) who investigate the *in-vitro* micropropagation of potato cultivar Kuroda, the increase of added BAP concentrations in MS medium induced an increase in the rate of shoot formation.

According to Salem and Hassanein (2017) who studied the effect of genotype on multiplication and micro-tuberization of potato *in vitro,* the highest number of microshoot was formed by cv. Hermes after cultivation in a medium supplemented with 1 mg.l^{-1} BAP + 0.5 mg.l^{-1} GA_3, whereas the least one was formed by cv. Spunta implying that the number of obtained microshoots is cultivar-dependent.

At the level of the multiplication phase, our results showed that the media P3 containing Kin 0.4 mg.l^{-1}, GA3 0.5 mg.l^{-1} and IBA 0.5 mgl^{-1} caused the highest number of shootlets/plant. Similar results were obtained by Emaraa *et al.* (2017) who supplemented their media with Kin (0.2 mg.l^{-1}) and naphtaleneacetic acid (NAA, 0.2 mg.l^{-1}) and obtained maximum number of shootlets/plant. Among the different cytokinins used by researchers when studying micropropagation of plants, Kin stands among the best to promote shoot formation and shoot length (Van-Staden *et al.,* 2008; Hoque, 2010). El Dessoky *et al.* (2016) also showed that the highest percentage of shoot initiation and multiplication were observed on MS medium containing Kin (0.1 mg.l^{-1}) and GA3 (5 mg.l^{-1}).

Concerning microtuber formation, this study revealed that the addition of BAP into the culture medium was more effective in inducing *in vitro* tuberization in comparison to kinetin. The highest average number (AN) of microtubers was produced when propagated shootlets were subcultured on M3 medium containing 5 mg.l^{-1} BAP. These results agree with others (Aksenove *et al.,* 2009; Hoque, 2010; Sota *et al.*, 2020) who also showed that BAP has greater potential for microtuberization than kinetin and had an effect on reduction of total sugar and subsequently have increased starch content (Sarkar *et al.,* 2006).

Furthermore, Liljana *et al.* (2012) observed that tuberization occurred only in the presence of BAP (2 mg.l^{-1}) + IAA (1 mg.l^{-1}). Wang and Hu (1982) and Badoni and Chauan (2010) also reported that the optimum condition for *in vitro* tuberization of virus-free potatoes were in a medium containing BAP at 10 mg.l^{-1}. Vural *et al.* (2018) used BAP (2.5 mg.l^{-1}) with NAA (0.5 mg.l^{-1}) in their *in vitro* micropropagation of potato and found that these cytokinins had a positive effect on micro tuber formation and can be recommended to use them commercially in mass production.

On the other hand, these cytokinins showed no significant effect on the average length (AL), average width (AW) and average weight (AWe) of microtubers. Our results contradict those of Aryakia and Hamidoghli (2010) who showed that BAP and Kin induced changes in microtuber size and weight in the *in vitro* microtuberization of two potato cultivars, Arinda and Diamant.

Prat (2004) reported that Kin played a significant role in creating sink during plant growth, and through

regulating the expression of a gene involved in the partition of assimilates towards the stolon as observed in potato. Our study reports that kin exerted no significant effect on growth, diameter and weight of microtuber and this is in agreement with Kefi *et al.* (2000) and Kanwal *et al.* (2006).

In general, a linear relation between size and weight of microtuber exists (Liu and Xie, 2001). Any factor that influences microtuber weight will directly influence microtuber size. The microtubers parameters AN, AL, AW, and AWe obtained differed based on the culture condition. Growth condition C2 proved to be better for microtuber formation, whereas condition C1 was better for AL, AW, AWe. Under C1 culture condition, most of the culture produced green microtubers and this could be due to the synthesis of the alkaloid solanine that has fungicidal and pesticidal properties and that is considered as one of the plant's natural defenses. Hoque has also shown that when potato tubers are exposed to light, they turn green and increase glycoalkaloid production (Hoque, 2010). On the other hand, culture condition C2 yielded brown-colored tubers (Fig. 1, I and J). These results are in agreement with those of Salem and Hassanein (2017) who showed that dark conditions were better for microtuber formation compared to a 16-h photoperiod. Similarly, Sakha *et al.* (2004) also reported that microtuber formation frequency is higher under dark than light conditions. In addition, García and Bolaños (2017) showed that under the light condition with a photoperiod of 16-hour light and 8-hour dark, the number of microtubers was lower, but their biomass was higher and greener. On the other hand, under the dark condition, the number of microtubers was greater, with a lower biomass average and cream color. Garner and Blacke (1989) Al-Hussaini *et al*. (2015), showed in periods of total darkness, the microtuber weight is reduced. Other study also concluded that the light condition of 8 hours may be of benefit to the *in vitro* tuberization and increased the size and uniformity of microtubers compared to the *in vitro* tuberization under dark conditions (Pruski *et al*., 2002). Thus, an appropriate combination of light and dark conditions with short days can synchronize and accelerate the initiation and development of microtubers as well as increase their numbers.

Finally, concerning the effect of sucrose on microtuber formation, lower sucrose concentration (6%) resulted in an increase in the average number of the produced microtubers. Whereas sucrose presence had no effect on the length, width and weight (Table 5). Our results agree with those of Fufa and Diro (2014) with respect to the effect of 6% sucrose on the AN of the microtuber. Aslam *et al.* (2011) also found that a medium containing 6% sucrose was optimal in terms of minimum time of induction, average tuber number and weight of microtubers per single nodal explant in cultivar Desiree. Imani *et al.* (2010) also reported that MS medium supplemented with 6% of sucrose yielded the maximum number and the highest AL and Aw of microtubers. In plant tissue culture, most plant requires an exogenous carbohydrates source because of the limited photosynthesis that occurred *in vitro* (Lian *et al.,* 2014). Tuberization is known to be regulated by carbohydrates availability such as sucrose which is the transported form of sugar required for starch synthesis (Abelenda *et al.,* 2019). Sucrose is essential for the *in vitro* tuberization as an energy source and a signal for microtuber formation (Donnelly *et al*., 2003; Fufa and Diro, 2013; MotallebiAzar *et al.,* 2013).

In this study, to obtain microtubers of a sufficient weight, potato microshoots were cultured for 7 days in 16-h photoperiod followed by 50 days in dark. This type of treatment was referred to as light/dark treatment (Table 1). It increased microtuberization by enhancing tuberonic acid synthesis that was shown to play an important role in *in vitro* tuber formation (Alisdair and Willmitzer, 2001).

In conclusion, the best combination for rapid microtuberization obtained in our study was 6% sucrose, 5 $mg.l^{-1}$ BAP for Spunta potato cultivar cultured under C2 culture condition was within 10 days. Hossain *et al*. (2015) who investigated the effect of sucrose, growth regulators and potato varieties ('Diamant' and 'Cardinal') on rapid microtuberization found that the best combination was 9% sucrose at 5 $mg.l^{-1}$ of 6-benzyl aminopurine cultivar within 6-8 days while Zakaria *et al.* (2008) who studied the optimum level of Benzyl Adenine (BA) and Chloro Choline Chloride (CCC) to obtain large-size microtubers of the potato cultivar Diamant showed that early microtuber induction occur by using 500 mg/l of CCC within 15.9 days or by using 10 mg/l of BA within 13.3 days.

In the present study, the effect of two growth regulators, Kinetin and BAP, and the presence of sucrose and different photoperiodic conditions on the performance of microtubers in the potato cultivar Spunta was evaluated. Microtubers were produced from *in vitro* grown plantlets regenerated in MS medium supplemented with kin 0.4 $mg.l^{-1}$, GA3 0.5 $mg.l^{-1}$ and IBA

0.5 mg.l^{-1}. The highest number of microtuber was obtained in M3 medium during C2 condition supplemented with 60 g.l^{-1} sucrose whereas C1 condition was better for AL, AW, and AWe of microtubers. The results indicated that microtuber induction of potato was highly dependent on sucrose, growth regulator and photoperiod conditions interaction. Production of large microtubers is important for successful utilization of microtubers in seed potato production. Hence, this study proposes an economical and reproducible method to obtain larger microtubers under laboratory conditions and it could be experimented to produce seed potato from *in vitro* grown tubers.

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References

ABELENDA J., BERGONZI S., OORTWIJN M., SONNEWALD S., DU M., VISSER R., SONNEWALD U., BACHEM C., 2019 - *Source-sink regulation is mediated by interaction of an FT homolog with a sweet protein in potato.* - Curr. Biol., 29(7): 1178-1186.

ABOU-JAWDAH Y., SOBH H., SAAD A., 2001 - *Incidence of potato virus diseases and their significance for a seed certification program in Lebanon.* - Phytopathologia Mediterranea, 40(2): 113-118.

AKSENOVE N., KONSTANTINOVA T., LOZHNIKOVA V., GOLYANOVSKAYA S., SERGEEVA L., 2009 - *Interaction between day length and phytohormones in the control of potato tuberization in the* in vitro *culture.* - Russian J. Plant Physiol., 56(4): 454-461.

AL-HUSSAINI Z., YOUSIF S.H., AL-AJEELY S., 2015 - *The role of sucrose and light duration on* in-vitro *tuberization for two cultivars of potato* Solanum tuberosum *L.* - Int. J. Current Microbiol. Appl. Sci., 4(2): 277-283.

ALISDAIR R., WILLMITZER L., 2001 - *Molecular and biochemical triggers of potato tuber development.* - Plant Physiol., 127(4): 1459-1465.

AL-SAFADI B., AYYOUBI Z., JAWDAT D., 2000 - *The effect of gamma irradiation on potato microtuber production* in vitro. - Plant Cell, Tissue Organ Cult., 61(3): 183-187.

Al-TALEB M., HASSAWI D., ABU-ROMMAN S., 2011 - *Production of virus free potato plants using meristem culture from cultivars grown under Jordanian environment.* - American-Eurasian J. Agric. Environ. Sci., 11(4): 467-472.

ARYAKIA E., HAMIDOGHLI Y., 2010 - *Comparison of Kinetin and 6- Banzyl Amino Purine effect on* in vitro *microtuberization of two cultivars of potato (*Solanum tuberosum *L.).* - American-Eurasian J. Agric. Environ. Sci., 8(6): 710-714.

ASLAM A., ALI A., NAVEED N., SALEEM A., IQBAL J., 2011 - *Effect of interaction of 6-benzyl aminopurine (BA) and sucrose for efficient microtuberization of two elite potato (*Solanum tuberosum *L.) cultivars, Desiree and Cardinal.* - African J. Biotechn., 10(59): 12738-12744.

BADONI A., CHAUHAN J., 2009 - *Microtuber: A source of germplasm conservation.* - Rep. Opin., 1: 69-71.

BADONI A., CHAUHAN J., 2010 - *Potato seed production of cultivar Kufri Himalini* in vitro. - Stem Cell., 1(1): 7-10.

BAMBERG J.B., MARTIN M.W., ABAD J., JENDEREK M.M., TANNER J., DONNELLY D.J., NASSAR AM.K., VEILLEUX R.E., NOVY R.G., 2016 - In vitro *technology at the US Potato Genebank. - In Vitro* Cell. and Dev. Biol. - Plant, 52(3): 213-225.

BASERA M., CHANDRA A., KUMAR V., KUMAR A., 2018 - *Effect of brassinosteroids on* in vitro *proliferation and vegetative growth of potato.* - The Pharma Innovation J., 7(4): 4-9.

BHUIYAN F., 2013 - In vitro *meristem culture and regeneration of three potato varieties of Bangladesh.* - Res. Biotech., 4(3): 29-37.

CHOUEIRI E., JREIJIRI F., WAKIM S., ISSA El KHOURY M., VALENTINI F., DUBLA N., GALLI D., HABCHY R., AKL K., STEFANI E., 2017 - *Surveys of potato-growing areas and surface water in Lebanon for potato brown and ring rot pathogens.* - Phytopathol. Mediter., 56(1): 87-97.

CLARK M., ADAMS A., 1977 - *Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses.* - J. General Virol., 34: 475-483.

DONNELLY D., COLEMAN W., COLEMAN S., 2003 - *Potato micro tuber production and performance: A review* - Am. J. Potato Res., 80: 103-115.

EL DESSOKY D., ATTIA A., ISMAIL I., EL-HALLOUS E., 2016 - In vitro *propagation of potato under different hormonal combinations.* - Intern. J. Adv. Res., 4(1): 684-689.

EMARAA H., HAMZA E., FEKRY W., 2017 - In vitro *propagation and microtuber formation of potato in relation to different concentrations of some growth regulators and sucrose* - Middle East J. Agric. Res., 6(4): 1029-1037.

FAO, 2019 - *FAO statistical databases FAOSTAT*. - FAO, Rome, Italy.

FUFA M., DIRO M., 2013 - *The effects of sucrose on* in vitro *tuberization of potato cultivars.* - Adv. Crop Sci. Technol., 1(114).

FUFA M., DIRO M., 2014 - *Microtuber induction of two potato (*Solanum tuberosum *L.) varieties.* - Adv. Crop Sci. Technol., 2(2).

FUGLIE K., 2007 - *Priorities for potato research in developing countries: Results of a survey.* - Am. J. Potato Res., 84: 353-365.

GARCIA J., BOLAÑOS J., 2017 - *Response to boric acid and light in the number and biomass of potato microtubers cv. "Floresta".* - Uniciencia, 31(2): 121-131.

GARNER N., BLAKE J., 1989 - *The induction and development of potato microtubers* in vitro *on media free of growth regulating substances.* - Annals Bot., 63(6): 663-674.

HAJARE S., CHAUHAN N., KASSA G., 2021 - *Effect of growth regulators on* in vitro *micropropagation of potato (*Solanum tuberosum *L.) Gudiene and Belete varieties from Ethiopia.* - Scientific World J., 2021: 1-8.

HOQUE M., 2010 - In vitro *tuberization in potato (*Solanum tuberosum *L.).* - Plant. Omics. J., 3(1): 7-11.

HOSSAIN M., KAWOCHAR M., MAHMUD A., SHOFIUR RAHAMAN E., HOSSAIN M., NASIRUDDIN K., 2015 - *Standardization of sucrose and 6-Benzyl aminopurine for* in vitro *micro tuberization of potato.* - Amer. J. Agricul. For., 3(2): 25-30.

HOSSAIN M.J., 2005 - In vitro *microtuberisation of potato obtained from diverse sources.* - Plant Tissue Cult. Biotech., 15(2): 157-166.

HUSSAIN I., CHAUDHRY Z., MUHAMMAD A., ASGHAR R., NAQVI S., RASHID H., 2006 - *Effect of chlorocholine chloride, sucrose and BAP* in vitro *tuberization in Potato (*Solanum tuberosum *L. cv. cardinal).* - Pak. J. Bot., 38(2): 275-282.

IMANI A., QHRMANZADEH R., AZIMI J., JANPOOR J., 2010 - *The effect of various concentrations of 6-Benzylaminopurine (BAP) and sucrose on* in vitro *potato (*Solanum tuberosum *L.) microtuber induction.* - American-Eurasian J. Agric. Environ. Sci., 8(4): 457-459.

JHA T., GHOSH B., 2005 - *Plant tissue culture - basic and applied.* - Universities press (India) private limited.

KANWAL A., ALI A., SHOAIB K., 2006 - In vitro *microtuberization of potato (*Solanum tuberosum *L.) cultivar Kuroda - A new variety in Pakistan.* - Inter. J. Agric. Biol., 8(3): 337-340.

KEFI S., PAVLISTA A., READ P., KACHMAN S., 2000 - *Comparison of thidiazuron and two nitroguanidines to kinetin on potato micrituberization* in vitro *under short and long days.* - Plant Regul., 19(4): 429-436.

LIAN M., PIAO X., PARK S., 2014 - *Mass production of* Lilium *bublets in bioreactors,* pp. 389-415. - In: PAEK K.Y., H. MURTHY, and J.J. ZHONG (eds.) *Production of biomass and bioactive compounds using bioreactor technology.* Springer Verlag, Dordrecht, Germany, pp. 709.

LILJANA G., MITREV S., FIDANKA T., MITE I., 2012 - *Micropropagation of potato* Solanum tuberosum *L.* - Electronic J. Biol., 8(3): 45-49.

LIU J., XIE C., 2001 - *Correlation of cell division and cell expansion to potato microtuber growth* in vitro. - Plant Cell, Tissue Organ Cult., 67(2): 159-164.

MAKKOUK K., KUMARI S., 1996 - *Detection of ten viruses by the tissue-blot immunoassay (TBIA).* - Arab J. Plant Protection, 14(1): 3-9.

MOA, 2012 - *Ministry of Agriculture. Recensement Generale*. - FAO/Project Recensement Agricole, Beirut, Lebanon.

MOTALLEBIAZAR A., KAZEMIANI S., YARMOHAMADI F., 2013 - *Effect of sugar/osmotica levels on* in vitro *microtuberization of potato (*Solanum tuberosum *L.).* - Russ. Agric. Sci., 39: 112-116.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bio assays with tobacco tissue cultures.* - Plant Physiol., 15(3): 473-497.

NARESH K., SEN D., SINGH V., KUMAR S., 2011 - *Effect of growing media on rooting and growth of patchouli (*Pogostemon cablin*) cuttings in subtropical humid region of Arunachal Pradesh.* - Environ. Ecol., 29(2): 567-569.

NIKITIN M.M., STATSYUK N.V., FRANTSUZOV P.A., DZHAVAKHIYA V.G., GOLIKOV A.G., 2018 - *Matrix approach to the simultaneous detection of multiple potato pathogens by real-time PCR.* - J. Appl. Microbiol., 124(3): 797-809.

NISTOR A., CAMPEANU G., ATANASIU N., CHIRU N., KARACSONYI D., 2010 - *Influence of potato genotypes on* "in vitro" *production of microtubers* - Romanian Biotechnological Letters, 15(3): 1-8.

PRAT S., 2004 - *Hormonal and day length control of potato tuberization*, pp. 538-560. - In: DAVIS P.J. (ed.) *Plant hormones: Biosynthesis, signal transduction, action*. Kluwer Acad. Pub., Dordercht, The Netherlands, pp. 802.

PRUSKI K., ASTATKIE T., NOWAK J., 2002 - *Jasmonate effects on* in vitro *tuberization and tuber bulking in two potato cultivars (*Solanum tuberosum *L.) under different media and photoperiod conditions. - In vitro* Cellular Developmental Biol. - Plant, 38(2): 203-209.

ROY B., 2014 - *Farmers' participatory quality seed production of field crops. A case study.* - J. Crop Weed, 10(2): 89-93.

SAKHA B., BHATIA A., BATRA V., CHAUDHARY V., BATRA P., KHURANA S., 2004 - In vitro *microtuberization in potato (*Solanum tuberosum *L.) cultivars.* - Indian J. Exp. Biol., 42(12): 1245-1247.

SALEM J., HASSANEIN A., 2017 - In vitro *propagation, microtuberization, and molecular characterization of three potato cultivars.* - Biol. Plantarum, 61(3): 427-437.

SARKAR D., PANDEY S., SHARMA S., 2006 - *Cytokinins antagonize the jasmonates action on the regulation of potato (*Solanum tuberosum*) tuber formation* in vitro. - Plant Cell, Tissue Organ Cult., 87(3): 285-295.

SOTA V., BEKHEET S., KONGJIKA E., 2020 - *Effect of growth regulators on micropropagation and* in vitro *tuberization of* Solanum Tuberosum *L. cv. Vermosh* - South Western J. Hortic., Biol. Environ., 11(2): 67-81.

VAN-STADEN J., ZAZIMALOVA E., GEORGE E., 2008 - *Plant growth regulators, II: Cytokinins, their analogues and inhibitors*, pp. 205-226. - In: GEORGE E.F., HALL M.A., and G.J. DE KLERK (eds.) *Plant propagation by tissue culture.* Springer, Dordrecht, Germany, pp. 502.

VURAL G., OZSAN T., GOZEN V., ONUS A., 2018 - In vitro *micro tuber formation in potato (*Solanum tuberosum *L.): is there any relation between methyl jasmonate, sugars, and explants?* - Inter. J. Biotech Trends Technol. (IJBTT), 8(1): 1-8.

WANG P., HU C., 1982 - In vitro *mass tuberization and virus-free seed potato production in Taiwan.* - Am. Po. J., 59(1): 33-37.

ZAKARIA M., HOSSAIN M., MIAN M., HOSSAIN T., UDDIN M., 2008 - In vitro *tuberization of potato influenced by benzyl adenine and chloro chline chloride.* - Bangladesh J. Agric. Res., 33(3): 419-425.

ZHANG Z., LI H., ZHOU W., TAKEUCHI Y., YONEYAMA K., 2006 - *Effect of 5-ami- nolevulinic acid on development and salt tolerance of potato (*Solanum tuberosum *L.) microtubers* in vitro. - Plant Growth Regul., 49(1): 27-34.