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RESEARCH

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# Factors affecting in vitro tuberization of potato

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## Abstract

**Background** Potato (*Solanum tuberosum* L.) is one of the most economically important annual vegetable crops. Microtubers can be produced, stored around year and directly transported to market without transferring to fresh media and without acclimatization. Thus reducing the field cycle to obtain sufficient number of seed potatoes and a high level of healthy materials. The aim of this study was to investigate effects of different combinations and concentrations of growth regulators i.e., kinetin (2.5 mg/l) and coumarin (20, 40 and 60 mg/l) alone or in addition to varied concentrations of sucrose (30, 60 and 90 g/l) on in vitro tuberization of potato (*Solanum tuberosum*) cv. Diamount.

**Results** The plantlets were propagated using single-node cuttings cultured on Murashige and Skoog medium containing 0.04 mg/l kinetin and 1.0 mg/l IAA. Shoots (6–7 nodes) from the previous step were cultured into medium supplemented with a factorial combination of sucrose concentrations (30, 60 and 90 g/l), coumarin concentrations (0, 20, 40, and 60 mg/l) and two concentrations of kinetin (0 and 2.5 mg/l). Our results showed that kinetin – induced medium have slightly effect to improve tuberization in vitro. But when kinetin was combined with sucrose, this effect was better when raised sucrose concentration from 30 or 60 to 90 g/l. The highest percentage of tuberization after 8 weeks, highest number of microtuber were obtained with high concentration of sucrose (90 g/l) together with dark condition, at 18–20 °C. Also, the highest concentration of sucrose significantly increased the fresh weight of microtuber. In case of coumarin, results revealed that it has important effect on tuber initiation especially with concentrations 20 mg/l and 40 mg/l; otherwise, the initiation period was minimized after adding kinetin. So, the plantlets must be continuously incubated in coumarin –induce medium plus kinetin for 8 weeks to affect the tuberization response. Also, all the other characters of tuberization process on coumarin-inducing medium could be improved by increasing sucrose concentration to 9 g/l.

**Conclusions** Generally, results pointed out that the treatment which consists of kinetin (2.5 mg/l) plus 90 g/l sucrose and 20 mg/l coumarin has the best characteristics of in vitro micro-tuberization.

**Keywords** *Solanum tuberosum L*, Sucrose, Kinetin, Coumarin, Microtubers

## Background

Potato (*Solanum tuberosum L.*) is one of the most important vegetable crops all over the world. It is the fourth largest staple crop next to rice, wheat and barley. In some countries, potato is considered the main daily food due to high nutritive value and their low price (Xin et al. 1998). Potato is an annual herbaceous plant, it is vegetatively propagated by tubers. Microtuberization of potato has been one of the successful methods to increase potato under in vitro conditions (Yu et al. 2000). Microtubers can be preserved for a long time and thus these could be an ideal propagation material (Fufa and Diro 2014; Mahdi et al. 2004). Also, they can be planted directly in the soil and produced for around-year. In vitro propagated potato plantlets are commonly used for the production of in vitro tubers in potato seed production programs.

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In vitro microtubers production was used to solve some problems such as virus-free potato seed storage, in vivo plantlets transplantation, conservation of important cultivars and handling healthy germplasm exchange (Kefi et al. 2000; Rosu et al. 2004; Kanwal et al. 2006). Microtubers can be produced, stored for around the year and directly transported to market without transferring to fresh media (Nhut et al. 2004) and without acclimatization (Jimerez et al. 1999). Thus reducing the field cycle to obtain a sufficient number of seed potatoes and a high level of healthy materials (Wróbel 2014). However, there are limitations in the production of microtuber due to the components of the culture environment and the low rate of photosynthesis process of the explants or plantlets. Tuberization is a complex physiological process controlled by several factors, such as environmental factors, nitrogen supply, growth regulators (PGRs), genotypes, growth nutrients, photoperiods, temperature, source of explant, potato cultivar and sucrose concentration (Ramawat and Merillon 2013). In vitro Potato microtubers derived from single-node cuttings is an efficient method for handling and storage compared to those obtained from sprouts (Liljana et al. 2012). Most studies pointed that tuberization is affected by hormonal control but the stimulus to tuberization is not unique and can be a single compound some of them having hormone activity. Although the physiological mechanism of potato tuberization between the various hormonal compounds that regulate the process is still unknown, several researchers used different growth regulators to stimulate microtuberization (Coleman et al. 2001; Tugrul et al. 2001). Potato microtubers induction depends on the combination of auxins and cytokinins (Tugrul and Samanci 2001; Hossain 2005). Some reports suggested that cytokinins have the best effect on potato microtubers formation (Wang and Xiao 2009) such as BAP (Kane 2011) or Kin (Hoque 2010) or coumarin (El-Sawy et al. 2007, 2015). Retardants or growth inhibitors are also needed to inhibit and suppress the activity of gibberellins so that the energy can be focused on forming potato tubers (Nuraini et al. 2018). Suppressing gibberellin activity accelerates the formation of tubers and ultimately increases their production (Kolachevskaya et al. 2019).

The objectives of the present study are to investigate the effects of kinetin, sucrose and coumarin on tuberization process and identify the best microtuberization media.

## Methods

### Source of plant materials

Virus-free potato plantlets cv. Diamount were obtained from plant Biotechnology Dep., Biotechnology Institute, National Research Centre, Cairo, Egypt.

### Micropropagation of plantlets

The plantlets were propagated using single-node cuttings. Ten explants were cultured in sterilized culturing jars containing 40 ml of standard medium. The medium contained 4.4 g/l Murashige and Skoog medium with vitamins ( Murashige and Skoog 1962), with vitamins, 30 g/l sucrose, 6 g/l agar, 0.04 mg/l kinetin and 1.0 mg/l IAA, the pH was adjusted to 5.8 -6 before adding the agar and autoclaving. The culture jars were closed with polyvinyl carbonate caps and sealed with household plastic foil and placed in a growth chamber set at 24 °C ± 1 and 16 h photoperiod for 4 weeks. The single–node explants were grown to rooted plantlets. Plantlets were cut again into single-node explants and were transferred into a fresh MS medium as described above.

The multiplication phase was routinely repeated every 4 weeks by subculturing single-node cuttings until the desired numbers of in vitro plantlets for the experiment was obtained (El-Sawy et al 2015).

### In vitro tuberization

Shoots (6–7 nodes) resulted from the previous step were cultured into solid MS medium supplemented with a factorial combination of four sucrose concentrations (30, 60 and 90 g/l), four coumarin concentrations (0, 20, 40, and 60 mg/l) and two concentrations of kinetin (0 and 2.5 mg/l). PH was adjusted to 5.7 before autoclaving for 20 min at 121° C. Each culture jar (250 ml) received 40 ml medium and contains five plantlet shoots. Cultures were incubated under completely dark conditions at 18–20° C.

All the experiments were repeated twice, three replicates per treatment with 5 explants for each replicate.

The following growth characters were recorded:

1. Tuberization growth curve during 8 weeks
2. Number of days to begin tuberization
3. Number of days to reach 50% tuberization
4. Percentage of tuberization after 8 weeks
5. Number of tubers per plantlet after 8 weeks
6. Fresh weight of microtubers (mg).

### Experimental design and statistical analysis

All experiments were set up in a factorial experiment based on a completely randomized design, and data were statistically analyzed by one-way analysis of variance (ANOVA) for testing the differences among treatments using least-significant-difference (LSD) test at $p < 0.05$ (LSD0.05) according to Gomez and Gomez (1984). The results were expressed using analysis of variance from which mean standard error values (mean ± SE) including

**Table 1** Effect of Kinetin on the development of in vitro potato microtubers

| Characters of microtuberization | Without kinetin | With kinetin |
|---|---|---|
| No. of days to begin tuberization | 12 ± 0.577[a] | 8 ± 0.577[b] |
| No. of days to reach 50%tuberization | 30.3 ± 0.723[a] | 24.5 ± 0.348[b] |
| % of tuberization after 8 weeks | 74.4 ± 1.131[b] | 81.8 ± o.608[a] |
| No of tubers per plantlet after 8 w | 1.4 ± 0.066[a] | 1.5 ± 0.066[a] |
| Weight of microtuber (mg) | 50.6 ± 0.457[b] | 62.6 ± 0.288[a] |

V values are means ± SE, LSD test at level ($p < 0.05$)

Values with the same superscript letters (a,b,c or d) are not significantly different from each other whilst, values with different superscript letters are significantly different

superscript letters were computed for comparison between treatments indicating the presence of significant difference at the 0.05 probability level.

## Results

### Effect of kinetin

Microtuberization process on kinetin–induced medium and non-kinetin –induced medium was evaluated after 8 weeks incubation period under complete dark condition at 18- 20 C°. The results are shown in Table 1 and Fig. 1, 81.8% of cultured plantlets produced microtubers when cultured on kinetin–induced medium (2.5 mg/l) after 8 weeks from culture. However, 74.4% of plantlets cultured on free-kinetin medium produced microtubers. These microtubers produced on kinetin-induced medium were heavier than those produced on the free medium by rate of 19.2%. The plantlets cultured on kinetin-induced medium began tuberization 8 days after cultivation, needed about 24.5 days to achieve 50% tuberization, but they needed 30.3 days on kinetin-free medium. Otherwise, the tuberized plantlets produced slightly number of microtubers (1.4 and 1.5/planlet). Our results showed that kinetin–induced medium had a slightly effect to improve tuberization in vitro of potato. Data in Fig. 1 showed that in both treatments the rate of tuberization was nearly closed for each. Tuberization is a complex developmental phenomenon including many physiologically regulated processes. Tuberization of potato plants would be better understood under in vitro conditions as several morphogenic regulators and environmental factors can be controlled.

### Effect of sucrose

The carbon source in culture media is primary factor for microtuberization. Many investigators have used different sugars with different concentrations in the medium. Sucrose is the most sugar have been used in in vitro tuberization. In this study, potato plantlets were

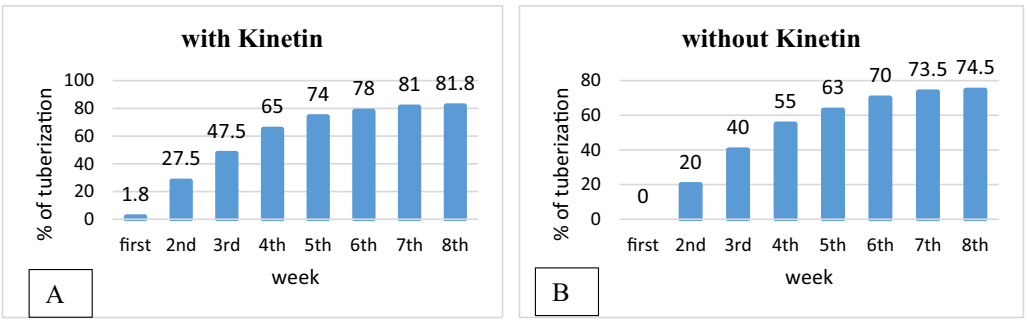



**Fig. 1** Tuberization frequency (%) on culture media with 2.5 mg/l Kinetin (**A**) or without Kinetin (**B**) every week

**Table 2** Effect of sucrose on development of in vitro potato microtubers

| Characters of microtuberization | Sucrose concentration (g/l) | | |
|---|---|---|---|
| | 30 | 60 | 90 |
| No. of days to begin tuberization | 16 ± 0.577[a] | 8 ± 0.577[b] | 6.5 ± 0.577[b] |
| No. of days to reach 50% tuberization | 39.6 ± 0.781[a] | 23.3 ± 0.260[b] | 19.4 ± 0.451[c] |
| % of tuberization after 8 weeks | 58 ± 3.403[c] | 88 ± 1.233[b] | 89 ± 2.791[a] |
| No of tubers per plantlet after 8 w | 1.3 ± 0.040[b] | 1.6 ± 0.036[a] | 1.6 ± 0.036[a] |
| Weight of microtubers | 26.8 ± 0.463[c] | 49 ± 0.577[b] | 66.3 ± 0.276[a] |

Values are means ± SE, LSD test at level ($p < 0.05$)

Values with the same superscript letters (a,b,c or d) are not significantly different from each other whilst, values with different superscript letters are significantly different

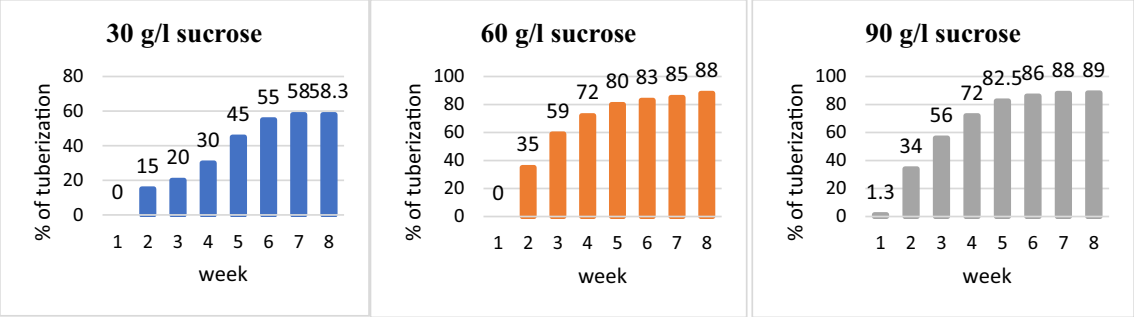



**Fig. 2** Tuberization frequency (%) under the effect of different sucrose concentrations every week

cultured on media containing 30, 60 and 90 g/l sucrose under dark condition at 18–20 °C. The results shown in Table 2 and Fig. 2, indicated that the cultured plantlets began to tuberize after 16, 8 and 6.5 days from culturing date depending on sucrose concentration 30, 60 and 90 g/l, respectively. These plantlets took about 39.6, 23.3 and 19.4 days to obtain 50% tuberized plantlets. This rate reached to 58%, 88% and 89% of tuberization after 8 weeks of culturing in the case of using 30, 60 and 90 g/l sucrose as shown in Fig. 2. Using 90 g/l sucrose gave the highest number of microtubers. Thus, it can be noticed that high sucrose concentrations with dark conditions and relatively low temperature (18–20 °C) can be used to produce microtubers. Also, the highest concentration of sucrose significantly increased the fresh weight of microtuber as high fresh weight (66.3 mg/l) was obtained by using 90 g/l sucrose as shown in Table 2. But it was lower in the case of using 60 g/l and 30 g/l sucrose (49 and 26.8 mg/l, respectively).

### Interaction between kinetin and sucrose

Our results pointed out that kinetin has slightly effect to improve microtuberization in vitro as mentioned before. This effect was better when sucrose concentration rises from 30 or 60 to 90 g/l in the medium as shown in Table 3 and Fig. 3. In our experiment, we investigated the effect of applying Kinetin at 2.5 mg/l in combination with different sucrose concentrations (30, 60 and 90 g/l). Results shown in Table 3 showed that effect of kinetin depends on sucrose content in the medium. Data in Table 3 and Fig. 3 indicated that increasing sucrose concentration in the medium either with or without adding kinetin promotes microtuberization in dark conditions at 18–20 °C. After 8 weeks, all cultured plantlets tuberized (100% tuberization) but the number of tuber /plantlet was 1.5 in case of adding kinetin with 90 g/l sucrose more than without kinetin (1.3 tubers/plantlet). Also, the fresh weight of microtuber in the case of using 90 g/l sucrose increased by 46.5%. While with 60 g/l sucrose was 51%. Our results pointed that kinetin has a higher inductive and stimulatory effect at intermediate with high sucrose means kinetin was shown to act mainly on tuber initiation thereby increasing tuber number. External sucrose is a very important factor since it provides the main source of energy under these experimental conditions. Furthermore, microtuberization has been affected by both high concentrations of sucrose and growth regulators. Although the important role of high sucrose in tuberization induction, it was not an acceptable factor for the formation process of tuber because of its high level in the culture medium.

**Table 3** The effect of interaction between sucrose and kinetin on development of in vitro potato microtubers

| Characters of microtuberization | Sucrose concentration (g/l) | | | | | |
|---|---|---|---|---|---|---|
| | 30 | | 60 | | 90 | |
| | With kin | Without kin | With kin | Without kin | With kin | Without kin |
| No. of days to begin tuberization | 18 ± 0.57735b | 25 ± 0.57735a | 8 ± 0.57735c | 8 ± 0.57735c | 8 ± 0.57735c | 8 ± 0.57735c |
| No. of days to reach 50% tuberization | 25 ± 0.57735b | 39 ± 0.5773a | 16 ± 0.5773e | 20 ± 0.5773c | 13 ± 0.5773f | 18 ± 0.5773d |
| % of tuberization after 8 weeks | 75 ± 1.258306 b | 67 ± 0.737111c | 100 ± 0.5773a | 75 ± 0.57735b | 100 ± 0.5 a | 100 ± 0.57735a |
| No of tubers per plantlet after 8 w | 1.2 ± 0.5773a | 1 ± 0.5773a | 1.3 ± 0.5773 a | 1.2 ± 0.5773 a | 1.5 ± 0.5773 a | 1.3 ± 0.5773 a |
| Weight of microtubers | 16.6 ± 0.288675e | 11.8 ± 0.1154f | 57.7 ± 0.3464c | 28.3 ± 0.3464b | 63 ± 1.73205a | 33.7 ± 0.17320d |

Values are means ± SE, LSD test at level ($p < 0.05$)

Values with the same superscript letters (a,b,c or d) are not significantly different from each other whilst, values with different superscript letters are significantly different

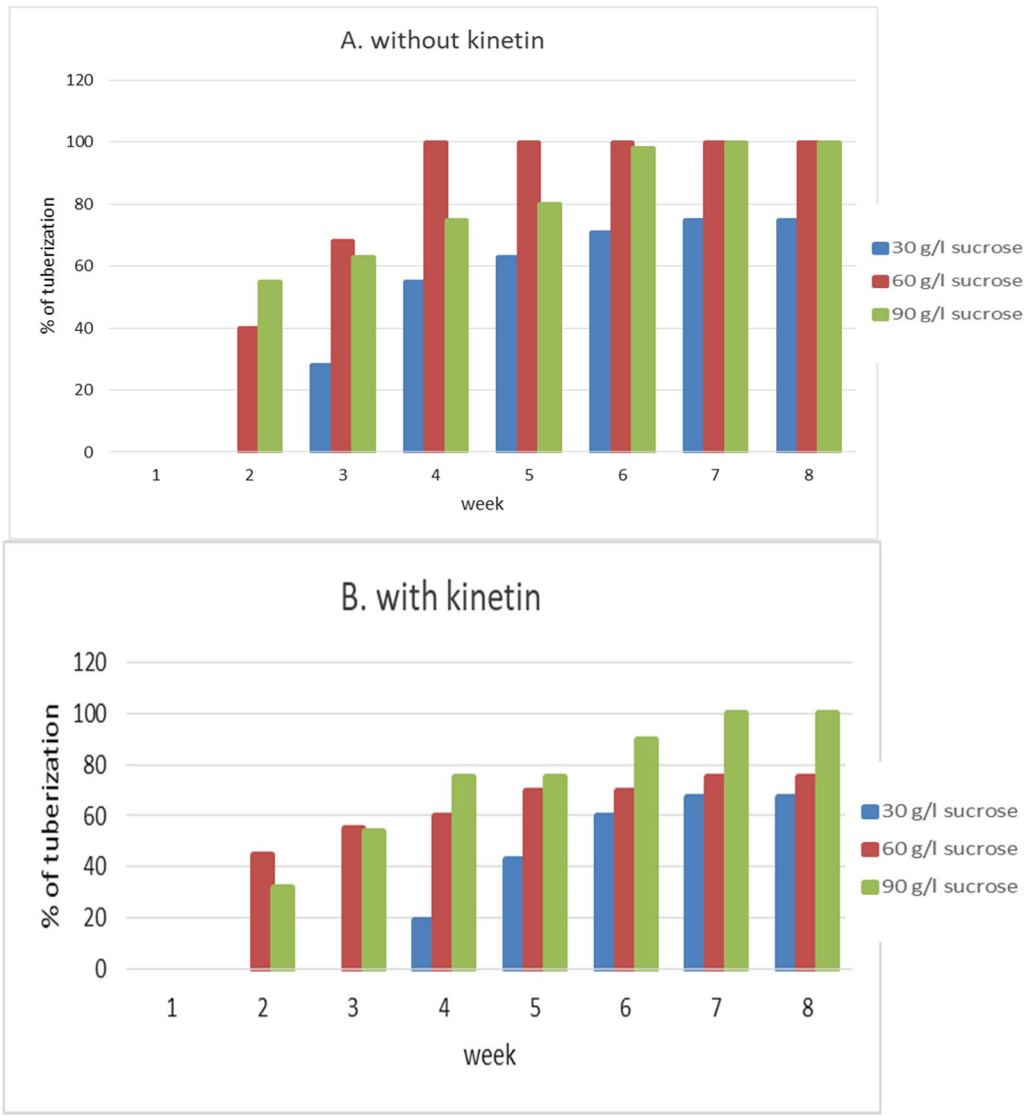



**Fig. 3** The effect of interaction between sucrose and kinetin on the tuberization frequency (%) every week

**Table 4** Effect of coumarin on development of in vitro potato microtubers

| Characters of microtuberization | Coumarin concentration ( mg/l) | | | |
|---|---|---|---|---|
| | 0 | 20 | 40 | 60 |
| No. of days to begin tuberization | 13.7 ± 5.666a | 11.3 ± 3.33a | 11.3 ± 3.333a | 13.7 ± 5.66a |
| No. of days to reach 50% tuberization | 30.7 ± 0.351b | 22.3 ± 3.21b | 25.3 ± 2.426b | 45 ± 5.507a |
| % of tuberization after 8 weeks | 65.2 ± 10.63a | 83.3 ± 8.81a | 80.6 ± 10.007a | 60 ± 5.773a |
| No of tubers per plantlet after 8 w | 1.2 ± 0.057a | 1.5 ± 0.152a | 1.5 ± 0.173a | 1.2 ± 0.1a |
| Weight of microtubers | 24.6 ± 6.333c | 69 ± 0.577a | 50.2 ± 0.986b | 27 ± 0.577c |

Values are means ± SE, LSD test at level ($p < 0.05$)

Values with the same superscript letters (a,b,c or d) are not significantly different from each other whilst, values with different superscript letters are significantly different

Also, the addition of the growth regulators in media for induction of microtuber makes it difficult to ensure the certain role of sucrose in tuberization.

### Effect of coumarin

The coumarin effect on tuberization is shown in Table 4. Tuberization was observed with 20, 40, 60 mg/l of coumarin concentrations but it is more effective at low concentration (20 mg/l). At 40 mg/l and 60 mg/l the effective rate decreased gradually compared with 20 mg/l. The tuberization percent was 65.2, 83.3, 80.6 and 60 at 0, 20, 40 and 60 mg/l of coumarin, respectively. It does mean that characteristics of the tuberization process on coumarin-induced media occurred with 20 mg/l as shown in Table 4 and Fig. 4. without any other growth regulators, especially cytokinin.

### Interaction between Coumarin and kinetin

The effects of kinetin and coumarin-induced tuberization are shown in Table 5 and Fig. 5 showed that kinetin slightly stimulated tubrization. During the course of our studies, we investigated four concentrations of coumarin (0, 20, 40 and 60 mg/l) added to the media plus kinetin at 2.5 mg/l for evaluation of tuberization process in potatoes under dark conditions at 18–20 °C. Tuberization was stimulated on coumarin-induced with this concentration with different stimulated rates depending on coumarin concentrations as mentioned before. The effect was more effective when added kinetin (2.5 mg/l) to the medium.

As shown in Table 4, one of our objectives in this study was comparison of coumarin effect on tuberization with or without addition of kinetin (2.5 mg/l) to

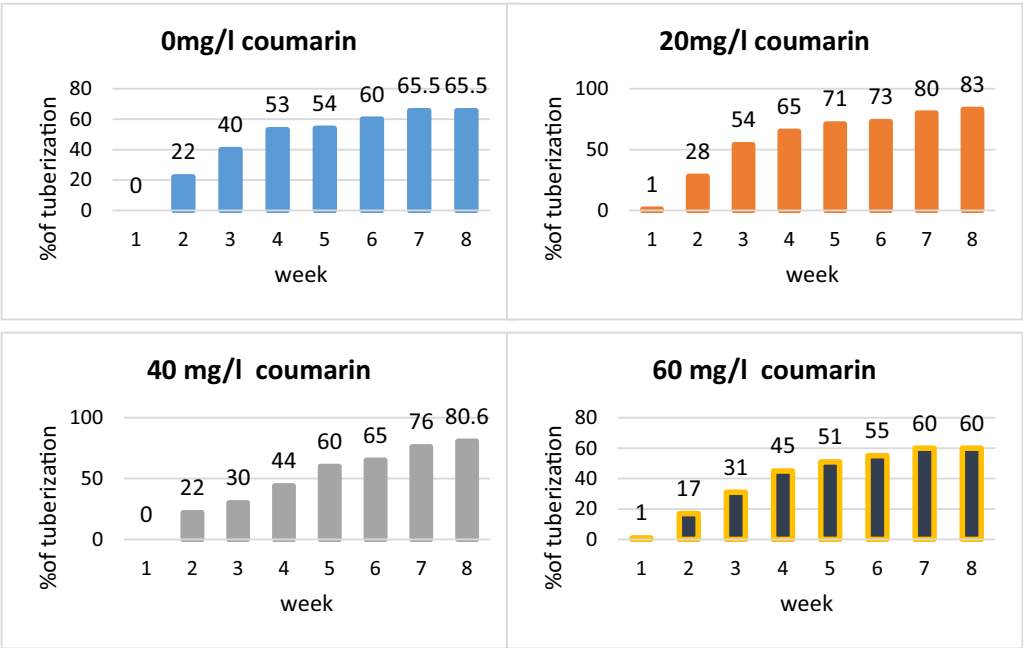



**Fig. 4** Tuberization frequency |(%) under the effect of different coumarin concentrations (0, 20, 40 and 60 mg/l) every week

**Table 5** Effect of interaction between Coumarin and kinetin: (2.5 mg/l) on development of in vitro potato microtuberization

| Characters of microtuberization | Coumarin concentration (mg/l) | | | |
|---|---|---|---|---|
| | 0 | 20 | 40 | 60 |
| No. of days to begin tuberization | 11.3 ± 3.333a | 6 ± 2a | 6 ± 2a | 8 ± 0.577a |
| No. of days to reach 50% tuberization | 18.6 ± 2.905b | 18 ± 1.527b | 22 ± 3.605a | 32.6 ± 8.685a |
| % of tuberization after 8 weeks | 87.2 ± 8.935a | 91.7 ± 8.33a | 83.6 ± 12.09a | 66.7 ± 13.059a |
| No of tubers per plantlet after 8 w | 1.3 ± 0.057b | 1.7 ± 0.145a | 1.6 ± 0.20ab | 1.3 ± 0.152b |
| Weight of microtubers | 45.8 ± 3.372b | 75.3 ± 2.91a | 46.6 ± 12.04b | 45.3 ± 5.126b |

Values are means ± SE, LSD test at level ($p < 0.05$)

Values with the same superscript letters (a,b,c or d) are not significantly different from each other whilst, values with different superscript letters are significantly different

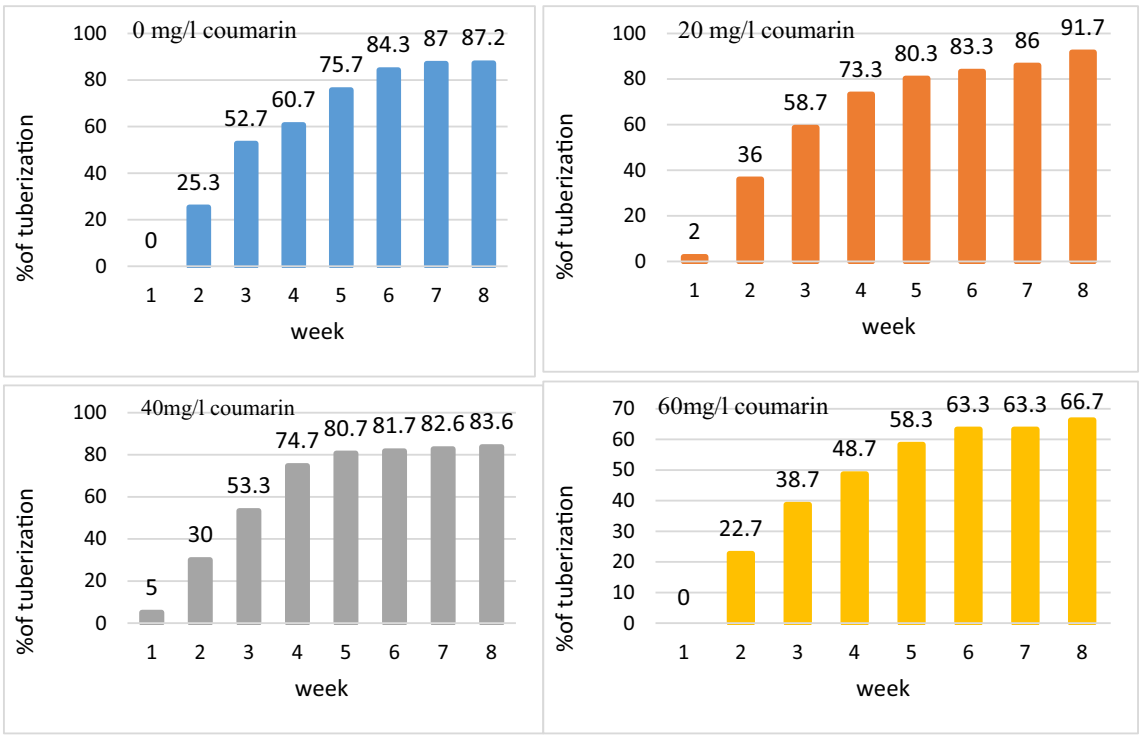



**Fig. 5** Tuberization frequency (%) under effect of interaction between Coumarin (0,20,40 and 60 mg/l) and kinetin: (2.5 mg/l) on the development of in vitro potato micro-tuberization

medium to induce tuberization. We found that plantlets tuberized in case of kinetin addition earlier than that without kinetin. This means that the initiation period was minimized after adding kinetin. The data of this study show that the plantlets must be continuously incubated in coumarin –induce medium plus kinetin for 8 weeks to affect the tuberization response (Table 5 and Fig. 5).

Our data pointed that axillary shoots formed tubers easily on media containing coumarin or kinetin, whereas other tested growth regulators were very inconsistent in stimulating tuberization.

## Effect of sucrose on coumarin-inducing media

Results shown in Table 6 indicate that the concentrations of sucrose of the culture media could affect coumarin in the tuberization process. So, the plantlets were cultured on the coumarin-induced media tuberization. These plantlets produced more tubers in the case of using 90 g/l sucrose than 60 g/l or 30 g/l (100, 100 and 66.7, respectively). Also, all the other characteristics of tuberization process on coumarin-inducing medium could be affected by sucrose concentration. It does mean that carbohydrate level in culture media for the plantlets could significantly modify the effect of nitrogen in the potato shoot media. Shoots cultured on high nitrogen media tuberized only in case of increasing carbohydrate level (sucrose concentration). The inhibition of high nitrogen of coumarin-induced tuberization could be changed by increasing the level of carbohydrates. Tuberization percent was compared in medium supplemented with 30, 60 and 90 g/l sucrose as shown in Table 6

### The optimal medium for tuberization

As mentioned before, our results showed that kinetin-induced medium has a slightly effect to improve tuberization in vitro. But when kinetin was combined with sucrose, this effect will be better when raised sucrose concentration to 90 g/l. Added to that, all the characteristics of tuberization process could be improved by adding coumarin at 20 mg/l. The plantlets were cultured on tuberized medium consisting of kinetin (2.5 mg/l) plus sucrose (90 g/l) and coumarin (20 mg/l) and incubated under the complete dark conditions at 18–20 C° gave the best characteristics of tuberization. The results are shown in Table 7.

## Discussion

Growth regulators are widely used for in vitro multiplication during plantlet production and for micro-tuberization. Some authors consider cytokinins to be

**Table 6** Effect of sucrose on coumarin-inducing media on development of in vitro potato micro-tuberization

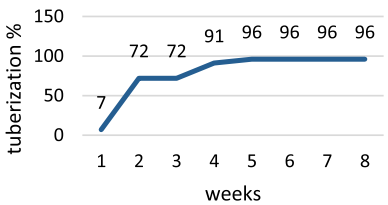
| Characters of microtuberization | Sucrose conc | Coumarin conc. (mg/l) | | | |
|---|---|---|---|---|---|
| | | 0 | 20 | 40 | 60 |
| No. of days to begin tuberization | 3 | 18 ± 0.577b | 18 ± 0.577b | 18 ± 0.577b | 25 ± 0.577a |
| | 6 | 8 ± 0.577c | 8 ± 0.577c | 8 ± 0.577c | 8 ± 0.577c |
| | 9 | 8 ± 0.577c | 8 ± 0.577c | 8 ± 0.577c | 8 ± 0.577c |
| No. of days to reach 50% tuberization | 3 | 25 ± 3.282e | 46 ± 1.527c | 39 ± 1d | 57 ± 0.577a |
| | 6 | 13 ± 1h | 21 ± 0.577f | 51 ± 1.527b | 27 ± 1.527e |
| | 9 | 18 ± 0.577fg | 15 ± 0.577gh | 27 ± 1.154e | 25 ± 0.577e |
| % of tuberization after 8 weeks | 3 | 75 ± 0.577b | 50 ± 2.886de | 66.7 ± 0.866c | 40 ± 2.886e |
| | 6 | 100 ± 0.577a | 60 ± 2.886cd | 60 ± 2.886cd | 100 ± 0.577a |
| | 9 | 75 ± 1.527b | 85.7 ± 8.256b | 100 ± 0.577a | 80 ± 11.547b |
| No of tubers per plantlet after 8 w | 3 | 2.3 ± 0.440a | 1 ± 0.577c | 1 ± 0.577c | 1 ± 0.577c |
| | 6 | 1.6 ± 0.115b | 1.7 ± 0.057abc | 1 ± 0.577c | 1.4 ± 0.115bc |
| | 9 | 1.3 ± 0.057bc | 1.7 ± 0.1abc | 2.1 ± 0.057abc | 1.8 ± 0.264abc |
| Weight of microtubers | 3 | 16.6 ± 0.288i | 55 ± 1.154d | 52 ± 1.154e | 6 ± 0.577j |
| | 6 | 33.7 ± 0.317h | 33.7 ± 0.317h | 33.7 ± 0.317h | 39.7 ± 0.115f |
| | 9 | 63 ± 1.732c | 98.8 ± 0.461b | 35.2 ± 0.115g | 106 ± 1.154a |

Values are means ± SE, LSD test at level ($p < 0.05$)

Values with the same superscript letters (a,b,c or d) are not significantly different from each other whilst, values with different superscript letters are significantly different

involved in tuberization. In general, kinetin advancement and increase in microtuberization are in agreement with these reports of cytokinin effects. Although, several authors attributed an important role to cytokinins such as kinetin (Coleman et al. 2001; Aksenova et al. 2009). Otherwise, Kinetin increases the number of microtubers due to its effect on cell elongation and tuberization (Romanov et al. 2000). However, addition of kinetin (2.5 mg/l) to the medium was less effective on microtuberization induction (Sota et al. 2020). Many researchers reported that sucrose is used for in vitro tuberization of potatoes, especially with high concentrations for enhancing this process. In the present investigation, the highest number of microtubers was produced using 9% sucrose. Bhojwani (2001) found that microtuberization of potatoes can be induced by transferring the shoots from MS medium containing 3% sucrose to the medium supplemented with 10% sucrose under dark conditions instead of light. Also, Sucrose at high concentrations between 6–12% was found to be the most critical stimulus for tuber induction, growth of potato tubers, increasing number of microtubers with incubation period length (El Fatih et al 2004, Wazir et al. 2015, Hossain et al. 2015 and Khan et al. 2018). Our results indicated that microtuberization in vitro can be formed in media containing 6% or 9% sucrose and 2.5 mg/l kinetin under dark conditions and occurred broadly with findings obtained by Hussey and Stacey (1984). Coumarin has long been known to be an inhibitor of growth processes in plants such as germination and root growth. However, it was clear that coumarin has stimulation effect on growth, compared with Indole acetic acid (IAA) but it has a different mode of action (Neuman 1959). Coumarin has physiological effect as it delayed the senescence process, maybe by blocking the loss of chlorophyll (Knypl 1967). Coumarin and cis-o-coumarinic acid lactone readily promote in vitro tuber formation of axillary shoots of

**Table 7** Characters of tuberization process on optimal medium

| Character | Value |
|---|---|
| No. of days to begin tuberization | 2 |
| No. of days to reach 50% tuberization | 14 |
| % of tuberization after 8 weeks | 96% |
| No of tubers per plantlet after 8 w | 1.5 |
| Weight of microtuber (mg) | 87.2 |
| Tuberization curve |  |

potato sprouts. Histological data by Reeve et al. 1969 proved that initiation and growth of tuber are due to cell enlargement and changes in the planes of cell division. In 1979, Stallknecht and Fransworth reported that for critical in vitro research on potato tuberization processes there should be a suitable balance between nitrogen as shoot growth stimulant and sufficient amount of carbohydrate, besides presence of a tuberization promoter like coumarin. As coumarin readily stimulates tuberization of potato axillary shoots, suitable level of coumarin must be continuously found in the culture medium for tuber initiation. Stallknecht and Fransworth (1982) reported that both coumarin and kinetin affect 100% tuberization of the in vitro cultured shoots. The obtained results here suggest that in vitro studies on tuberization processes of potatoes, the balance between the nitrogen stimulated growth and a sufficient supply of carbohydrate ( sucrose), besides tuberization stimulus are available like coumarin.

## Conclusions

Microtuberization is a method could be used in potato seed production programs. The optimal factors for microtuberization have been reported, including kinetin (2.5 mg/l), sucrose (90 g/l) and coumarin (20 mg/l) under complete dark conditions at 18–20 C°.

#### Abbreviations

| | |
|---|---|
| IAA | Indole acetic acid |
| MS | Murashige and Skooge |
| BAP | Benzylamino purin |

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#### Author contributions

AE designed the study, organized and supervised the experimental work and wrote the article. ND performed the experiments, collected data, performed the analysis, drafted the manuscript and wrote the article. All authors have read and approved the manuscript.

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#### Availability of data and materials

All data generated or analyzed during this study included in this published article (and its supplementary information files).

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

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