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Optimization of Photoperiod, Carbon Source and Plant Hormones for Effective Micro-Tuberization in Potato

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Authors Contribution

S. R. Bharath :
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Abstract

Potato micro-tuberization is an effective and efficient in-vitro technique for production of micro tubers through single node cuttings. This technique accelerates the production of high quality disease free potato seeds faster and cheaper than the other methods. However, micro-tuberization is regulated by several key factors such as photoperiod, lower temperature, carbon sources and plant hormones. In the present study, we showed that, short day condition induces tuberization with more efficiency when sucrose is used as a source of carbon than maltose. Further, 50g/l of sucrose was found to be very effective, while increased concentration was rather lethal for growth and micro-tuberization in potato. Although, both BAP @ 2ppm and kinetin @ 1ppm were found to be effective as a source of cytokinin to induce micro-tuberization, kinetin can be a better source of cytokinin as its requirement is only 1 ppm as against 2 ppm of BAP. Taken together, single node cuttings cultured on MS media supplemented with 50g/l sucrose and 1 ppm of kinetin and exposed to a short day condition of 8 hrs. light with 16 hrs. of dark period would be an ideal situation for efficient induction of micro-tubers in potato.

Keywords : Micro-tuberization, Carbon source, Plant hormones, Short days, Sucrose, Kinetin

In-vitro production of tubers through culturing of nodal cuttings is often referred to as Micro-tuberization (Badoni and Chauhan, 2010). Micro tubers have many advantages that make them ideal planting material for producing high quality seed potatoes. Compared with in-vitro plantlets, micro tubers are more robust, easier to handle and can be stored for a longer period (Mc-Cown and Joyce, 1991). In fact, micro tuber production technology offers a wide range of advantages of small space requirement, ease of transport and storage and accelerates the production of high quality disease free potato seeds faster and cheaper than other methods (Ranalli, 1997; Nistor *et al*., 2010 and Raveesha, 2014). In India, the advances in micro tuber productionare considered as second 'green revolution' in agriculture and are expected to make farming more efficient, profitable and environmentally safe in addition to helping the farmers economically, socially and commercially.

Potato micro-tuberization is rather influenced by several *in-vitro* tuber inducing factors like media composition, short day photoperiodic conditions (8h/16h) (Nistor *et al*., 2010), low temperature (18-21^0 C), sucrose or maltose, phyto-hormones like 6-BAP, kinetin and others (Mamiya *et al*., 2020). Many researchers have reported that, MS basal medium is quite effective and efficient for micro-propagation and micro-tuberization in potato (Hussey and Stacey, 1981; Roselli *et al.,* 1987; Aburkhes *et al.,* 1991; Gopal *et al*., 1998 and Ozkaynak and Samanci, 2005). In addition, the short day photoperiods highly influence potato micro-tuberization and would promote aerial stolons and tubers from axillary meristems (Macwan *et al*., 2017;

Ali *et al*., 2018 and Kondhare *et al*., 2021). Besides photoperiod, low temperature (18-21 ^{0}C) seems to be very crucial factor that promotes miro-tuberization in potatoes under in-vitro conditions (Nistor *et al*., 2010 and Macwan *et al*., 2017). A slight increase in temperature would inhibit potato micro tuber formation (Wang and Hu, 1982 and Salem and Hossain, 2017). In addition, altering the media composition with sucrose will further increase the micro-tuberization process. Sucrose is the main tuber inducing stimulus and signaling molecule for micro tuber induction in potato. Therefore, supplying a sufficient amount of sucrose as a carbon source would stimulate plant growth and development, tissue proliferation and increases micro tuber weight and diameter (Perl *et al*., 1991; Khuri and Moorby, 1995; Yu *et al*., 2000; Donnelly *et al*., 2003 and Nistor *et al*., 2010). Maltose is also serving as another important carbon source for the development of a healthy and vigorous plantlets from the nodal explants (Rahman *et al*., 2015). Like sucrose, maltose would also regulate the formation of micro tuber number, tuber weight and micro tuber diameter (Altindal and Karadogan, 2010). However, which carbon source would favor micro-tuberization more effectively needs further examination.

Phytohormones like BAP and kinetin also play a major role in micro-tuberization in potatoes. BAP is the potential option to increase micro tuber number, size, weight and tuber diameter (Fufa and Diro, 2014; Rahman *et al*., 2015; Dessoky *et al*., 2016; Borna *et al*., 2019 and Meenakshi, 2020). However, even kinetin also shown to regulate micro-tuberization under *in-vitro* conditions from single nodal cuttings (Dessoky *et al*., 2016 and Olga *et al*., 2022). The sucrose and kinetin together play a critical role in micro tuber size and tuber weight (Ali *et al*., 2018). Therefore, the main objective of the present study is to standardize the photoperiodic conditions with most effective carbon source and hormonal combination for effective plant growth and micro-tuberization in potato. As many factors regulate micro-tuberization in potato, it is necessary to standardize each of these factors for effective micro-tuberization.

Material and Methods

Planting Material

The experiments were conducted at the Plant Tissue Culture Laboratory, Department of Crop Physiology, UAS, GKVK, Bangalore. The virus-free planting material was procured from the Central Potato Research Institute (CPRI), Shimla and the College of Horticulture, Bengaluru campus, UHS, Bagalkot.

In-vitro Micro-Propagation of Potato and Induction of Micro-Tuberization

Potato micro-propagation is a very important tissue culture technique to multiply plantlets within a short period of time (Pruski *et al.,* 2002). However, multiplication of plantlets in-vitro requires standardization of media, carbon source, plant hormones including regulation of photoperiod. In this context, the said conditions / parameters were standardized. Towards multiplication of plantlets, five important potato cultivars namely, Kufri-Jyoti (KJ), Kufri-Himalini (KH), Kufri-Chipsona-1 (KC-1), Kufri-Chipsona-3 (KC-3) and Kufri-Chipsona-4 (KC-4) were used. As standardization of micro-propagation protocol is a pre requisite for micro tuber production in potato, the micro-propagation protocol was standardized with an overall objective of producing micro tubers in potato.

Standardization of Media Composition

Single nodal explants made from 21 days old potato plantlets were inoculated into a growing MS media. The media composition for micro - tuberization of potato plantlets consist of 75 per cent of basal MS medium supplemented with standardized concentration of sucrose and the plant hormone and the pH was adjusted to 5.8. To this, 5g/l agar was added which served as a solidified agent. The media was autoclaved at 121 ^{0}C for 15 min and after two hours, media was poured into the tissue culture bottles to inoculate the single nodal explants. After solidification of media, single nodal explants were inoculated under the laminar air flow chamber (LAF) and the inoculated bottles were kept exposed to standardized photoperiodic treatment. After

21 days of inoculation, the bottles were observed for *in-vitro* grown plantlets and the growth parameters were recorded in them. Towards inducing micro-tubers, the plantlets were allowed to grow in the same growing condition for some more days and finally, the number of tuber inducing plants and the micro tuber number and size were recorded under each treatment.

Carbon Sources for Induction of Micro-Tuberization in Potato

Carbon source is very important for robust plant growth and micro-tuberization. In this regard, two carbon sources namely, sucrose and maltose @ 50g/l of each was used in the media separately to examine which carbon source favoring robust plant growth and micro-tuberization. Our preliminary experiment indicated that, sucrose is a better source of carbon than maltose and hence, the sucrose concentration was further standardized to examine whether or not the altered carbon source brings in better micro-tuberization in potato. As sucrose is serving as an important carbon source and also a micro tuber inducing stimulus (Nistor *et al.,* 2010), different concentrations of sucrose were used which include 30g/l (T1), 40g/l (T2), 50g/l (T3), 60g/l (T4), 80g/l (T5) and 100g/l (T6) along with the normal MS media.

Standardization of Hormone Source for Effective Micro-Tuberization

Besides media, carbon source and photoperiod, plant hormone is also equally important for effective micro-tuberization. In this context, different cytokinin sources namely, kinetin and BAP were used in the MS media and examined which cytokinin source induces effective micro-tuberization in potato. Accordingly, the cytokinin source which induced more micro tuber was considered as standardized hormone source.

Standardization of Photoperiodic Conditions

Different photoperiodic treatments were given to the cultured single node cuttings to standardize the photoperiodic condition. These treatments include complete dark (T1), complete light (T2), short days (T3) and long days (T4). Complete dark treatment was given in a dark chamber where, complete darkness was maintained all through the day and during the entire growing / culturing period. However, complete light treatment was given through light chambers using LED lights with an intensity of 4000 Lux. Short days were created for about 8 hours of light treatment with 16 hours of darkness using an automatic timer. Similarly, long days were also created by exposing the bottles to artificial light for 16hrs and dark for 8hrs through timer regulation.

Multiplication of Potato Plantlets *In-vitro*

After standardizing various elements of micro-propagation in potato, five different cultivars as mentioned above were cultured in-vitro and plantlets were multiplied. A flowchart depicting the standardization of various elements of micro-propagation of potato under in-vitro condition is given in flowchart 1.

Induction of Micro-Tuberization in Potato

As our main objective is to produce micro tubers in potato, single node cuttings of Kufri Jyoti, one of the leading cultivars of potato in India with high multiplication rate, robust growth and wider adaptability including disease resistance was used. Single node cuttings made from 21 days old plantlets were inoculated into the MS-media supplemented with 50g/l of sucrose and 1 ppm kinetin. Later, the cultured bottles were kept in short day condition of 8h photoperiod (4000 lux) and 16 hr. of dark all through the experimental period and observed for micro-tuberization. The inoculated bottles were kept inside the BOD incubator where the temperature was maintained at 18 ^{0}C with an RH of 80 per cent. At the end of 45 days, initiation of micro tubers was observed and recorded. The flowchart depicting the steps followed for micro-tuberization is given in flowchart 2.

Statistical Analysis

Data on plant height, number of nodes, no. of leaves, micro-tuber number, weight and tuber diameter were collected during harvest. Further, all the collected data

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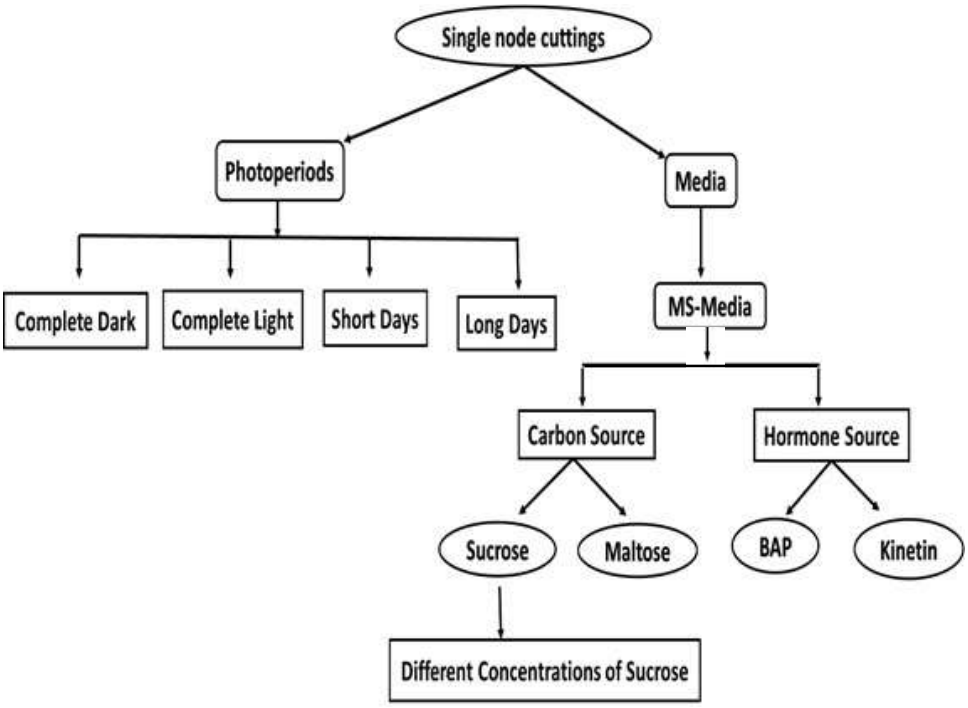
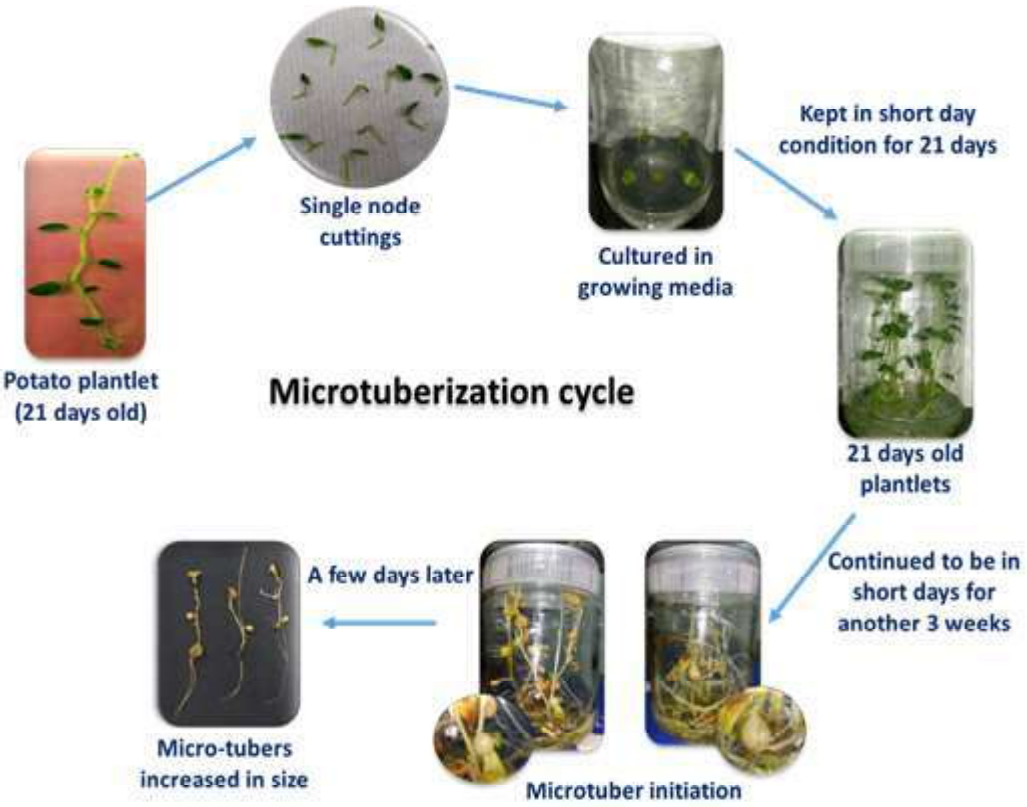


Flowchart 1: A Flowchart depicting standardization of various elements for micro-propagation in potato



Flowchart 2: Schematic representation of different steps involved in micro-tuber production in potato

were analyzed by analysis of variance and the means were compared according to DMRT (Duncan's Multiple Range Test) and the mean separation was done at 5 and 1 per cent level of significance.

Results and Discussion

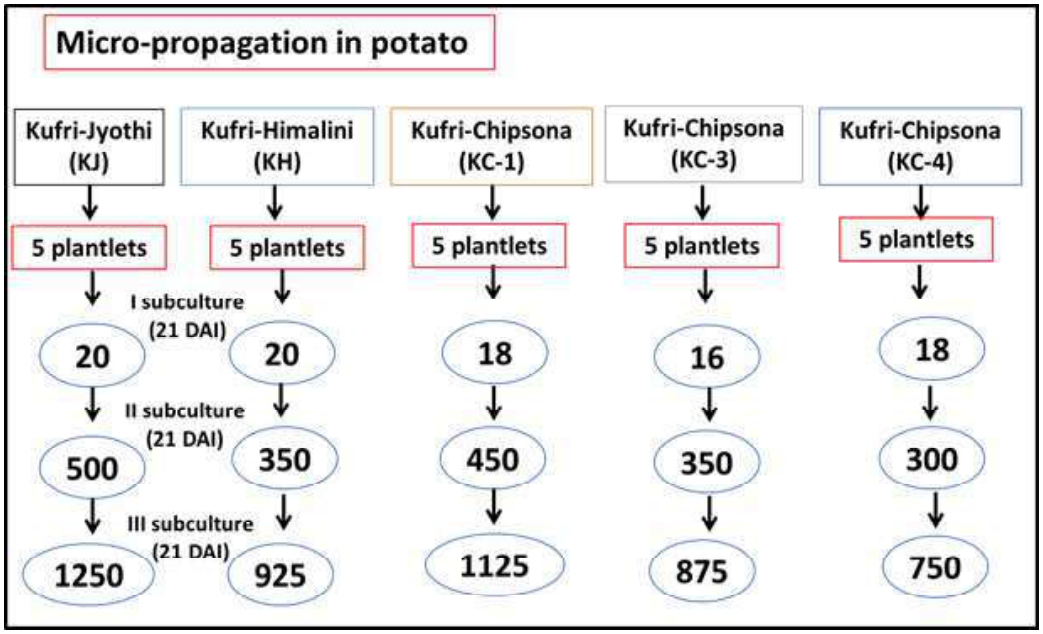
Potato micro-propagation is regulated by several factors which include photoperiod, media composition, carbon source and even plant hormones. Therefore, in the present study, the influence of the said parameters was evaluated for micro tuber induction in potato.

Mass Multiplication of Potato Plantlets

Initially, mass multiplication of potato plantlets free from pests and diseases were taken up to generate the required number of plantlets for inducing micro-tuberization. Accordingly, mass multiplication was taken up in 5 varieties of potato. Initially, 5 plants each were taken from each of the varieties and sub cultured the single node cuttings for 21 days and end of which, the plantlets generated were again sub cultured to produce more number of plantlets (Flowchart 3). Accordingly, at the end of 3rd sub culturing, sufficient number of plantlets were produced. Interestingly, Kufri Jyoti, one of the leading varieties (Srikant Tengli and Mohan Raju, 2022) produced significantly higher number of plantlets of 1250 while, Kufri Chipsona had only 750 plantlets to suggest the variation in number of plantlets generated at the end of each sub culturing. It appears that, Kufri Jyoti responds very well to sub culturing and mass multiplication compared to the other varieties (Flowchart 3). Such differential response was also noticed by Pavithra (2020) while working with different cultivars of potato, where she found significant variation in number of plantlets generated at each cycle due to higher rate of mortality in some cultivars (Flowchart 3).

Effect of Carbon Sources on Growth and Micro-Tuberization in Potato

Although maltose and sucrose serve as carbon source for in vitro grown plantlets, sucrose seems to be very effective as a source of carbon as it is not only triggered the vegetative growth, but even the micro-tuberization in potato. Single node cuttings cultured under in-vitro media with sucrose as a



Flowchart 3: Mass multiplication of different cultivars of potato under in-vitro conditions and their success rate

Note: Single node cuttings were used in every sub-culturing steps
DAI-Days after inoculation

source of carbon showed significantly higher number of nodes, leaf number and even the root length. In addition, more than 80 per cent of the plantlets produced micro tubers as against only 25 per cent with maltose as carbon source. Further, the number of micro tubers per plant and also the fresh weight of micro tuber was significantly higher when sucrose was used as a carbon source instead of maltose. The results therefore suggest that, sucrose seems to be the ideal carbon source than maltose for effective growth and micro-tuberization in potato (Table 1 and Plate 1).

The importance of sucrose as a source of carbon for effective micro-tuberization has been reported by several other workers. Accordingly, increased biomass, micro tubers, micro tuber dry matter was promoted with sucrose as a source of carbon compared to the other sources of carbon (Gopal *et al.,* 2004 and Altindal and karadogan, 2010). In several earlier studies, it has been reported that, concentration of sucrose decides the efficiency of micro-tuberization in potato. In one of the studies, Hossain *et al.* (2017) have reported that, the micro-tuberization and micro tuber number was highest in 8 per cent sucrose and as the sucrose concentration increased, they observed decreased micro-tuberization. Similar observation was also made by others where they showed improved micro-tuberization when the sucrose concentration increased from 3-8 per cent in the media (Omokolo *et al.,* 2003 and Macwan *et al.,* 2017).

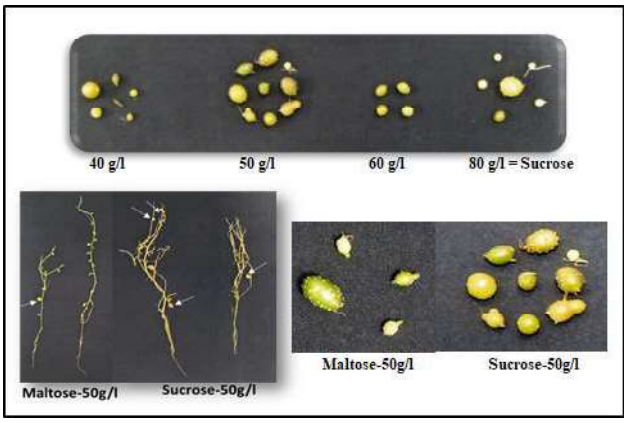


Plate 1: Plant growth and micro-tuberization as influenced by different carbon source

Sucrose is rather serving as energy source and osmotically active compound and at higher concentration, it serves as a signaling molecule for micro tuber formation (Perl *et al*., 1991, Donnelly *et al*., 2003 and Gibson, 2005). According to Garner and Blake (1989), the use of 8 per cent sucrose would increase the tuber initiation, number and weight of micro-tubers compared to 4 per cent sucrose concentration. However, further increase in the concentration of sucrose up to 12 per cent caused a delay in tuber initiation resulting in smaller tubers.

In this direction, when we examined the concentration of sucrose on plant growth and micro-tuberization, we found that, the plant growth, number of nodes, number of leaves and root length increased up to a

TABLE 1

Effect of different sources of carbon on growth and micro-tuberization in potato (Var. Kufri Jyoti)

Treatments	Plant height (cm)	No. of nodes	No. of leaves	Root length (cm)	% Micro-tuberizing plants	Number of micro tubers /plant	Micro tuber fresh weight (g)
Maltose-50g/l	13.67 [a]	21.89 [b]	19.89 [b]	7.39 [b]	25.00 [b]	0.31 [b]	0.09 [b]
Sucrose-50g/l	12.33 [b]	36.56 [a]	32.78 [a]	8.10 [a]	83.33 [a]	1.18 [a]	0.18 [a]
CD (P<0.01)	0.67 **	0.32 **	0.44 **	0.23 **	1.14 **	0.038 **	5.37 **
SE(m)	0.17	0.08	0.11	0.06	0.28	0.009	1.33
SE(d)	0.24	0.12	0.15	0.08	0.40	0.013	1.89
CV (%)	2.22	0.48	0.72	1.28	2.71	2.197	2.70

** Significant at 1% level

TABLE 2

Effect of different concentrations of sucrose on various growth parameters in in-vitro grown potato plantlets (Var. Kufri Jyoti)

Treatments	Plant height (cm)	No. of nodes (no.)	No. of leaves (no.)	Root length (cm)
T_1-Sucrose-30g/l	8.28 ^{d}	19.11 ^{d}	15.67 ^{e}	6.78 ^{bc}
T_2-Sucrose-40g/l	10.17 ^{b}	25.73 ^{c}	20.89 ^{c}	6.50 ^{c}
T_3-Sucrose-50g/l	11.83 ^{a}	34.67 ^{a}	32.74 ^{a}	7.78 ^{a}
T_4-Sucrose-60g/l	9.29 ^{c}	33.67 ^{b}	33.44 ^{a}	6.93 ^{b}
T_5-Sucrose-80g/l	8.22 ^{d}	34.50 ^{ab}	31.44 ^{b}	6.67 ^{bc}
T_6-Sucrose-100g/l	7.78 ^{d}	16.54 ^{e}	18.78 ^{d}	6.12 ^{d}
CD (P<0.01)	0.68 **	0.85 **	1.23 **	0.33 **
SE(m)	0.22	0.28	0.40	0.11
SE(d)	0.31	0.39	0.56	0.15
CV (%)	4.13	1.74	2.69	2.74

** Significant at 1% level

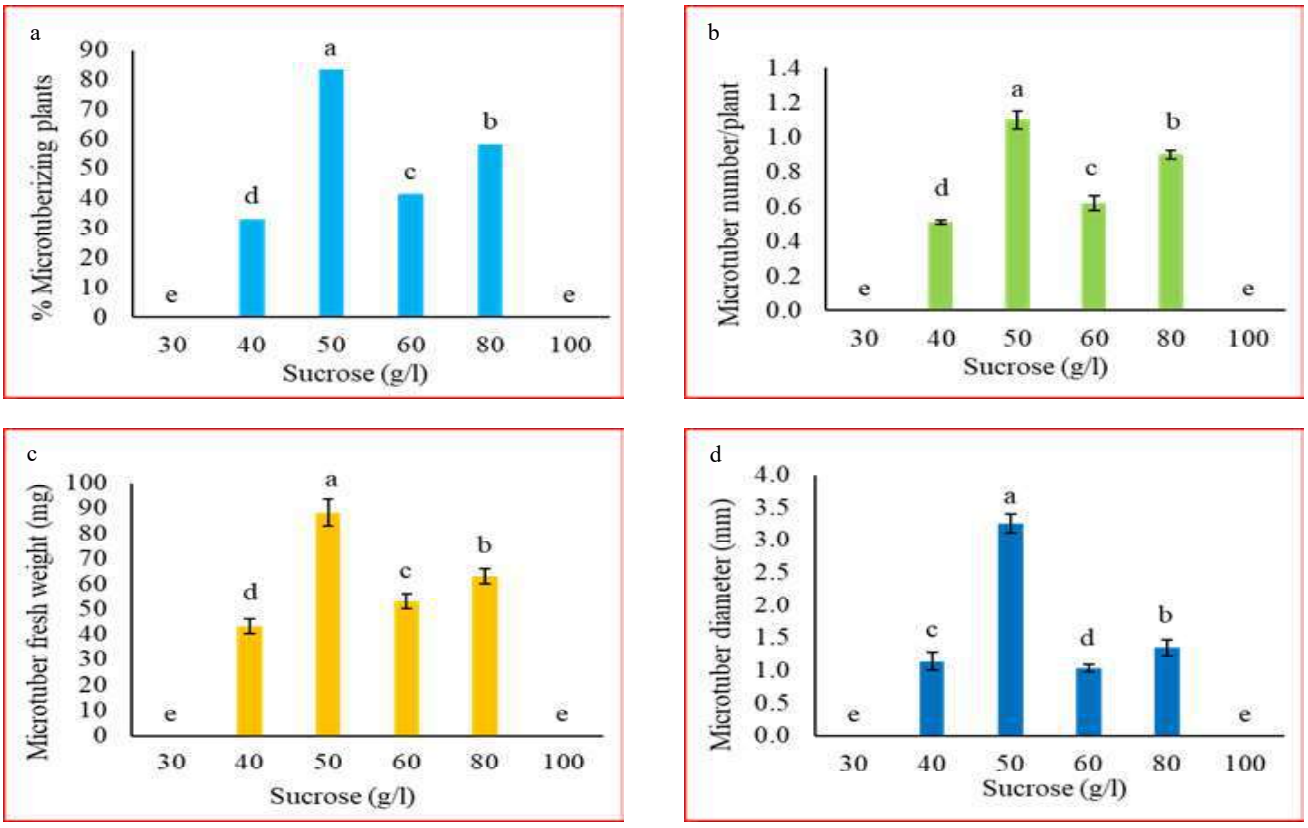


Fig. 1: Effect of different concentrations of sucrose on micro-tuberization in potato (Var. Kufri Jyoti); a) % Micro-tuberizing plants b) Micro tuber number/ plant, c) micro tuber fresh weight and d) Micro tuber diameter

concentration of 50 g/l from 30g/l (Table 2). Further, the number of tuberizing plants and the number of micro tubers per plant with tuber fresh weight and diameter increased gradually with increased concentration of sucrose (Plate 1 and Fig.1). However, with increase in sucrose concentration in the media beyond 50g/l, a reduction in said parameters was observed which corroborates with the results of earlier workers. Very interestingly, significantly very high number of micro tuberizing plants coupled with increased micro tubers per plant as well as fresh weight and diameter observed in plants grown in a media supplemented with 50g/l of sucrose compared with the rest of the concentrations. However, at a very high concentration of 100 mg/l of sucrose, we did not observe any tuberizing plants suggesting that, the higher concentration of sucrose becomes lethal for micro-tuberization in potato (Fig.1).

Effect of Cytokinin on Micro-Tuberization in Potato

Cytokinin plays an important role in micro-tuberization in potato. In this regard, different sources of cytokinins namely, BAP and kinetin were included in the growth media separately. While the BAP was used at a concentration of 1, 2, 4 and 6 ppm, the kinetin was used at a concentration of 0.5 and 1.0 ppm separately. The results of the study indicated that, 2 ppm BAP was found to be effective in increasing the micro-tuberization with higher number of micro tubers per plant coupled with more fresh weight and diameter compared to control and other concentrations of BAP (Fig 2). Similarly, 1 ppm of kinetin was found to be effective than 0.5 ppm and interestingly, the response was similar to that of BAP at 2 ppm. At 1 ppm of kinetin, nearly 79 per cent

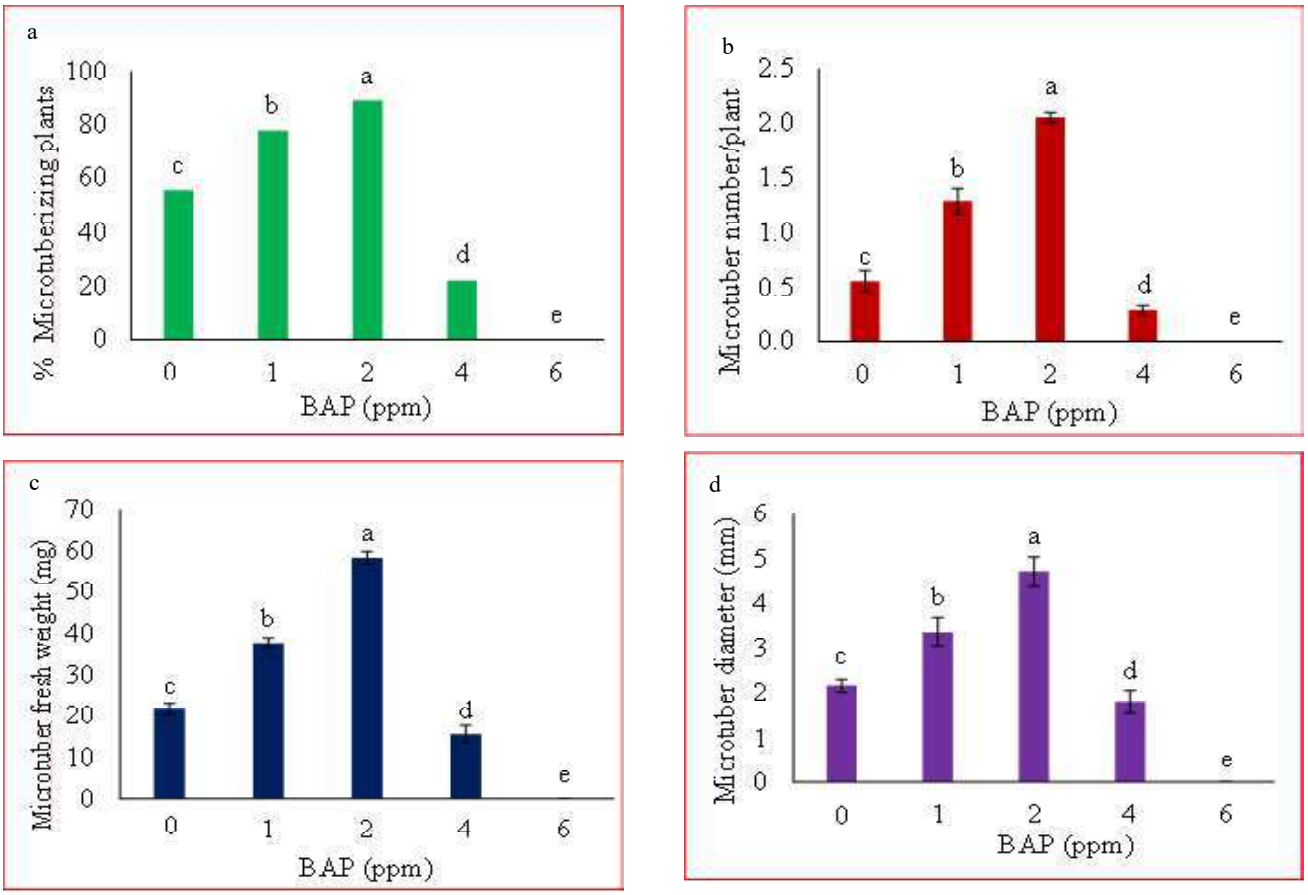


Fig. 2: Effect of 6-BAP on micro-tuberization in potato; a) % Micro-tuberizing plants b) Micro tuber number per plant c) Micro tuber fresh weight and d) Micro tuber diameter

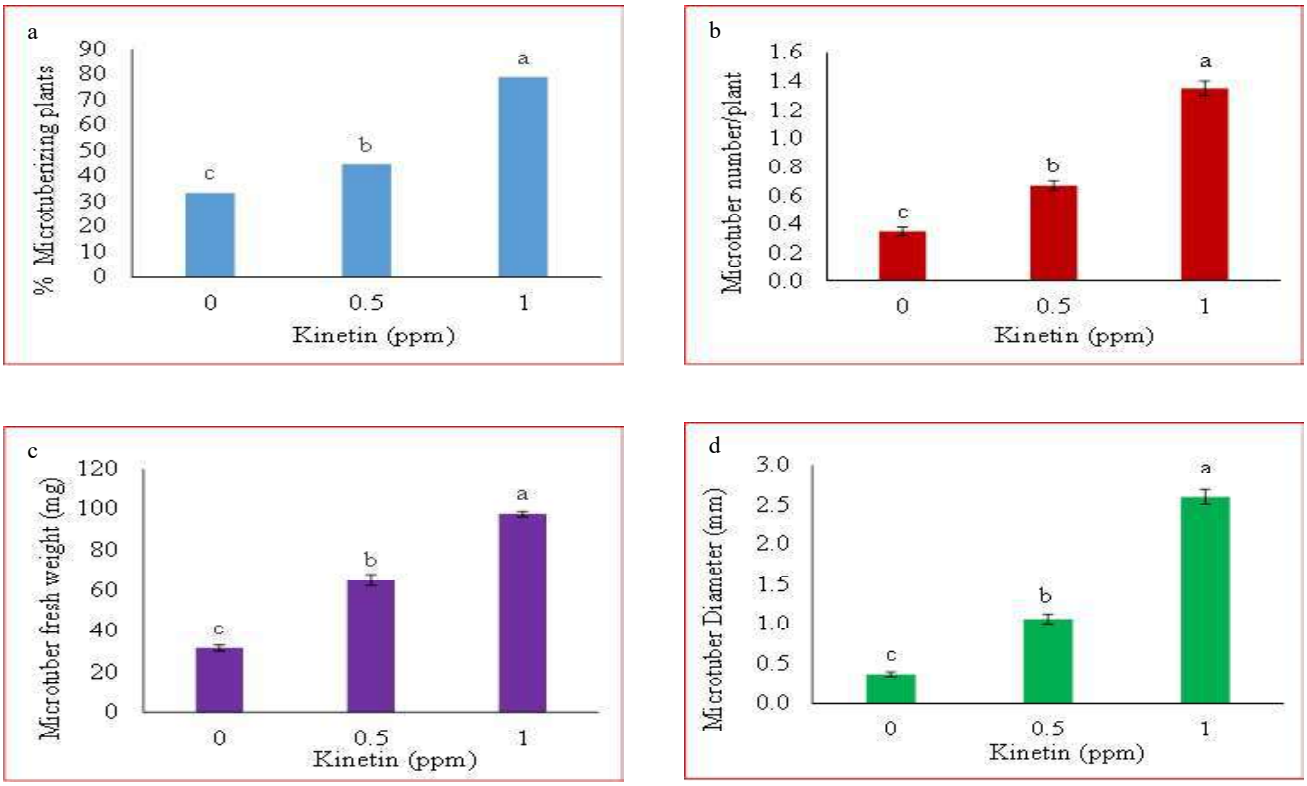


Fig. 3: Effect of Kinetin on micro-tuberization in potato; a) % Micro tuberizing plants b) Micro tuber number per plant c) Micro tuber fresh weight and d) Micro tuber diameter

of micro-tuberizing plants was observed with significantly higher fresh weight and diameter of micro tubers compared to 0.5 ppm of kinetin (Fig 3). The results therefore suggest that, 1 ppm of kinetin is effective for micro-tuberization in potato.

In contrast to our observations, others have found BAP as a good source of cytokinin for effective micro-tuberization. Accordingly, cultured medium supplemented with 2.25 mg/l (Meenakshi, 2020) and 5 mg/l of BAP (Dessoky *et al*., 2016) showed higher percentage of tuberizing plants as well as micro tubers suggesting the other sources of cytokinin would also do the job of inducing micro tubers in potato. Our study however indicates that, 1 ppm of kinetin would be sufficient to bring in the desired effect on micro tuberization in potato.

Effect of Photoperiod on Micro-Tuberization in Potato

Micro-tuberization in potato is influenced by photoperiod. While some reports indicated a good response in complete darkness, yet others showed the requirement of short days or longer days for micro-tuberization. In this regard, the effect of photoperiod on micro-tuberization was examined by exposing the single node cuttings of potato under *in-vitro* condition to either complete darkness or complete light or short days or long days. The results indicated that, both complete darkness or complete light for 24 hours a day did not show any response for micro-tuberization although they put on some plant height. However, short day photoperiod of 8 hrs. of light and 16hrs of dark period showed a higher micro-tuberization under hormonal treatment. Accordingly, under control condition without hormone treatment, the number of micro tubers produced per plant was 0.56 as against 1.29 tubers observed under hormonal treatment which is significant. Further, the average fresh weight and diameter of micro tubers was 108 mg and 3.88 mm respectively for the micro tubers produced under hormonal treatment, while, it was 81.6 mg and 1.92 mm respectively for control (Table 3).

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TABLE 3

Effect of photoperiod on micro-tuberization in potato (Var. Kufri Jyoti)

Treatments	Plant height (cm)		No. of micro tubers/plant		Average fresh weight of micro tubers (mg)		Average diameter of micro tubers (mm)	
	Control	Hormone	Control	Hormone	Control	Hormone	Control	Hormone
T_1- Complete Dark	19.10 [a]	9.26 [b]	0.00 [b]	0.00 [c]	0.00 [b]	0.00 [c]	0.00 [b]	0.00 [c]
T_2- Complete Light	8.56 [c]	9.43 [a]	0.00 [b]	0.00 [c]	0.00 [b]	0.00 [c]	0.00 [b]	0.00 [c]
T_3- Short Days	9.02 [b]	8.50 [c]	0.56 [a]	1.29 [a]	81.6 [a]	108.0 [a]	1.92 [a]	3.88 [a]
T_4- Long Days	9.24 [b]	5.92 [d]	0.00 [b]	0.69 [b]	0.00 [b]	98.0 [b]	0.00 [b]	3.06 [b]
CD (P<0.01)	0.33 **	0.11 **	0.004 **	0.012 **	0.37 **	1.35 **	0.037 **	0.08 **
C X H	1.046		0.037		0.016		0.491	
SE (m)	0.11	0.04	0.001	0.004	0.12	0.45	0.012	0.03
SE (d)	0.16	0.06	0.002	0.005	0.17	0.63	0.017	0.04
CV (%)	2.15	1.06	1.97	1.74	1.34	1.94	5.65	3.53

** Significant at 1% level

Under long day conditions, micro-tuberization did not take place in control treatment. However, with the intervention of hormones, tuberization did take place with low efficiency both in number of micro-tuberizing plants as well as fresh weight and tuber diameter. The results therefore suggest that, short day condition induces micro-tuberization in potato. Very interestingly, when micro-tuberization is not possible under long day conditions, with the intervention of cytokinin, the plants could be made to induce micro-tuberization although the number and fresh weight and diameter is significantly lower compared to the plants exposed to short day conditions (Table 3).

Induction of tuberization in dark or at low light intensities with a light period of 8 hrs has been shown by several earlier workers (Struik and Wiersema, 1999; Dobrfinski, 2000 and Martlnez-Garcfa *et al*., 2002). However, in contrary to this, Macwan *et al*., (2017) have reported that, light conditions of 16 hrs. photoperiod was found to be the best at a temperature of 18 °C for tuberization with micro tubers showing increased weight and size. Similarly, cultures incubated at 20 °C either in dark for 8 weeks or two weeks in 16-h photoperiod followed by 6 weeks in dark, a combination of both dark and 16h-photoperiods promoted micro-tuberization in potato (Salem and Hassanein, 2016) suggesting both short days and long days would influence micro-tuberization in potato. However, in our study, we found that, short days favored micro-tuberization with no tuberization under long days.

In-vitro micro-tuberization is an effective way of producing disease free quality planting material in potato which not only saves space significantly, but tubers can also be stored for longer time. However, it is influenced by several factors and the present study revealed that, micro-tuberization was found to be effective when single node cuttings were cultured on MS media supplemented with 50g/l of sucrose and 1mg/l of kinetin and keeping the cultured bottles in short day conditions of 8 hrs. light with 16 hrs. of dark period.

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